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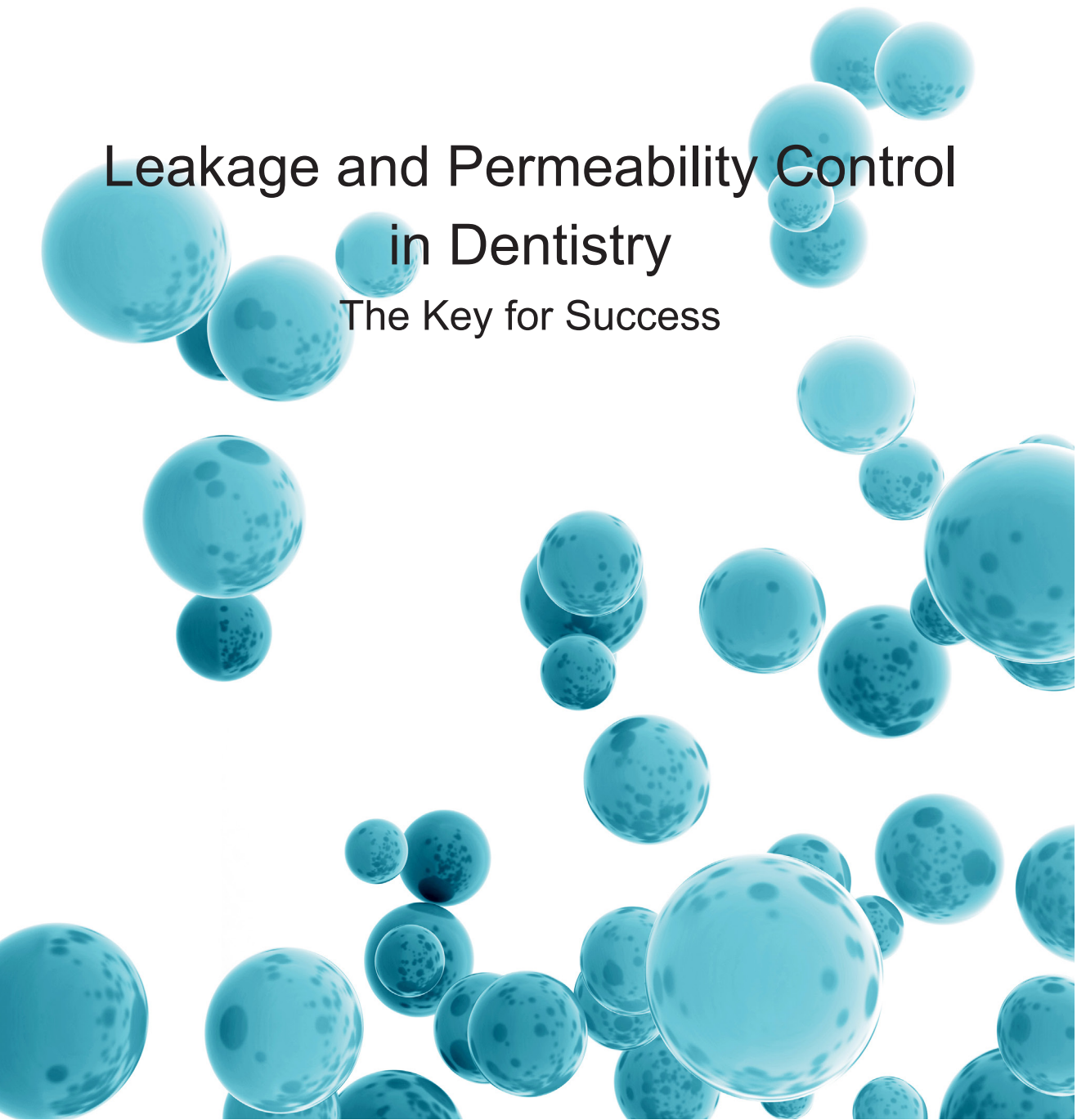
ANAS AL-JADAA

# Leakage and Permeability Control in Dentistry

## The Key for Success



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## The Key for Success

ACADEMIC DISSERTATION
To be presented, with the permission of
the Board of the School of Medicine of the University of Tampere,
for public discussion in the small auditorium of building M,
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UNIVERSITY OF TAMPERE

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UNIVERSITY OF TAMPERE

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This PhD is dedicated to the soul of my father, who taught me to never give up. To my mother who's her support and encouragement inspired me throughout my life.

# CONTENT

## List of original publications

This thesis is mainly based on the following articles, referred to in the text by their Roman numerals.

I. Laboratory validation of a new gas-enhanced dentine liquid permeation evaluation system.
Al-Jadaa A, Attin T, Peltomäki T, Heumann C, Schmidlin PR.
Clin Oral Investig. 2014 Dec;18(9):2067-2075.

II. Comparison of three *in vitro* implant leakage testing methods.
Al-Jadaa A, Attin T, Peltomäki T, Schmidlin PR.
Clinical Oral Implants Research. 2015 Apr; 26(4):e1-7.

III. Impact of Dynamic Loading on the Implant-abutment Interface Using a Gas-enhanced Permeation Test *In Vitro*.
Al-Jadaa A, Attin T, Peltomäki T, Heumann C, Schmidlin PR.
Open Dentistry Journal. 2015 Mar; 31(9):112-119.

IV. Evaluation of a novel repetitive gas-enhanced permeation test for restoration leakage determination after thermo-mechanical loading
Al-Jadaa A*, De Abreu D*, Attin T, Peltomäki T, Heumann C, Roger Schmidlin P.
Acta Odontologica Scandinavica.. 2015 Sep; (16):1-8. [Epub ahead of print]
DOI: 10.3109/00016357.2015.1085090

*These authors contributed equaly to the accomplishment of the publication

## List of abbreviations

| | |
|---|---|
| DNA | Deoxyribonucleic acid |
| GEPT | Gas Enhanced Permeation Test |
| IAI | Implant-Abutment Interface |
| SEM | Scanning Electron Microscopy |
| μ-CT | Micro-Computed Tomography |
| FUM | Fluid Medium |
| EDTA | Ethylenediaminetetraacetic acid |
| PVC | Polyvinylchlorid |
| B3i | Biomet 3i Implant System |
| AT | Astra Tech Implant System |
| NB | Nobel Biocare Implant System |
| RCT | Root Canal Treatment |
| UCI | Upper Central Incisors |
| MRLM | Mesial Roots Lower Molars |
| WV | Water Volume (ml) |
| IQR | Interquartile Ranges |
| SD | Standard Deviation |
| ANOVA | Analysis of Variance |

## Abstract

The history of causality between oral microbiota and oral diseases returns back in its roots to 1884. Though the theory was non-specific, oral diseases were related to the overall accumulation of dental plaque. Since the establishment of dentistry as a separate health care profession in the late 19th century, it concentrated on the treatment of oral diseases and prevention of their occurrence by preventing plaque accumulation in ecological niches. The idea of eliminating artificial ecological niches to eliminate the accumulation rate, by increasing the used materials adaptation appeared with the first leakage test in 1912. Since then, leakage testing models were developed to investigate this phenomena. The acceptance of these models over the years has changed due to their shortcomings in addition to the application of improper methods/materials which led to faulty conclusions.

The aim of this thesis is to develop a testing method which can overcome the disadvantages of the previously known leakage and permeability methods and at the same time can be applied in different dental disciplines. More specifically, the first study used a tooth model with different dentinal wounds sizes, to evaluate the new method for its repeatability, detection limit as well the correlation of the infiltrated fluid volume to the pressure difference change over time. A second study that utilised extracted third molars with class I preparation were used to verify the influence of bonding on the sealability of different restorations and at the same time to compare the new system to the well-known SEM marginal surface analysis as well as the Fuchsin permeation test. In another study, root canals with simple and complicated root canal anatomies were used to correlate the measured leakage values as determined with the new method to the root canal volumes sealed with a root canal filling. For the last two studies, three implants systems of different designs, but almost the same dimensions, were used to compare the new method measured values to the substrate (endotoxin like) and bacterial leakage

tests. These were also used to investigate the influence of thermo-mechanical loading on implants leakage.

The idea of the new testing method is based on measuring the pressure difference change established between two chambers with the sample held in between, the capability of the sample to maintain a tight seal between the chambers contributes to the sample's leakage indirectly. Simultaneously, the permeated fluid volume through the sample is measured as a direct indicator of the sample leakage status.

The results showed a high repeatability, low detection limit, a high correlation of the penetrated fluid volume to the rate of difference change over time and a proper response of the measured permeation in correlation to the dentinal wound size. It also proved the embedding used to be reliable over time with almost no change in its efficiency after multiple measurements. The importance of bonding in preventing leakage was clearly noticeable when testing different restorative materials and protocols. Correlation between different tests applied was in the favour of the new method to the gold standard (Fuchsin penetration test) over the traditional SEM marginal surface analysis. The different implant systems tested showed consistent performance patterns for both testing conditions (under static conditions and under dynamic conditions), where non-significant changes in their measured leakage values could be noticed after the thermo-mechanical loading. The new method showed a consistent correlation to the bacterial leakage patterns as indicated by the day at which leakage was observed under all tested conditions. This correlation was missing once comparing both testing methods to the substrate (endotoxin like) leakage testing method.

The new method, proved itself to be reliable and correlates well to the most acceptable leakage/permeation testing methods.

# Tiivistelmä

Tutkimuksen tarkoituksena oli kehittää saumavuotoa testaava menetelmä, jota voitaisiin soveltaa hammaslääketieteellisessä materiaalitutkimuksessa.

Uuden testausmenetelmän perusideana on mitata paine-eroa kahden kammion välillä, kun testattava näyte on asetettu kammioiden väliin. Materiaalin täydellisen saumatiiviyden ollessa kyseessä kammioiden välisen paine-eron tulisi säilyä muuttumattomana. Samanaikaisesti näytteen läpi kulkevan nesteen tilavuus mitataan indikoimaan näytteen saumatiiviyttä. Täydellisen tiiviyden ollessa kyseessä nesteen virtausta ei tapahdu.

Ensimmäisen tutkimuksen tarkoituksena oli tutkia uuden menetelmän mittausten toistettavuutta, havaintotarkkuutta sekä nestemäärän ja paineen muutosten välistä yhteyttä ajan funktiona. Näytteinä käytettiin hampaita, joihin oli tehty erisuuruisia dentiiniin ulottuvia kaviteetteja. Toisessa tutkimuksessa käytettiin testausmateriaalina poistettuja viisauden-hampaita, joihin tehtiin standardoidut kaviteetit ja jotka täytettiin eri materiaaleilla ja menetelmillä. Saumatiiviyden tutkimuksessa uutta menetelmää verrattiin pyyhkäisy-elektronimikroskoopilla (SEM) sekä fuksiini-värin imeytymisellä saataviin tulosiin. Endodonttisessa tutkimuksessa käytettiin saumatiiviyden tutkimisessa hampaita, joissa oli joko yksinkertainen tai monimuotoinen juurikanavan anatomia. Hampaisiin tehtiin juurentäytteet ja näitä verrattiin juurikanavan volyymiin nähden. Kahdessa viimeisessä tutkimuksessa tutkimusmateriaalina käytettiin kolmen eri valmistajan implantteja, jotka olivat kooltaan lähes samanlaisia, mutta poikkesivat rakenteeltaan. Implantti-abutmentti saumatiiviyttä tutkittiin uuden menetelmän lisäksi kemiallisella ja bakteeritestillä sekä altistamalla implantit lämmölle ja mekaaniselle rasitukselle.

Kehitetyn menetelmän havaittiin tuottavan samat tulokset toistomittauksissa, olevan havaintotarkkuudeltaan hyvä sekä havaitsevan nestemäärän ja paineen muutosten välisen yhteyden ajan funktiona luotettavasti.

Tulokset osoittivat myös, että testausmateriaalien kiinnitys oli luotettava ja mittausten toistaminen tuotti lähes identtiset tulokset. Sidostamisen tärkeys estää sauman vuotamista tuli selvästi esille tutkittaessa eri restoraatiomateriaaleja ja -menetelmiä. Verrattaessa uutta menetelmää SEM:llä tai fuksiinilla saataviin tuloksiin havaittiin uuden toimivan ainakin yhtä hyvin kuin nämä perinteiset testausmenetelmät. Tiiviin juurikanavan täytön merkitys etenkin monimuotoisissa juurissa tuli selvästi esille korrelaationa juurikanavan täytön laadun ja sauman vuodon välillä. Eri valmistajien implanttijärjestelmät osoittivat toimivan samalla tavalla molemmilla menetelmillä tutkittaessa ja sekä staattisessa että dynaamisessa testissä. Ei merkitsevä muutos havaittiin, kun implantit altistettiin lämpömekaanisesti. Lisäksi uusi menetelmä osoittautui korreloivan toistettavasti bakteerivuototestin kanssa.

Yhteenvetona voidaan todeta, että väitöskirjatyössä kehitetty saumatiiviyttä testaava menetelmä on luotettava ja korreloi hyvin aikaisemmin käytettyjen menetelmien kanssa.

# 1. Introduction

The oral cavity - the most proximal part of the gastro-intestinal track - is inhabited with a large variety of microbiota, which are responsible for oral health in the case of an adequately balanced ecology, but also disease, especially when pathogenic species become predominant. The main pathological entities in this context are periodontitis and caries. Whereas periodontal therapy basically relies on the re-establishment of a good oral hygiene, the first defence line against caries disease lies in prevention and strengthening the tooth structure through fluoride application (Arnold 1948), or the use of non-fermentable sugars, such as xylitol (Twetman 2009). In case of disease development (caries) and loss of tooth structure, i.e. decay, the therapy of choice, still focuses on the repair or restoration. The latter is primarily accomplished by the use of direct restorations (i.e. resin composite, glass ionomer cement, compomer cement) and/or indirect restorations (ceramic, gold and/or base metal alloys). If teeth reached an end stage of vitality, ending up non-vital, tooth hard tissue can be preserved by applying root canal treatment (RCT). In worse scenarios where teeth are lost, fixed or removable prosthetic appliances (i.e. bridges, removable dentures), using abutment teeth or implants for retention (i.e. artificial roots) are required for oral rehabilitation. Most available materials and techniques in this context inevitably create one or more interfaces. Due to the chemo-mechanical and physical differences between natural tooth structure and dental materials used, the interface is considered highly susceptible for pathological changes, especially at the interface between the remaining tooth structure and the overlying reconstructions (Hickel and Manhart 2001, and Manhart *et al.* 2004). In case of dental implants, an evidence correlating peri-implantitis to the so-called implant-abutment interface was established, this interface was found to be susceptible for bacterial inoculation, which may jeopardize the health of adjacent supportive tissues by inducing inflammation (Becker *et al*, 1990).

In order to optimize clinical performance and to reduce biological risks by bacterial re-colonization and thus a new disease initiation/progression, the establishment of an optimal integrity between different materials and components remains an important focus in dental research. Testing methods allowing for comparison of different materials as well as different rehabilitation techniques, are mandatory to increase our understanding of adverse factors influencing treatment prognoses and outcomes. While clinical studies are cost-intensive, time-consuming and sometimes ethically questionable, laboratory studies still provide a suitable alternative.

## 1.1. Laboratory testing of permeability and leakage

The therapy of dental caries has a long tradition and therefore it is not surprising that several techniques and systems have been developed in the last decades to improve the adaptation of dental restorations materials to reduce the risk for potential sequels i.e. recurrence of caries and further loss of tooth structure.

To assess restoration integrity, leakage or permeation tests are most frequently used to evaluate restoration adaptation (Güngör *et al.* 2014). These tests are based on studying the penetration phenomena through interfaces and gaps (Kidd, 1976), cracks or dentinal structure, e.g. dentinal tubules. The applied leakage test methods in dentistry can be principally categorised based on the penetrating substrate/assessment method in the following subclasses:

- Fluid penetration: Based on quantitative determination of fluids penetrating through a sample within a certain period of time.

- Microbial leakage: Provides mainly a qualitative indication of tightness at interfaces by assessing the penetrating bacteria, by means of size and number of penetrating microbe.
- Marker penetration: Determines the penetrated fluid volume indirectly by measuring the concentration of a detectable marker. Potential target substrates include glucose, endotoxins and radioactive ions (Crisp and Wilson 1980)
- Dye penetration: Visualizes leakage pathways using fluorescent dyes, colorants, e.g. basic fuchsin.
- Gap measurement: Determines the quality of interface by visualization using high-resolution radiographs, scanning electron microscopy (SEM) and μ-CT.
- Gas penetration: Measures leakage gas pressure loss using gas flow through presumably untight samples.

Most *in vitro* leakage testing models have not delineated clinical implications and more importantly, most leakage models are not universally applicable and accepted due to some experimental limitations (De-Deus *et al.* 2012).

The capability of some testing set-ups of investigating only at a single time point, while the sample should be sacrificed or disassembled in order to test and measure, is another shortcoming of such tests. In addition, the results of such test protocols do not allow for studying and comparing different materials as well different treatments applied to identical sample at different testing times/intervals.

Due to these mentioned limitations, the need for re-designing a new testing platform seems to be a necessity to reliably measure leakage based on the advantages offered by the currently available testing methods.

Gas penetration method has lately been described to quantitatively determine leakage in dental implants (Romieu *et al.* 2008). This method appears to be simple and reproducible, however, the validation of the method and standardized measurement conditions have not been determined and described to

date. One possible disadvantage of this method is the use of gas as the penetrating substrate, which does not necessarily corresponds to clinical situation in the oral cavity. However, if adequately modified and controlled, the method may be fast, non-destructive and may allow repeated evaluation sequences after different treatments steps, e.g. loading or wear conditions. Therefore, gas penetration method may represent an ideal tool for the investigation of leakage in a variety of dental materials. In addition, liquid percolation may also be assessed at the same time, which allows for more clinically relevant assessment

## 1.2. Dentine permeability

Dentine has a unique tubular structure which represents an effective evolutionary adaptation to improve the biological function of teeth. This structure not only enables withstanding the mastication forces by transducing bite pressures into tensile forces in the collagen matrix (Kishen *et al.* 2000) but also allows stimulus transmission by fluid-filled dentinal tubules to the underlying pulp (Brännstrom *et al.* 1967) and supports the alarming protective function of the pulpal nervous system. The pulp-dentin complex represents a sophisticated sensitive organ. Exposure of dentinal tubules can lead to hypersensitivity or – if adjacent to infectious pathological processes like caries (West *et al.* 2013) - may open a pathological pathway, leading to the initiation of pulpal and periradicular changes (Chogle *et al.* 2012). Effective protection of dentinal tubules therefore thus has a pivotal role in clinical dentistry.

The observation of fluid permeation through dentinal tubules of extracted teeth led to various *in vitro* models assessing dentinal wound models (Brännstrom *et al.* 1967 and Spreter *et al.* 1951). The most well-known and accepted method for dentine permeability is the fluid shift method introduced by Brännstrom *et al.* in 1967. This model has been digitized to measure the infiltrated fluid volume in

patients *in vivo* (Ciucchi *et al.* 1995). This progress significantly helped studying the effects of different stimuli, which can be directly applied to vital teeth. Disadvantages of the method are long testing time and lack of information regarding the initial status of the embedding quality around the tested sample. Possible leakage due to embedding failure cannot be excluded. Modifications of this basic method using substrates (e.g. larger molecules) penetrating through dentinal tubules (Pashley *et al.* 1977) are less acceptable. This is due to the possibility of blockage of the dentinal tubules once insoluble or larger substrates are used, resulting in a false negative readings (Pashley and Livingston 1978).

Inspired by this method, modified versions were developed to test the leakage in restorations, implants at their abutment-implant interface as well as in root canal treatments (RCT). The versatile split-chamber model design to test infiltration of isotopes was a revolution in leakage and permeability testing (Outhwaite *et al.* 1974). With its simple design, it allowed the positioning and testing of dentinal disc specimens. In the 1980's, Derkson and co-workers introduced – based on the fluid shift model of Brännstrom - their pressurized fluid transport model, which was aimed to test the sealing capacity around restorations (Derkson *et al.* 1986). Later, it was adapted to test the sealing potential of root canal fillings (Wu and Wesselink, 1993).

Visual assessment of a dye spread is meaningless in the dentine permeability testing, because dentinal tubules are usually open and the method mostly shows a complete staining of the whole sample.

The hydrodynamic theory is still widely accepted to explain dentine sensitivity (Pashley *et al.* 1996), which supports the fluid infiltration method to be considered the gold standard in dentine permeability/leakage testing. Regardless of the wide acceptance of this theory, the available testing models based on it, do exhibit some disadvantages. These include long testing periods and a mounting set-up difficulties to allow for repeatable measurements, lacked an internal control and

entrapment or the reaction of permeating substrates within the samples. Additional potential bias, is the embedding process, which was underestimated for a long time while utilizing adhesive materials (epoxy resins, waxes, etc.) (Rechenberg *et al.* 2011). These materials were never adequately tested for their capability withstanding these testing conditions. This had been influenced by a lack of an internal quality control as well as an initial status validation.

These limitations of one of the most acceptable permeation/leakage testing methods, do call researchers to explore and develop new testing protocols.

## 1.3. Marginal adaption in restorative dentistry

A high quality of adhesive restoration's adaptation, the so-called marginal integrity, is mandatory for long-term clinical success, specifically of direct restorations (Krämer *et al.* 2000). The shrinkage - resulting from polymerization stresses - represents a major challenge hampering the interface quality and may therefore jeopardize the restoration's success due to gap formation (Botha and De Wet 1994, and Griffiths *et al.* 1999). It has been demonstrated that bacteria and/or bacterial by-products may follow the path of dentinal tubules making their way to the pulp, given respective inaccuracies at the restoration margins (Goldman *et al. 1992*). This represents the main mechanism through which bacteria and its by-products can reach the pulp and initiate pulpal inflammation, hypersensitivity or even pulpal death (Goldman *et al. 1992*), or if restricted to the superficial aspects may cause marginal discoloration and later secondary caries (Krejci and Lutz 1991). Therefore, the improvement of restoration quality in terms of enhanced materials and techniques remains an important aspect of preclinical research and development. The critical screening and validation, especially *in vitro* prior to clinical application, therefore remains an important topic in dental research.

The evaluation of nano- or micoleakage of dental restorations is defined and classified based on the type of the penetrating substrate, e.g. air, bacteria, fluid, molecules or ions penetration (Kidd 1976). The air permeation test goes back to 1912 (Harper 1912). To achieve the goal, air was compressed through the roots apices while bubble formation at the restoration-tooth interface, while immersed in a water path, was observed under the microscope to confirm the restoration tightness. Compressing dyes applying by the same principle, and have also been used to validate the sealability of restorations (Derkson *et al.* 1986). The results achieved using these methods were qualitative and expressed filling tightness until signs of leakage were observed.

Another option to assess the performance of a restoration is the evaluation of surface margin quality given that gap formation and leakage starts at the surface. In this context, replica techniques were established to screen the restoration margins circumferentially under higher magnification using Scanning Electron Microscopy (SEM) (Blunck and Roulet 1989). The margin quality was thereby studied and described based on pre-defined assessment criteria, which qualified the marginal restoration quality to assess the margin continuity, with or without specified deterioration like enamel or restoration fractures. The shortcoming of this method is its limitation to assess the surface conditions only. To compensate for this limitation, dye penetration models were established to visually assess the subsurface penetration pathways and depths passing the intra-coronal surface defects (Schmidlin *et al.* 2008). To allow for this percolation assessment, sections of the samples were judged according to a scoring system (Going 1972). However, the latter technique bears also limitations: only single evaluation can be carried out, while sectioning of the specimens is required. This probably leads to loss of some information about the penetration tracks in tooth during the sectioning process.

Despite the fact that marginal adaptation testing offers information regarding only the occlusal interface quality, it remains an important area of study

interest, because, if defective, bacteria inoculate the predilection sites and increase the susceptibility of secondary caries propagation and development (Lundin *et al.* 1990).

Unfortunately, clinical performance of adhesively placed restorations do not necessarily correlate to the marginal adaptation tests (Heintze and Zimmerli 2011). However, the importance of such *in vitro* tests arises from their role in screening materials and techniques while comparing their performance prior to the clinical use. Still, it should be kept in mind the possible limitations of all these testing set-ups.

## 1.4. Root canal therapy and filling

The controversy about the efficiency and value of leakage testing in root canal treated teeth, has been initiated two decades ago and remains a continued issue of controversy. So far, this debate did not openly discuss all problematic aspects and how to solve them but instead, seems to be blocked by some endodontic communities. Some scientific journals even abandoned all submissions regarding this important topic (JOE Editorial Board, 2007). The controversy dates back to 1993, when the efficacy of endodontic leakage testing was questioned for the first time (Wu and Wesselink, 1993). In Wu and Wesslink investigation, a fluid infiltration method was used and the authors found the rate of leakage decreasing over time. The authors therefore concluded that substrate infiltration may be influenced by the entrapment of the used substrate throughout the path of leakage, resulting in blockage of this path. In addition, it was reported that the temperature increase may have facilitated and/or even have enhanced leakage values. Another important observation was the gas bubble entrapment, which was claimed to retard the leakage testing process. This observation led the authors to suggest applying vacuum on the counter part to overcome this problem.

Another used method allowing for testing leakage through obturated root canal is the bacterial leakage method in a two-chamber set-up. This model was first adopted in the field of endodontics in 1980 (Goldman *et al,* 1980). Since then, many studies were established based on this model, and leakage was indicated to happen within weeks (Torabinejad *et al*, 1990). These findings did not corroborate with histological studies indicating no bacterial presence in the apical canal portion, even if the root fillings were exposed for a long period of time, provided that the filling was properly made (Ricucci and Bergenholz 2003, Ricucci *et al*, 2009). Systematic testing of this method (Rechenberg *et al*, 2011) indicated a possible bias resulting in a false positive detection of leakage phenomenon. The leakage is influenced by routes considered always to be properly sealing, such as sample embedding. An improper embedding may result in an over estimation of leakage by allowing additional gaps and pathways.

Three-dimensional Micro Computed Tomography (μCT) is a new method assessing root canals *in vitro* at different stages throughout the course of treatment (Paqué *et al*. 2012). This technique can assess the volume removed or added to root canal space. It allows for a volumetric quality assessment of the root canal filling by means of its capability in occupying the space within the root canal system. This method cannot express leakage of the sample *per se*, but can properly indicate the quality of the root canal filling, i.e. showing a tight seal or defective root filling.

In summary, there is no doubt that the current leakage testing methods in endodontics are still lacking a proper set-up, and can still neither exclude possible leakage routes nor assess the initial status of tested samples. In addition, leakage testing *in vitro* must correspond to *in situ* findings of properly obturated root canal treated teeth. Recent editorial at the International Endodontic Journal sent an open invitation to investigators encouraging to establish new experimental models to rank root fillings qualities in terms of techniques and materials in a reliable and reproducible way (De-Deus 2012). Therefore, shortcomings of classical root canal

leakage testing methods must induce the search for new and advanced testing methods, to overcome previously mentioned problems.

## 1.5. Dental implants and their restoration interface

### 1.5.1. Leakage of implants under static conditions

In oral rehabilitation of missing teeth, implants are highly successful nowadays and show high survival rates (Bazrafshan and Darby 2013, van Velzen et al. 2014, Merheb et al. 2015, Moraschini et al. 2015). However, implants still do encounter some biological, technical and prosthetic challenges in the short and long-term.

The biological challenge of implants is the establishment of a stable and healthy hard and soft tissue integration. However, the complexity of indigenous flora and bone quality may interfere with this goal (Mombelli *et al.* 1987). The bacterial influence on implant supporting tissue was always correlated to the capability of plaque to retain at rough and even smooth surfaces or at niches, like the implant-abutment interface (IAI). This retention of plaque provide the potential to accumulate bacteria and their biproducts thus result in soft tissue inflammation, called mucositis (Ericsson *et al.* 2012). Furthermore, this pathological status can develop to end up in bone resorption, called peri-implantitis (Broggini *et al.* 2006). The first study to correlate implant failure to bacterial inhabitation was carried out by DNA analysis (Becker *et al*, 1990) of samples cultivated from failed clinical cases. The previous mentioned study have detected moderate levels of bacteria on the surface of investigated failed implants. The implants presented with an increase in implant mobility, an increase in probing depth and an incidence of peri-implant bone loss indicated by radiolucency. The position and the quality of IAI are strongly believed to play a determining factor in bone loss around implants

(Piattelli *et al.* 2003). The introduction of new materials like zirconia to dental implants has further increased the concerns about leakage at the IAI. A recent study comparing the sealing capability of titanium and zirconia abutments has revealed an overall larger marginal gab at the IAI in zirconia abutments (Smith and Turkyilmaz 2014).

In an ideal situation, implants should provide a perfect seal at their IAI to overcome or limit any biofilm formation limiting inflammatory reactions at the adjacent supporting tissues. It has been suggested that a tight interface presents a well-adapted surface, which does not allow biofilm entrapment (Baggi *et al* 2013).

Due to the possible influence of IAI in inducing complications and even failures, their study remains an important focus in implant dentistry research.

To assess this phenomenon, many *in vitro* models have been established. While hypothesising that leakage at the IAI might increase the risk of bacterial-based pathological changes, tight seal assessment, seems to be an important criteria to evaluate and compare in different implant systems and designs. The long history of leakage testing in other dental fields has inspired researchers to adopt and modify available methods to test implants leakage based on similar principles.

The bacterial leakage at the IAI as a testing method is the most applied and accepted method, thus can be considered the gold standard in implant leakage testing (da Silva-Neto *et al.* 2012). The reason behind is the suggested pathological causality between bacteria and peri-implantitis (Mombelli *et al.* 1987). Many *in vitro* models have been established based on the assumption to test for different bacterial species capability of penetrating at the IAI and to cause inflammation to a different degree. The assessment took place in many forms such as cell cultures (Quirynen *et al.* 1994), checkerboard DNA- DNA hybridization (do Nascimento *et al.* 2009) and turbidity tests (Dias *et al. 20012*).

Visual assessment of the gap at the IAI utilizing radiographs, SEM or other optical means is a simple direct method to detect and assess the adaptation accuracy of two implant parts (Meleo *et al.* 2012). This testing method, however, cannot be relied on to assess leakage, because the continuity of the observed gap and its depth are difficult to judge, and measurements are mostly semi-quantitative and based on 2D-analyses only.

Molecular seal (Harder *et al.* 2010) and dye penetration tests (Park *et al.* 2012) represent more sophisticated methods to assess the leakage status of implants. In 2008, Romieu and co-workers (Romieu *et al.* 2008) introduced a new prospect in implants leakage and permeability testing using a model with dual pressure chambers. By continuous recording of the change in air pressure difference between two chambers, a ratio of the pressure drop could be used to delineate a curve. This curve was considered to indicate air leakage through the mounted samples over time. However, this assessment was carried out under dry conditions, which does not necessarily correspond to the physiologic conditions in the oral cavity, which represents a significant shortcoming of this test method.

While most leakage testing set ups provide qualitative information about the seal status, the substrate spectrophotometry detection may provide a quantitative method to determine permeating fluid volume (Harder *et al.* 2010). The accuracy of this method is still compromised by the detection limit and repeatability, which can be jeopardized by a potential substrate entrapment. Also, the need for a long testing periods and the necessity to dissemble abutments, allows only one point assessment over time. This results in the critical fact that leakage status of tested implants, end-up in changed assemblies after the second mounting and evaluation phase. In this aspect, the gas permeation method may provide a more accurate and repeatable non-destructive approach.

Due to significant clinical implications and the need to control and prevent inflammatory bone loss, implant leakage testing even under static conditions *in vitro*

requires further development, investigation and validation in order to assess and screen the seal status of the IAI reliably.

### 1.5.2. Leakage of implants under thermo-mechanical loading

Many implant designs were developed and claimed by manufacturers to increase and enhance the tightness at the IAI. The designs were thought to increase the stability of two-piece implants, especially under clinical and functional situations. Laboratory research mainly concentrated on the ability of microorganisms to penetrate at the IAI harbouring the adjacent supporting structure, through forming a non-cleansable focal source of infection (Mombelli *et al.* 1987). Some studies showed leakage to be a dynamic process, which could not be detected under static conditions only (Steinebrunner *et al.* 2005, Koutouzis *et al.* 2011). Implants do experience different physical conditions under functional loading like a pumping effect due to the vertical forces in the occlusal direction and luxation forces in the axial occlusal direction. In general, however, implant leakage studies under thermo-mechanical loading, were limited to the implants seal performance under loading without considering the preloading status of mounted implants again. The need to disassemble the abutments to cultivate samples of the inner implant chamber illustrates limitation of implant testing at different stages (Koutouzis *et al.* 2011). It also limits the comparison of treatments, as each step presents a different performance resulting in the change of leakage status due to multiple tightness applications (Do Nascimento *et al*, 2009). A testing protocol allowing assessment of implant leakage status at any time point and after different treatment conditions utilizing identical implants is still missing. The lack of information about the leakage development in the course of implanting process, may potentially lead to false conclusions about the reason behind the leakage. None of the available studies can explain underlying causes of leakage, whether it is due to mechanical failure due to thermo-mechanical loading or simply because of misfits from the beginning due to manufacturing problems. To fully understand

the true underlying processes, a thorough analysis of the implant performance under static and dynamic conditions without disassembling the mounted implants at any time is required. This will allow determining more exactly the influences of pre-loading conditions and thermo-mechanical loading on the overall implant sealing performance.

# 2. Aims of the thesis

## 2.1. General aim

The main goal of this thesis is to develop a leakage and permeation testing set-up applicable for various dental materials, namely, restorations, root fillings and dental implants. The device should be non-invasive, reproducible and allow accurate multi-disciplinary leakage and permeability testing in dentistry.

## 2.2. Specific aims

### 2.2.1. Validation of the GEPT (*Gas Enhanced Permeation Test*) system

To validate the testing GEPT device with regard to accuracy, reproducibility and leakage-free embedding of samples. The correlation between measured values for the identical tested samples has to be proven (Study I).

### 2.2.2. Restorations leakage testing

To compare three restoration leakage testing set-ups, the developed gas enhance permeation test (GEPT), SEM marginal analysis and dye penetration test (Study II).

### 2.2.3. Root canal filling leakage testing

To compare root canal filling sealing performance in simple root canal anatomy to the sealing capacity in complicated canals. In addition, the leakage values will to be correlated to the corresponding root canal filling quality assessed by μCT evaluation.

### 2.2.4. Implant leakage under static conditions

To compare two commonly used implant leakage testing methods, the microbial and the molecular leakage detection to the GEPT under standardized static conditions (Study III).

### 2.2.5. Implants leakage under thermo-mechanical loading

To test different implants designs for their sealing performance under dynamic conditions, taking in consideration their preloading static seal conditions (Study IV).

# 3. Hypotheses of the study:

## 3.1. Validation of the GEPT system

- The embedding process results in tight samples and causes no false-positive measurements.

- The measurements are repeatable for identical samples and result in reproducible values.

- The system presents a low detection limit to assess permeation.

- The liquid volume collected during the permeation measurement correlates to the gas pressure difference changes over time.

## 3.2. Restorations leakage testing

- A restoration with poor adaptation at the restoration interface will result in more leakage as compared to a given gold standard (e.g. adhesively placed inlay)

- GEPT evaluation of the restoration leakage correlates to currently used surface and subsurface evaluation techniques, e.g. marginal SEM analysis and dye penetration test.

## 3.3. Root canal filling leakage testing

- Root canals with complicated anatomy are more difficult to be properly obturated and result in increased leakage values

- The observed leakage corresponds to the quantitative 3D root canal filling quality evaluation.

## 3.4. Implants leakage under static conditions

- GEPT is as effective in assessing leakage when compared to the most acceptable implants leakage testing methods, e.g. bacterial and molecular leakage evaluation.

## 3.5. Implants leakage under thermo-mechanical loading

- Tight implants under static conditions maintain their sealing capacity under loading conditions (or deteriorate).

- Different implant designs may influence the performance and stability under dynamic loading conditions.

# 4. Materials and Methods

## 4.1. Development of a new leakage testing device

The new testing device called gas enhanced permeation test is based on the principles of split chamber model, fluid infiltration and gas pressure difference measurement.

### 4.1.1. Technical details

The split testing chamber consists of two custom-made plexiglass parts, which are tightened together with three solid screws (Fig 1). This design allows the embedded specimens to be fixed in between the two parts, easy removal and replacement. To ensure tight seal around the mounted sample, a rubber O-ring with an outer diameter of 22 mm, an inner diameter of 15 mm and a thickness of 3.5 mm is used. The lubricated O-ring with silicone grease (Molykote 111 compound, DOW Corning GMBH, Germany) aimed to enhance the sealability between the two chambers. As a result two fully separated chambers holding the sample in between is formed. The lower chamber allows collection of the infiltrated fluid, through an eppendorf attached to an adaptor fixed to the outside at the terminal end of the chamber. The two chambers are controlled and stabilized using two valves. The valves are closed once desired pressure is reached and during the whole testing period.

Because gas pressure is highly sensitive to temperature changes, temperature is controlled in the following manner: The main permeability/leakage unit (Fig 2, a) is installed in an isolation chamber (Fig 2, b), where temperature is constantly held at 35°C. Furthermore, the chamber is placed in a second larger experimental box (Fig 2 c), where temperature is kept at 31°C. Room temperature is stable at 25°C.

A pressure difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) have two inlets; one for positive pressure and the other for negative pressure. This device is connected through tubes to the upper and lower chambers just before the valves and allows for real-time measurements.

**Figure 1**

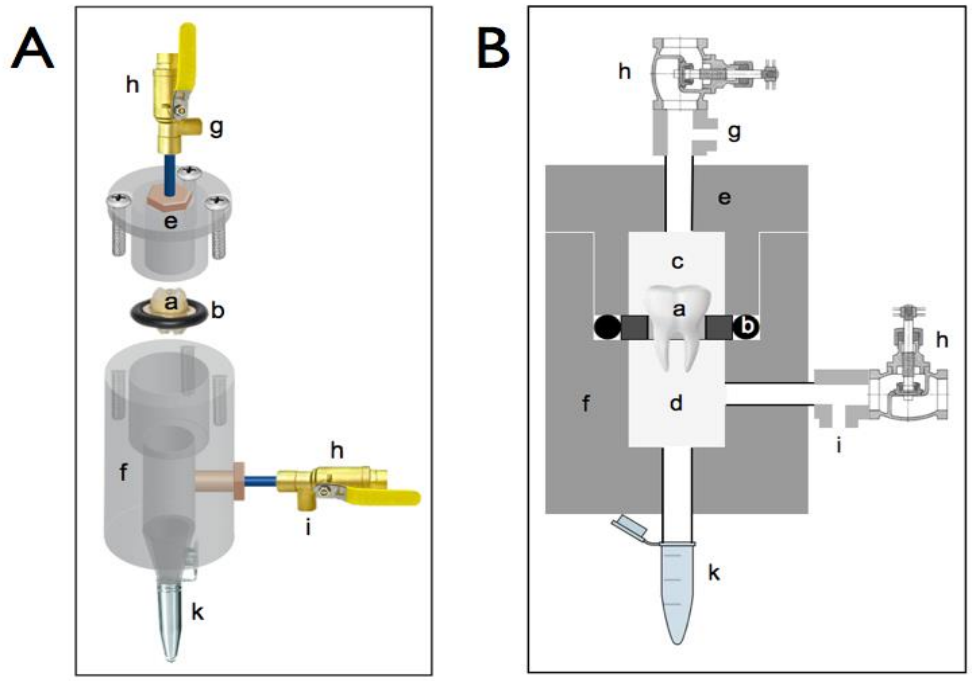


***Figure**: Split chamber with the two valves connected to control pressure on both sides*
*Exp. **A**: 3D graph, Exp. B: enhanced schematic drawing showing the position of the mounted tooth in testing chamber. The parts are matched in both drawings. (**a**) A tooth sample mounted in a disc carrier. (**b**) O-Ring. (**c**) Positive pressurized chamber. (**d**) Low pressurized chamber. (**e**) Split chamber cover. (**f**) Split chamber body. (**g**) Positive outlet attached to the pressure difference measuring device. (**h**) Securing valves. (**i**) Negative outlet attached to the pressure difference measuring device. (**k**) Eppendorf tube to collect permeating fluid.*

The measuring device reports measurements to a computer-unit running a proprietary program (V 4.2 SP2, Testo AG, Germany). A specimen is placed in the O- ring at the designated position. Subsequently, 2.5 ml of a pre-pressurized ($N_2$ gas 860 hPa) 0.9% NaCl solution is added on top in the upper chamber. The cover

is repositioned, and the three screws tightened utilizing a torque-controlled screwdriver. A positive pressure is then applied to the upper chamber ($N_2$ gas to 860 hPa). Simultaneously, the lower chamber is negatively pressurized down to minus 170 hPa. The resulted effective pressure difference between the two chambers accounted for 1030 hPa. Given the hypothesis that there was a connection between the two chambers, i.e. leakage through the sample, the pressure difference would change. The penetration of the saline through the leakage site will create more space in the upper chamber and results in a positive pressure drop in the upper chamber. Simultaneously, pressure in the lower chamber would increase. Total effect will present as a reduction in pressure difference between the two chambers. The process would continue until pressure is equalized in both chambers, i.e. the difference will reach 0 hPa. The rate, by which the pressure changes, indicates the effective amount of leakage. Pressure difference measurements are started and continued over 40 min at a rate of 1 measurement/s. The resulted data, is plotted to produce a curve, which represents the rate of pressure change expressed as a drop in pressure difference over time. From a preliminary study, it was concluded that the slope between the two pressure values at two fixed time points (1200 s and 2400 s) can be defined to calculate a slope representing the leakage status of tested sample.

$$\text{Slope} = \frac{P2 - P1}{T2 - T1} \text{ hPa/min.}$$

P2: Pressure difference at time point 40 min.

P1: Pressure difference at time point 20 min.

T2: Time point 40 min.

T1: Time point 20 min.

All results are expressed as positive values for the statistical analysis for the ease of understanding, as should show a positive correlation with the infiltrated fluid volume.

**Figure 2**

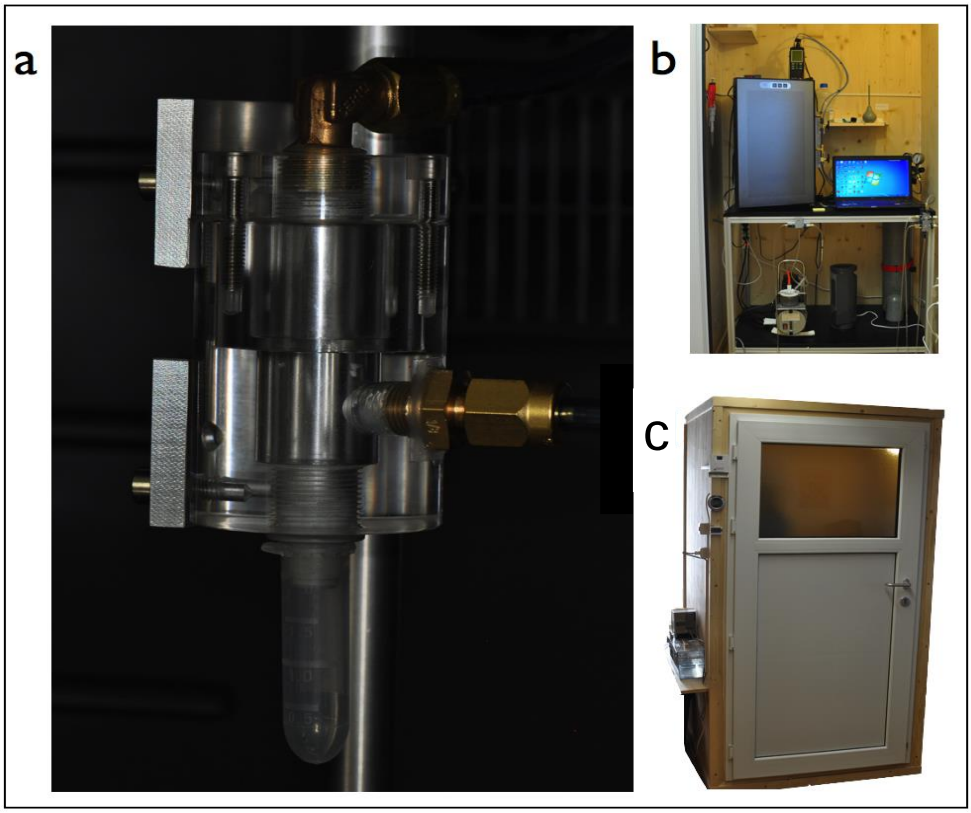


***Figure**: Stepwise temperature control; **a**) Split chamber mounted in the testing inner isolation room. **b**) Inner Isolation chamber. **c**) Outer Isolation room.*

These fixed time points to detect the slope were decided from preliminary observations of repeated measurements of the same sample, where it was found to be reproducible. The leakage chamber design, which allowed for re-measuring the samples at different time points, allowed for testing leakage/permeation at the before-treatment point. This value represented the tightness of the embedding is

considered as the baseline (the zero point), which had been subtracted from any measured value corresponded to any treatment, i.e. the actual value that represented the leakage status could be calculated.

To confirm the leakage to occur as hypothesized, the infiltrated physiological saline solution was collected and weighed to calculate the volume that permeated the specimen. Its correlation to the calculated slope value was found to be positive.

## 4.2. Sample preparation

### 4.2.1. Tooth samples

#### *4.2.1.1. Embedding*

Two variations of teeth were used as natural specimens. Intact teeth in the case of filling assessments, and sectioned teeth with build-ups to judge leakage in root fillings in certain root canal anatomy of interest. Where sectioned molar teeth vs. single rooted front teeth were used, teeth length was adjusted to 18 mm by cutting the crown from the occlusal side with a slow speed diamond saw (0.4 mm, Struers GmbH, Birmensdorf, Switzerland) under water-cooling (Fig 3, B). Sectioning at the furcation area to obtain the targeted section was performed with a diamond disc (Super-Flex 911HH, Busch & CO., Engelskirchen, Germany). A cylindrical coronal build-up of 11 mm diameter and 10 mm height was cast in a custom made Teflon mould. This build-up covered the coronal 7 mm of the tooth (Fig 3, C). The coronal part was first conditioned, after sealing the canal opening with a cotton pellet, with a Clear fill bonding system (Clearfil SE Protect, Kuraray America Inc., USA). The build-up followed using the Luxa Core build-up material (Luxa Core Automix, DMG, Hamburg, Germany). Samples were then light cured

for 5 min in a light cure chamber (Spectramat, Ivoclar Vivadent, Schaan, Liechtenstein).

**Figure 3**

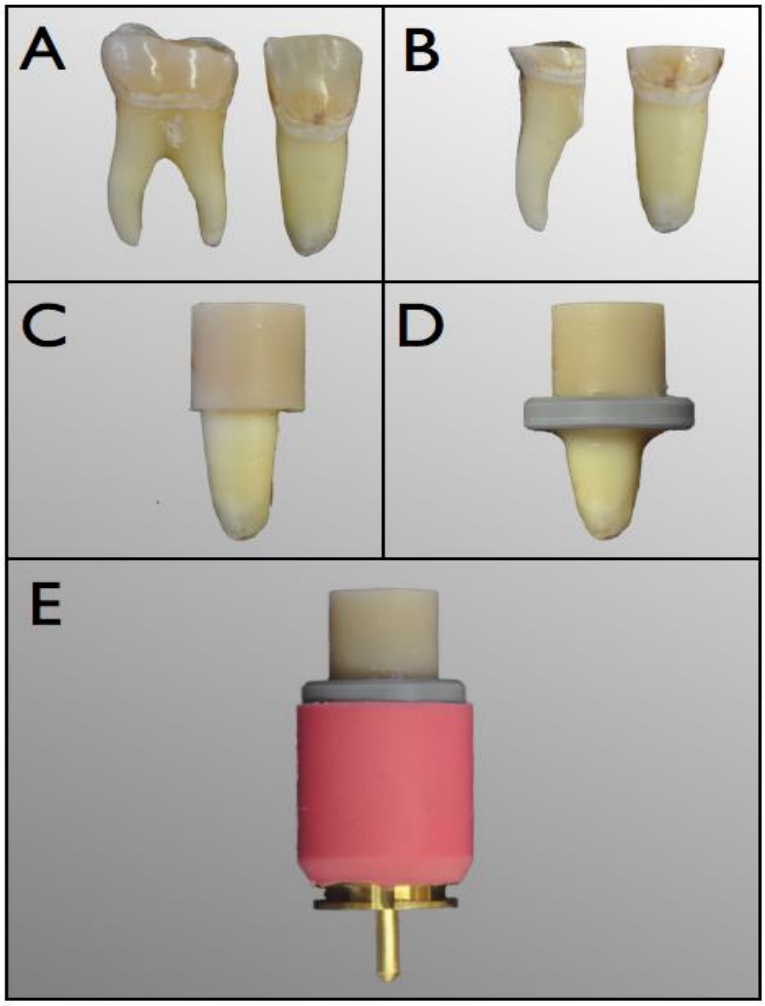


***Figure**: Samples preparation for the root filling quality assessment.*
***A**. Samples selection, **B**. Adjusting the length to 18mm as well sectioning of roots with the anatomy of interest, **C**. Core build up, **D**. Embedding in the PVC rings, **E**. Mounted in the rubber carriers to carry out the μ-CT scans.*

All teeth samples (full and sectioned) were embedded in custom-made brass/PVC rings based on the study design and requirement. PVC rings were used when μ-CT scans of embedded samples are planned in the study, to avoid

scattering effect resulting from metals. The rings had an outer diameter of 15 mm, an inner counterpart of 10 mm, and a thickness of 3 mm. The rings were conditioned by grit-blasting on their inner surface using 50-μm aluminium oxide (Benzer-Dental AG, Zurich, Switzerland). The teeth were then embedded with a light-curing nail build-up material kit (Sina, Shenzhen Cyber Technology Ltd, Guangdong, China). This material was proved to perform better than any dental adhesive material in pretest. The nail build-up gel material consists of a primer, a gel, and a glaze. The teeth as well the rings (on their inner surface) were primed and subsequently light-cured for 2 min in a light cure chamber (Spectramat, Ivoclar Vivadent, Schaan, Liechtenstein). The parts were then held together in position for this purpose in a rubber carrier made of a silicone putty material (Optosil, Heraeus Kulzer GmbH, Hanau, Germany) (Fig 4). The gel was applied in one increment on the top side to fill the space between the ring and sample and was light cured for 4 min The sample was then turned in an upside down position, and the gel was optimized and extended on the root surface, before being light cured for another 4 min (Fig 4, b). Care was taken not to allow excess material formation on the upper or lower surfaces of the ring. A glaze layer to strengthen and eliminate any imperfections was applied to both upper and lower gel surfaces and finally light cured for another 4 min. This embedding method was used for all included tooth samples.

#### *4.2.1.2. Restoration cavity preparation*

Class-I preparations of all different dimensions in this study were drilled in a parallelometer on a XY table (Cendres & Metaux SA, Biel, Switzerland) after mounting teeth in brass rings. The drilling was accomplished utilizing a diamond bur with a grit size of 80 μm (Bur 837 KR, 8614, Intensive SA, Grancia, Switzerland).

**Figure 4**

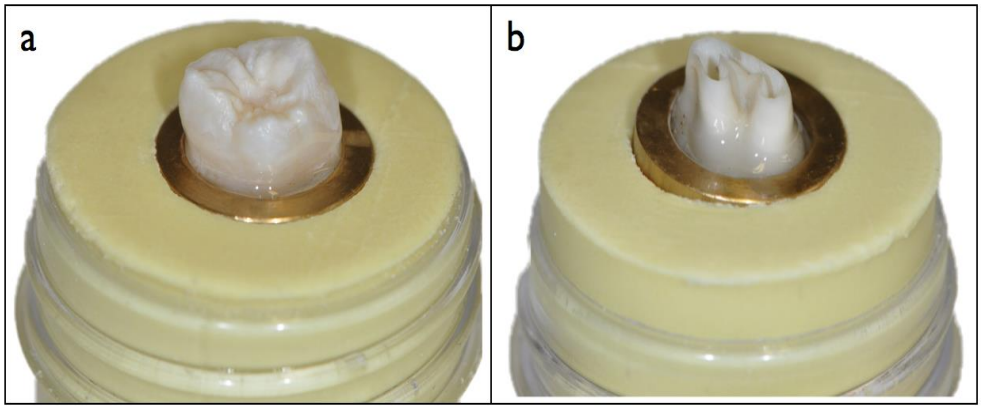


***Figure**: Embedding; **a**) Embedding from coronal side, **b**) Embedding from apical side*

### *4.2.1.3. Tooth filling and restoration*

#### *Resin composite filling without bonding*

This treatment was aimed to present a non-sealed type of filling. The cavities were restored with resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) but without any surface conditioning procedures, i.e. without any etching, priming and bonding. The application of the resin composite material took place in two horizontal increments, which were polymerized each for 20 s. at 800 mW/cm² (Bluephase LED G2, Ivoclar Vivadent, Schaan, Liechtenstein). Marginal finishing was achieved using specially designed finishing burs (Intensiv SA, Grancia, Montagnola, Switzerland) and polishing discs (Sofflex discs, 3M ESPE, Seefeld, Germany). To optimize this finishing procedure, the whole process was carried out under a stereomicroscope (Stemi 1000, Zeiss, Oberkochen, Germany).

***Resin composite filling with bonding***

This treatment was meant to present a regular treatment simulating clinical situation. Enamel was selectively etched for 1 min with 35% phosphoric acid (Ultra Etch, Ultradent, South Jordan, Utah-USA) followed by thorough water rinsing for 40 s. After air drying, a self-conditioning, maleic acid containing primer (Syntac Primer, Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 15 s and gently air-dried before a second primer applied for 20 s (Syntac Adhesive, Ivoclar Vivadent, Schaan, Liechtenstein). Air was gently applied and an unfilled bonding resin (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 20 s and light-cured for 40 s (Bluephase LED G2, Ivoclar Vivadent, Schaan, Liechtenstein). Resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) was applied in three horizontal increments, which was polymerized individually for 20 s each. Finishing and polishing was made as previously mentioned.

***Ceramic inlay***

Ceramic inlays were fabricated using a chair side Cerec 4D system (Sirona Cerec Blocs, VITA Zahnfabrik, Bad Säckingen, Germany) with a leucite reinforced glass-ceramic material (IPS Empress CAD Multi, Ivoclar Vivadent, Schaan, Liechtenstein).

Cavities were conditioned according to the same etch-and-rinse protocol and adhesive system described previously (Syntac Classic, Ivoclar Vivadent, Schaan, Liechtenstein). Ceramic inlays were acid-etched with hydrofluoric acid (Vita Ceramics Etch, Vita Zahn Fabrik, Bad Säckingen, Germany) on their bonding surface for 1 min. After extensive water spray rinsing, a silane coupling agent was applied (Monobond Plus, Ivoclar Vivadent) for 1 min and the inlay was dried. An unfilled bonding resin was applied (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein) to the bonding fitting surface without light curing. Resin composite filling material (Filtek Supreme XT, 3M ESPE), pre-warmed to 37°C (AdDent Inc.,

Danbury, USA), was then applied to the inlay fitting surface and in the cavity. The inlay was first positioned by finger pressure followed by ultrasound (mini Piezon, EMS, Nyon, Switzerland) for 10 s to enhance the final placement, using the thixotropic effect. Excess material was carefully removed and light polymerization was applied from 5 surface aspects for 1 min each, from the occlusal, mesial, distal, buccal and oral direction, respectively.

#### *4.2.1.4. Root canal preparation and filling*

Teeth to undergo root canal treatment leakage testing were all unified in their working length (including the build-up) to 21 mm. The root canal preparation took place under the magnification of a stereomicroscope (Stemi 1000, Zeiss, Oberkochen, Germany), the access cavity was opened through the crown with a high speed handpiece (Sirius, Micro Mega, Besancon, France) provided with a diamond bur having a grit size of 80 μm (Bur 837 KR, 8614, Intensive SA, Grancia, Switzerland). Canals were located, negotiated with an iso 10 H-file (dentsply, Dentsply-Maillefer, Ballaegues, Switzerland) until the file tip observed at the root apex. Working lengths was confirmed by a standard X-ray technique (Heliodent Plus, Sirona, Germany) utilizing a digital receptor scanned with a digital X-Ray scanner (Digora Optime, Scanora, Soredex, Tuusula, Finland) and viewed on a screen with the aid of X-ray viewing program (Scanora, Soredex, Tuusula, Finland). The canals were then prepared with a chemo-mechanical preparation approach, utilizing Pro-taper rotary system (Pro-taper universal, Dentsply-Maillefer, Ballaegues, Switzerland) run on a rotary motor (Endo-Mate TC2, NSK, Tochigi, Japan). Irrigation utilizing a side vented needle (Max-i-Probe; Hawe-Neos, Dentsply, Gioggio, Switzerland) to the working length, with 1 ml, NaOCl 1% after each file size preparation took place. Canals were prepared to file size F3. A final irrigation with 5 ml EDTA 17%, followed. Canals were dried and a build-up was established as described in section 4.2.1.1. .

The root canal filling was performed after the build-up on top of each tooth was established. The access cavity was re-established, canals were recapitulated with irrigation with 5ml EDTA 17 % to the full working length. The root canal filling was made by implementing a continuous wave condensation technique: Master point gutta-percha was fitted to the full working length (F3 GP, Dentsply-Maillefer, Ballaegues, Switzerland). The fitting of the master point was confirmed with a standard X-ray. The canal was then dried with paper points (ISO size 30, 0.04 taper, Dentsply-Maillefer, Ballaegues, Switzerland). An epoxy resin, root canal sealer (MM Seal, Micro Mega, Besancon Cedex, France) was then mixed on a glass plate. The master point was immersed into the sealer and placed to the full working length in the canal. With the aid of vertical thermal plugger (Xtra Fine, 0.04 taper, System B, Sybron Endo, California, USA) the master point was cut up to 3-4 mm form the apex. And a Gutta-percha back fill was achieved (Obtura III, Sybron Endo, California, USA).

In between treatments, the access cavity was secured with a cotton pellet and a temporary filling material (Cavit, 3M ESPE, Seefeld, Germany). All samples when not in analysis were kept in a humid box and at a temperature of 37 °C (Heraeus UT6420, Thermo Fisher Scientific, Dreieich, Germany).

A newly graduated dentist who was not aware of the study aims was taught the above and performed all root canal treatments.

### *4.2.2. Implants and abutments*

#### *4.2.2.1 Implants embedding*

Three all titanium implant systems were selected (Table 1) representing variation in the platform design but with nearly same dimensions. Astra Tech implants system (AT) has a taper lock and an internal hexagonal mating surface design. Biomet 3i implants system (B3i) present a flat-to-flat interface design with

an internal hexagonal mating surface and Nobel Biocare implants was have a flat-to-flat with a trilobe mating surface.

Before being tested these implants were mounted in PVC discs. The disc had a diameter of 15 mm and a thickness of 3 mm (Fig 5). The implant diameter was measured at the level of 1 mm from the implant abutment interface (IAI). A reduced drill with a diameter of 0.2 mm from the measured diameter was performed in discs in which the implants to be mounted, using a parallelometer. The dimensions were 3.3 mm (B3i), 4.0 mm (NB) and 3.8 mm (AT). The implants were screwed to a final position displayed 1 mm of the IAI above the disc top surface. An extra measure to ensure perfect sealing at the disc-implant interface was achieved by grit-blasting with 50 μm aluminum oxide from the apical side (Benzer-Dental AG, Zurich, Switzerland), followed by conditioning and sealing using a commercially available nail buildup gel material (Sina, Shenzhen Cyber Technology Ltd, Guangdong, China).

**Table 1:** Implants under investigation

| | Astra Tech | Nobel Biocare | Biomet 3i |
|---|---|---|---|
| Description | Astra Tech™ OsseoSpeed™ TX/S | Nobel Replace® Tapered Platform Switch | OSSEOTITE® Tapered Certain® PREVAIL® |
| Size | 4.0x15 mm | 4.3x16 mm | 4.0x15 mm |
| Item No. | 24944 | 36895 | XIITP4315 |
| Abutment | TiDesign 3.5/4.0-1.5 mm | Esthetic Abutment NP - 3mm | GingiHue® - 2 mm |
| Abutment item No. | 24285 | 36824 | IMAP32G |
| Screw | Uncoated Screw | Uncoated Screw | Gold Coated Gold-Tite® Screw |
| Screw item No. | Included with Abutment | Included with Abutment | IUNIHG |

Implants systems in test. Parts used and their codes.

**Figure 5**

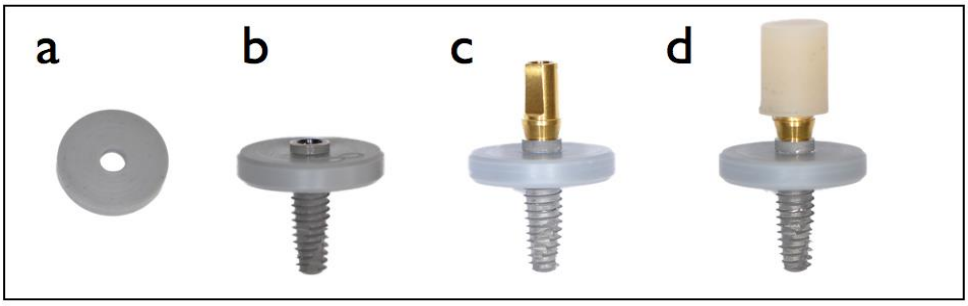


***Figure**: Samples embedding for GEPT test.*
***a**. Drilled discs. **b**. Blank abutments screwed in the discs. **c**. Abutments fixed. **d**. Standard core build-up.*

#### *4.2.2.2 Core build-up*

Implant was held in a straight Kelly hemostat (Hu-Friedy Mfg. Co., Chicago, USA) and the abutment was positioned and tightened to the implant using the manufacturer recommended screw, utilizing a torque control wrench according to the manufacturer torque recommendations. Preparation and conditioning of the abutment took place by grit-blasting with 50 μm aluminum oxide while the platform being protected with a punched metal matrice. The screw channel was sealed and protected with Teflon strip, which was tightly packed. A standardized resin composite build-up (6mm diameter and 10 mm height) (Luxa Core Automix, DMG, Hamburg, Germany), extending to the abutment restoration finish line was casted in a Teflon mold. To enhance the build-up bonding, the grit-blasted abutment part was conditioned with Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein) and an adhesive system (Clearfil SE Protect, Kuraray America Inc., USA).

## 4.3. Validation of the GEPT system

While the GEPT system presents a new approach to study leakage, it was necessary to test for tightness/sealability, repeatability, detection limit, correlation between the measured outcomes and the capability of the embedding procedures in maintaining a tight seal after multiple measurements with no or minimal changes. For that purpose a solid metal disc, embedded resin composite discs, and third molars were used. The solid metal disc had the same dimensions as the embedding brass rings (3 mm thick and had a diameter of 15 mm) was considered as gold standard for tightness, as no interfaces and no embedding imperfections can be involved. The metal disc had also the exact thickness and outer dimensions of the embedding brass rings used in the set-up (Fig 6, Exp. A). This approach was used to measure possible internal system leakage resulted from all joints and

connections. Hypothetically, this test should result in no leakage/negligible leakage and serve as internal system tightness control.

To provide a non-porous biomaterial/tooth surrogate, resin composite discs with a 7 mm diameter and a 3 mm thickness were prepared. The discs were fabricated with the aid of a teflon mold to cast the resin composite discs out of a dual cure resin composite build-up material (Luxa Core Automix, DMG, Hamburg, Germany). Suggesting no leakage to occur given adequate sealing around it, these samples are expected to show close tightness characteristics as the solid metal disc.

Extracted third molars selected from the department's pool. Third molars were used due to their availability and because they are characterized of having widely open dentinal tubules. All teeth has been extracted for reasons not related to the current study (patients aged 18-20 years). Written informed consent was obtained by all donors according to the recommendation of the Swiss Academy of Medical Science (Salathe 2010). Personnel handling the teeth applied all necessary precautions for infection control. Ethical guidelines were followed (World Health Organization 2003), and anonymisation was performed in accordance with state and federal law (Human Research Act HRA). The included teeth had to be sound and caries free, and another pre-requisite was that the roots were not fully developed ensuring proper pass to the pulp chamber and allowing for retrograde pulp extirpation. Samples were stored in 0.2% thymol at a temperature of 5°C for no longer than one year before use.

### 4.3.1. Sealing efficiency and repeatability evaluation

To assess device tightness at the junctions, efficiency of embedding technique and repeatability of the measurement, leakage of the metal disc, three resin composite discs and three intact third molars embedded as described in section (4.2.1.1) were repeatedly tested with the GEPT (Fig 6, a). Measurements

were carried interchangeably between samples and each sample was tested eight times at different time points.

Furthermore, dentine permeability of three embedded third molar teeth were measured after inducing three different preparations in dentine (class I preparations; 2 mm × 5 mm and a depth of 2 mm from the fissure level and full occlusal surface preparation). The full occlusal preparation had completely removed the occlusal enamel and supporting dentin until the CI preparation floor was reached. All preparations were made using a tapered diamond bur (Number 8117, Intensiv SA, Montagnola, Switzerland) attached to a parallel drill holder (Cendres & Metaux SA, Biel, Switzerland). To ensure that the repeated measurements had no effect on the embedding, teeth were restored to fully seal dentinal tubules. The restoration took place, after conditioning (Clearfil SE Protect, Kuraray America Inc., USA), using CAD/CAM onlays (Sirona Cerec Blocs, VITA Zahnfabric, Bad Säckingen, Germany) cemented with Multilink (Ivoclar Vivadent, Liechtenstein). All samples were tested eight times for each stage (Fig. 6, Exp. A&B). To assess the potential influence of storage on the embedding and permeability, the repeated measurements of each sample after each preparation were carried out on different days. In the meanwhile, samples were kept in physiologic saline at room temperature.

**Figure 6**

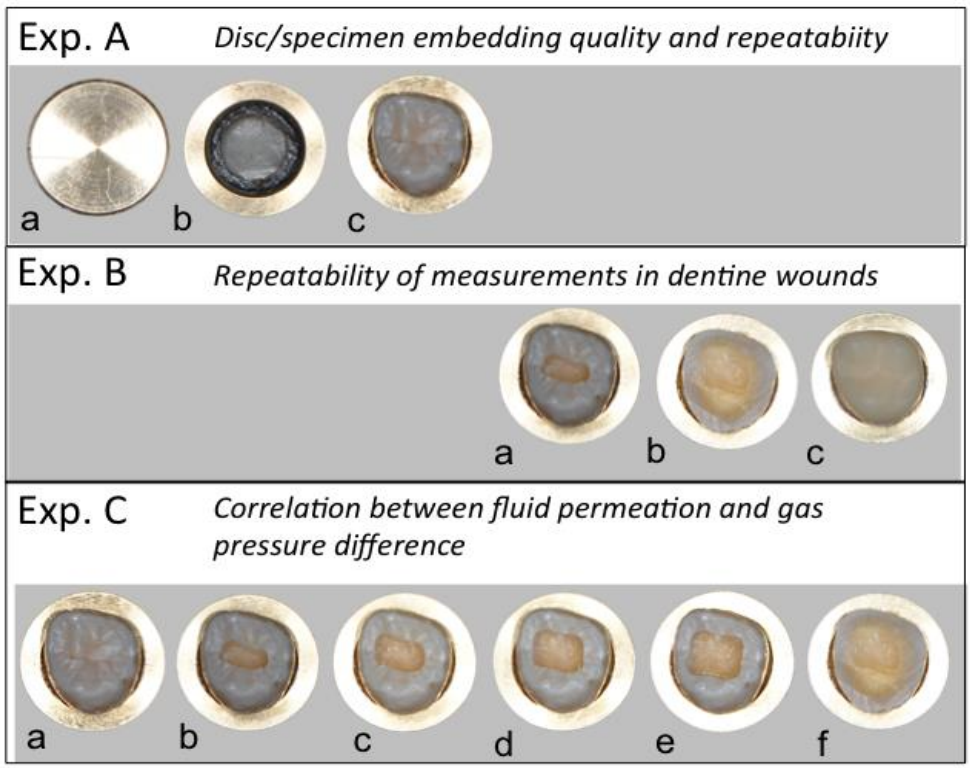


***Figure*** *- Exp.* ***A****: Disc/specimen embedding quality and repeatability; One full metal disc (a,no embedding), three embedded resin composite discs (**b**) and three embedded third molars (**c**); eight consecutive measurements in each sample*
*- Exp.* ***B****: Repeatability of measurements in dentine wounds; Three molars (of Exp.* ***A****) with 2x5 mm (**a**) and full occlusal preparation (**b**) as well as consecutive restoration (**c**); eight consecutive measurements in each sample*
*- Exp.* ***C****: Correlation between fluid permeation and gas pressure difference; Six third molars (**a**) with step-wise increasing preparation size of 2×5, 3×5, 4×5, 5×5 mm (**b**-**e**) and full preparation (**f**); one measurement per sample.*

### 4.3.2. System detection limit and correlation between pressure difference and fluid permeation

To assess correlation between the two quantitative primary outcome parameters of the device, i.e. gas pressure difference change and liquid permeation, six additional third molar teeth from the department's collection of extracted teeth

were selected (molars 4-9). The embedding was first assessed before treatment and the baseline values were determined. The resulting measured curves were used to determine the method detection limit, which was defined as the minimum measured permeability value that could be observed in a sample with confidence. Consecutive preparations were induced in all specimens while increasing invasiveness and dimensions at each subsequent preparation (2×5, 3×5, 4×5 and 5×5 mm and a depth of 2 mm from the fissure level). The final preparation presented as a full occlusal trimming, which was performed as described under section 4.3.1 (Fig. 6, Exp. C). After each preparation step, the pressure difference change was measured utilising the GEPT (Fig 7, B). The effective leakage value of the treatment was calculated by subtracting the base line slope value from the slope value obtained after treatment.

In addition, the permeating saline through each specimen was collected in the tube attached to the apparatus. The liquid volume was determined by calculating the weight difference of the tube before and after the experiment using a precision scale (Mettler AT261 Delta Range, Greifensee, Switzerland). The correlation between the pressure difference change and the corresponding fluid infiltration for each measurement was established.

**Figure 7**

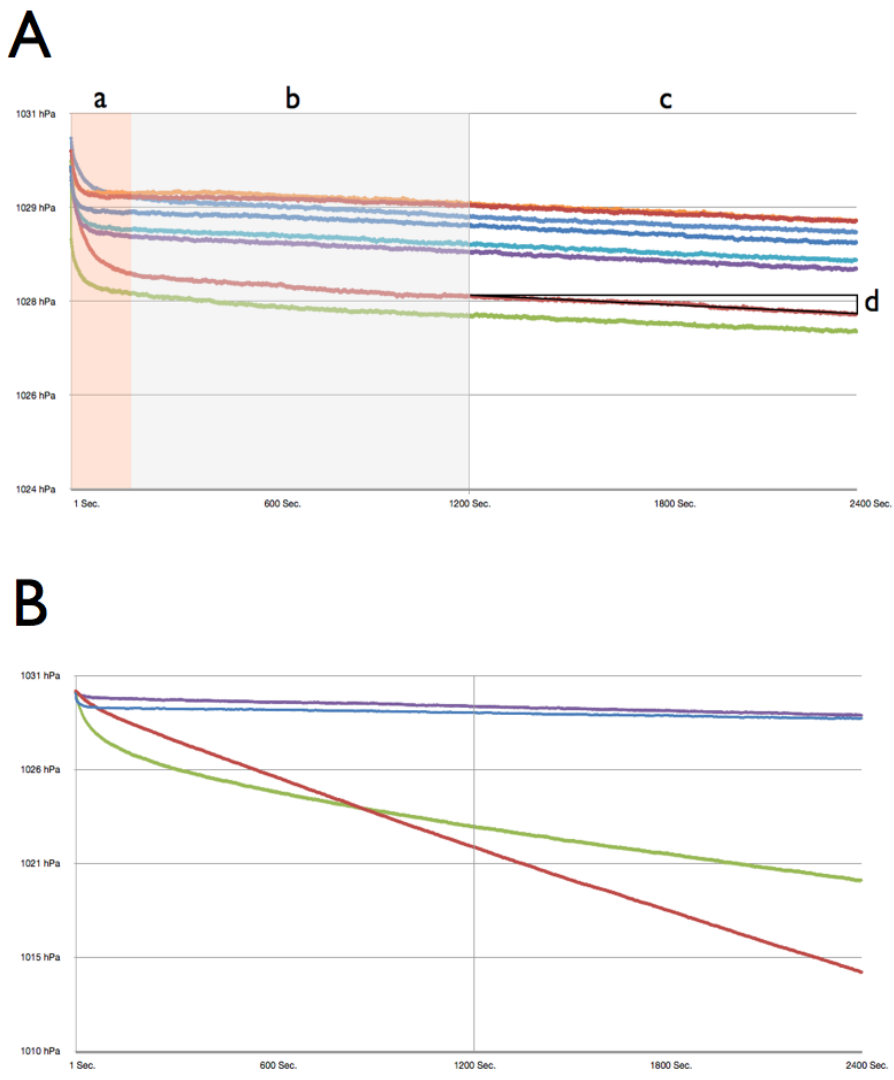


***Figure*** *– Exp.* ***A****: A represantative graph of a tested sample with 8 repeated measurements for its baseline permeability (hPa/min): (**a**) The gas compensation curve (each pressurized gas will behave unstable for a period of time). (**b**) System stabilization curve, which is related to temperature compensation. (**c**) The permeability curve which is related to the sample permeability status. (**d**) The permeability slope.*
*- Exp.* ***B****: A representative graph showing the permeability curves of a sample tested for multiple treatments.* ■ *Baseline curve.* ■ *After CI I preparation.* ■ *After full occlusal preparation.* ■ *After Cerec onlay restoration.*

## 4.4. Leakage evaluation using GEPT, compared and correlated to other leakage evaluation tests

### 4.4.1. Restorations leakage testing

Thirty-five extracted third molars were selected from the department collection. The teeth were extracted from 18-20 years old patients for reasons not related to the current study. The teeth were sound, caries free and had an open apex with fully developed roots to ensure a proper pass to the pulp chamber and to allow for retrograde pulp extirpation. The teeth were stored in 0.2% thymol at a temperature of 5°C for no longer than one year.

Thirty teeth were randomly assigned to three test groups (A1, A2, and B). Class-I preparations (6 mm long in mesio-distal direction, 3 mm wide in bucco-lingual direction and 2 mm deep; measured from the middle fissure level) and were prepared as described in section 4.2.1.2 (Fig 8, C). In the group A1, the teeth were restored with resin composite without bonding (n=10), in the group A2 with resin composite with bonding (n=10) and in the group (B) was restored with ceramic inlays with bonding (n=10). All restorations were carried out according to the protocol in section 4.2.1.3. Group of intact teeth (n=5) without preparation, served as controls (C).

GEPT measurements for all samples were carried out at the following time points:

a) At baseline before any treatment established, i.e. after embedding, to assess tight sealing.

b) After preparation to determine the maximal leakage through the dentin wound.

c) After restoration to measure the restoration leakage value.

d) After thermo-mechanical stress to study the effect of thermo-mechanical loading on the restoration integrity.

**Figure 8**

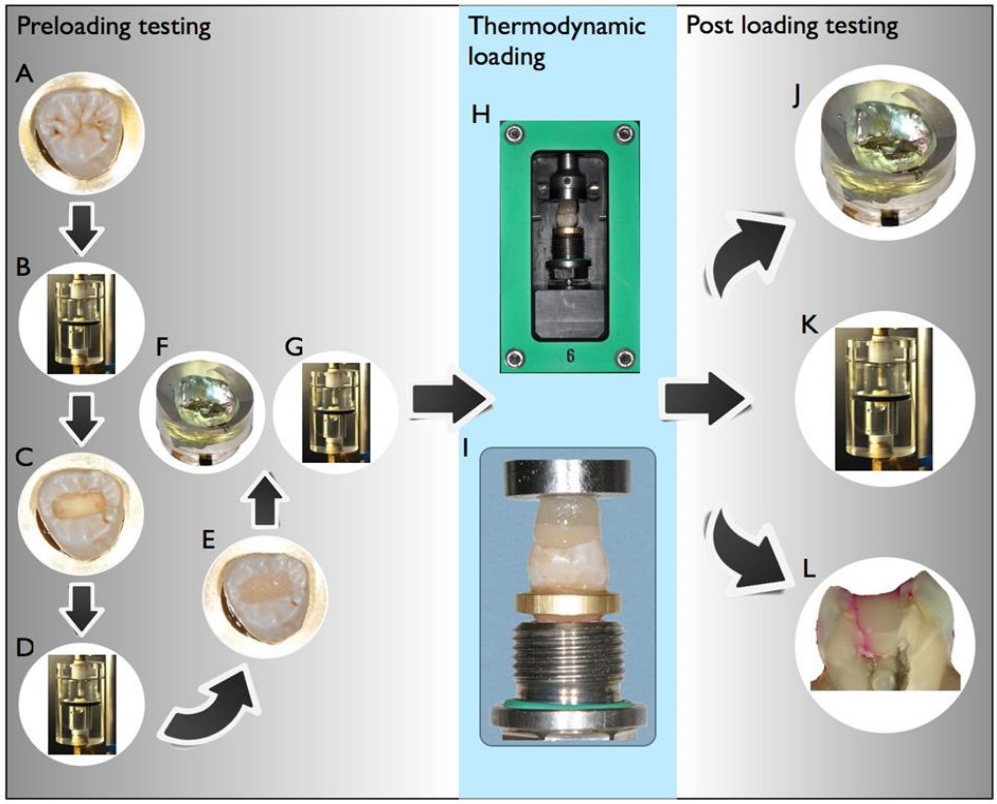


***Figure:*** *Overview of the different restorations testing phases: After mounting of the samples. (**A**), first GEPT measurements were taken (**B**) and preparations were drilled (**C**). GEPT was re-assessed (**D**) and restorations were placed (**E**). Leakage was determined with SEM (**F**) and GEPT (**G**). Thermodynamic loading was performed in a loading chamber (**H**,**I**) and the final evaluation was made with SEM (**J**), GEPT (**K**) or dye penetration testing (**L**)*

To test for the thermo-mechanical stress effect on restorations, samples were transferred to special carriers and mounted without interrupting the disc mounting integrity (Figure 8, H & I). For this purpose, stainless steel carriers with an internal one side opened cylindrical compartment (Diameter of 11 mm and a

depth of 12.5 mm) was developed. This compartment was filled with a heavy body impression material (3M ESPE Pentamix 2, 3M Deutschland GmbH, Seefeld, Germany). To maintain a safety space between the disc and its carrier, a 1 mm high separator made of rubber, was placed between the embedding disc and the carrier during the mounting process. It was removed later. The created space ensured any luxation of the disc to be avoided and thus, the stress to be transported only to the root ensuring no deterioration of the mounting integrity.

Full occlusal contact among the restoration was established by fabricating antagonists made of resin composite material (Filtek Supreme XT, 3M ESPE). These antagonists were individualized for each sample separately.

The samples and their antagonists were mounted in a computer-controlled thermo-loading loading and subjected to thermo-mechanical loading; 1'200'000 loadings at 20 N/cm² and 3'000 thermal cycles (Krejci et al. 1990).

#### *4.4.1.1. SEM evaluation of the restoration interface*

The restoration integration quality of samples tested in section 4.4.1 was determined before and after the thermo-mechanical loading. This allowed for studying the effect of thermo-mechanical stress on the restoration survival.

The occlusal surfaces were cleaned with alcohol, intensively rinsed with water spray and finally dried with air. Impressions of the occlusal surface were obtained using low viscosity, addition silicone impression material (President plus jet light body (Coltene, Altstätten, Switzerland). This process took place after the restorations placement as well after the thermo-dynamic loading. The impression material was allowed to fully set for 24 h. The impressions were then poured with epoxy resin (Stycast 1266, Emerson & Cuming, Henkel Eleotronlo Materials, Westerlo, Belguim) and allowed to set for an another 24 h. The casts were trimmed and mounted on SEM holders (SCD 030, Balzer Union AG, Liechtenstein). The

mounted samples were then left to dry for another 24 h. The casts were coated with 90 nm gold layer with the aid of sputtering device (Oerlikon Balzers Coating AG, Balzer, Liechtenstein) under 0.08 mbar pressure and a current of 45 mA over 3 min.

The replica were studied and analysed under a 200-fold magnification for the integrity, i.e. a gap presentation. A gap was defined as a defect in the continuity between tooth and restoration surface characterized by including a non-detectable floor (Figure 9, B). The total margin analysis of the restoration was carried out in steps with a scanning electron microscope (SEM; Carl Zeiss Supra 50 VP FESEM, Carl Zeiss, Oberkochen, Germany). The restoration total quality was expressed for each sample individually as a percentage of discontinuity, i.e. the percentage of restoration defective margin (Blunck *et al.* 1986). One blinded and calibrated operator carried out the marginal analysis two assessments. The operator repeatability to measure same sample was tested and found at different time's intervals (2 weeks) to be 91%. The marginal assessment was carried out based on the following criteria:

- Perfect margin: No visible interruption of the interface continuity, i.e. no levels difference visible (Fig 9, B-a).

- Marginal gap: the interface shows discontinuity, e.g. cracks or gaps (Fig 9, B-b).

- Non-assessable areas were defined as any deviation from the above-mentioned criteria. Such as bubble presentation at the margin, obstruction of margin with smear (Fig 10).

The assessment was made for all negative replicas representing the before and after thermo-mechanical loading.

**Figure 9**

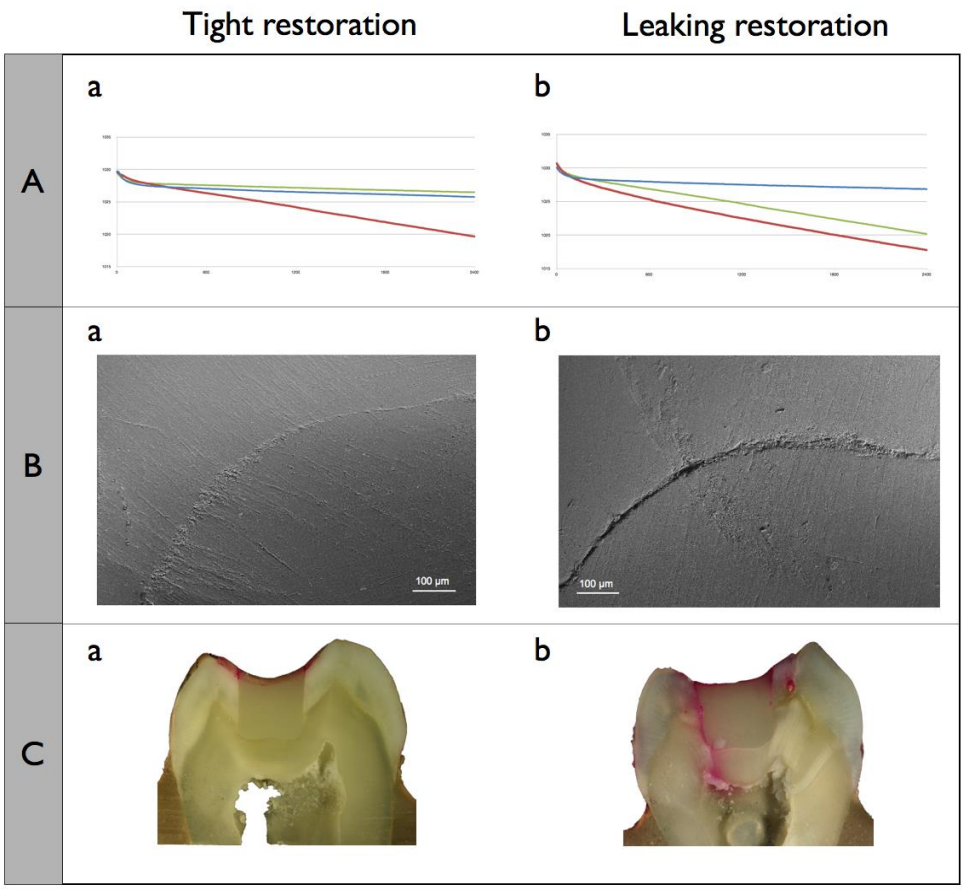


***Figure**: Illustration of the results of the three test methods (left: "non-leaking", right: "leaking"): GEPT evaluation with representative baseline pressure curves*
*(**A**); blue = baseline, red = after preparation and green = after restoration, (**B**) SEM margin analysis and (**C**) Fuchsin dye penetration test.*

**Figure 10**

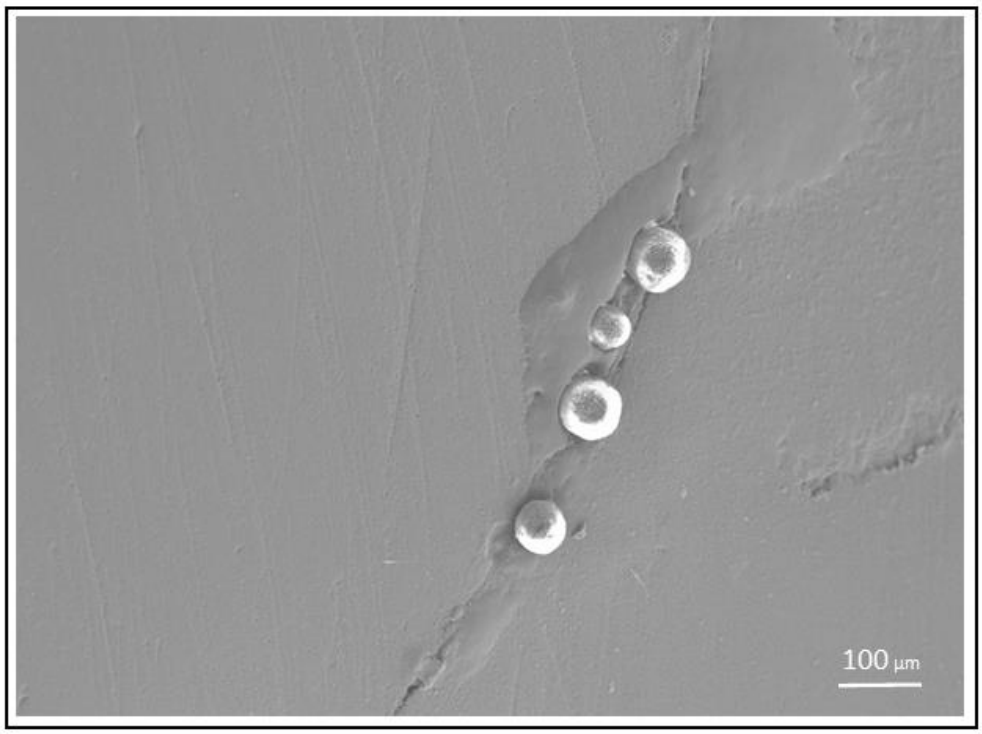


***Figure**: Non-assessable areas presented as bubbles and/or smear obscuring the direct visibility of the tooth-restoration margin.*

### *4.4.1.2. Dye penetration evaluation of restorations*

At the very final stage of restoration tightness and integrity evaluation as described in the section 4.4.1.1, the dye test took place. When this evaluation required samples to be sectioned, the samples could be evaluated only at the end and at only a single time point. The samples were carefully demounted from embedding rings. The teeth were circumferentially sealed up to the surrounding 1 mm around the restoration margins with nail varnish and were immersed in 0.5% basic Fuchsin stain solution for 20 h.

The samples were sliced under kerosene cooling in bucco-lingual direction utilizing a slow speed diamond saw (0.4 mm, Struers GmbH, Zweigniederlassung, Switzerland). Out of each sample a total of four sections could be prepared for evaluation. The sections were photographed at a 25-fold magnification and digitized. Samples were evaluated to the dye penetration at the restoration-tooth interface. They were classified dichotomously into "non-leaking" (=0) when the dye stopped before reaching the pulp chamber or "leaking" (=1) when the dye reached the pulp chamber (Figure 9, C). All sections were evaluated independently by two blinded investigators. In case of disagreement sections were reassessed and discussed until an agreement was reached.

### 4.4.2. Root canal filling leakage testing

For the purpose of studying the influence of root canal anatomy on the root filling quality and consequently studying the influence of its tightness, teeth with two different root canal morphologies were selected. The first group consisted of upper central incisors (UCI) with a single root canal (n=12), while the second group included mesial roots of lower molars (MRLM), containing two canals and an isthmus between (n=12) (Fig 3, A). None of the teeth had previous root canal treatment, carious or cracks in their roots. They all had a fully formed apex and were extracted for reasons not related to the study and preserved in thymol 0.2% at 5°C for no longer than 1 year. The teeth were pre-scanned utilizing μ-CT device (μ-CT 40:Scano Medical, Brüttisellen, Switzerland) to confirm their suitability to the study purpose. Standard root canal preparations were carried out (section 4.2.1.4). The samples were either sectioned/fully embedded after establishing the build-up as described in section 4.2.1.1. The samples were tested to determine their GEPT baseline and the permeating saline volume. Subsequently, the root canals were filled based on the technique described (section 4.2.1.4). The first μ-CT scans was achieved to calculate the total volume in the lower 11 mm of canal. To standardize treatment, only the lower 11 mm of the prepared canals were

filled. The access cavity was secured with a cotton pellet and sealed with a temporary filling material (Cavit, 3M ESPE, Seefeld, Germany) and left to dry in a humid box at 35°C for 24 h. Temporary fillings were removed and μ-CT scans repeated. Leakage status was determined again using the GEPT. At the end of testing, the last free 2 mm of the apex were sealed with a resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) after a standardized conditioning and bonding (Syntac Classic, Ivoclar Vivadent, Schaan, Liechtenstein). Subsequently, the GEPT was assessed for the last time to ensure that the leakage measured was related to suggested path through the whole root canal filling length and not related to dentinal tubules connected to possible insufficiencies of the embedding at the outer root surface.

#### *4.4.2.1. μ-CT analysis of root canal treatment*

The root canals fillings were tested with the GEPT (section 4.4.2) and evaluated for the 3D root canal filling quality. It was hypothesized that a filling compromising 100% of the root canal volume, would form a tight seal and prevent leakage. To allow multiple measurements, individual custom-made carriers made of heavy-body rubber impression material (3M ESPE Pentamix 2, 3M Deutschland GmbH, Seefeld, Germany) were established for each sample (figure3, E). The rubber carriers were glued to scanning electron microscopy stubs (014001-T, Bal Tec AG, Balzers, Liechtenstein). This set-up allowed for easy sample removal and repositioning of samples at almost the same position at each test stage. Each sample was scanned after embedding (after root canal preparation) utilizing a high-resolution μ-CT scanner (μ-CT 40: Scano Medical, Brüttisellen, Switzerland) at an isotopic resolution of 20 μm, 70 kV and 114 μA (medium resolution). This resulted in 600-800 slices for each root scan. The lower 11 mm of the root below the mounting disc, presented the area of interest. The scan was repeated at 70 kV and 114 μA with an isotropic resolution of 10 μm (i.e. a high resolution set up) after the root filling were made. To compensate for possible inaccuracy in repositioning by

the established carriers, superimposition was established using special software (IPL Register 1.01beta, Scano Medical, Brütisellen, Switzerland). Volumes of root canals before and after root canal filling were calculated with the aid of a specially developed software (IPL V5.06B, Scano Medical, Brütisellen, Switzerland) (Figure 11).

**Figure 11**

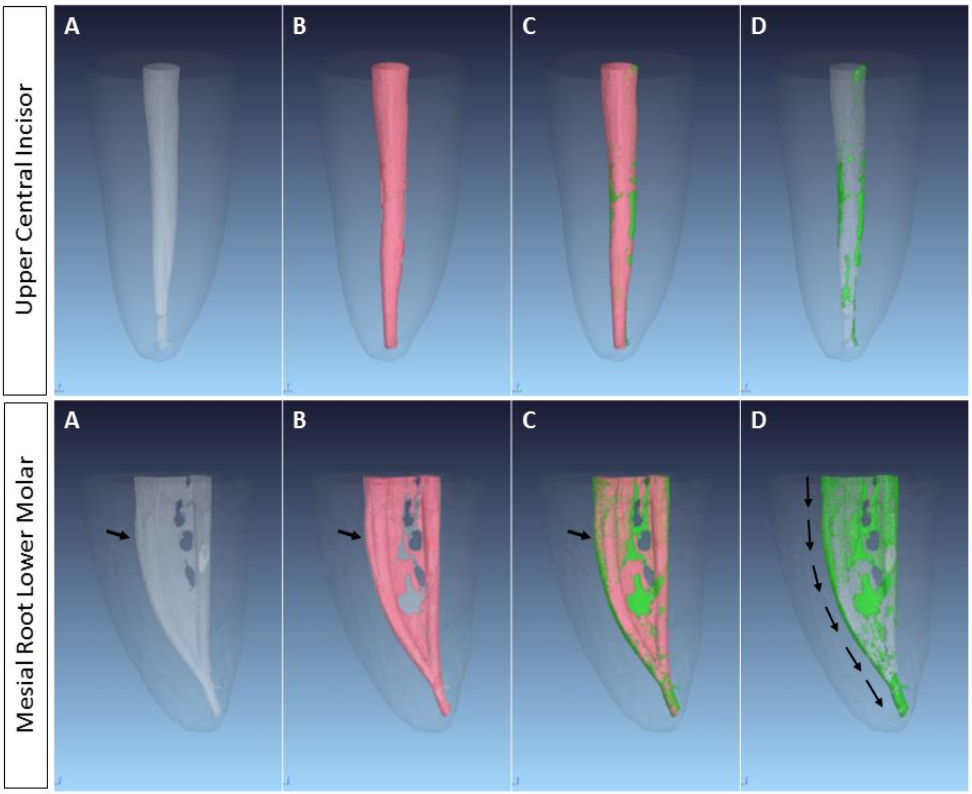


***Figure:*** *Steps for 3D root canal treatment analysis.*
***A****) Root canal scan after preparation,* ***B****) Root canal scan after filling,* ***C****) Superimposition of both scans and* ***D****) Calculation of unfilled space in the root canal system by subtracting the filled volume from the total canal space.*

The root filling (Gutta-percha and sealer) was identified and the volume was calculated as following: the voxels defined in the preoperative (before root filling) as soft tissues, fluids and air presented the total canal volume. The voxels,

which were shown to be filled with a radio-opaque material in the postoperative scan (after root filling), were considered to be filled with the root filling material. Counting these voxels allowed for volume calculation by multiplying in one voxel volume. The root filling volume was presented as a percentage to the total canal volume, out of which the remaining unfilled root canal volume (root canal filling defect) could be calculated.

### 4.4.3. Implants leakage under static conditions

Three different previously described implants designs (Table 1) with a sample size of 20 each (n=16, tested samples, and n=4, controls) were tested by all tests under static conditions i.e. GEPT, molecular leakage and bacterial leakage (Fig 12). First, implants were embedded as described and measured with the GEPT to determine their baseline values. Second, an inside-outside connection was established by drilling a hole from the apical direction to the internal implant compartment using a 1 mm hard metal drill at a speed of 1100 rpm under extensive continuous water-cooling. A parallelometer was used to hold the implants in an inverted position. Care was taken not to harm the internal threads. For this purpose, the distance required not to reach the thread openings was precisely calculated, and drilling took place only to that depth. In the control implants, the drilling was performed without getting access to the thread space. It was aimed to study the potential deleterious effect of drilling on the embedding integrity, which was previously assessed. Core build-ups were then fabricated (section 4.2.2.2) and the implants were tested again as described. Finally, the baseline slope was subtracted from the slope after build-up to calculate the absolute leakage slope. The saline flow was recorded again as well.

**Figure 12**

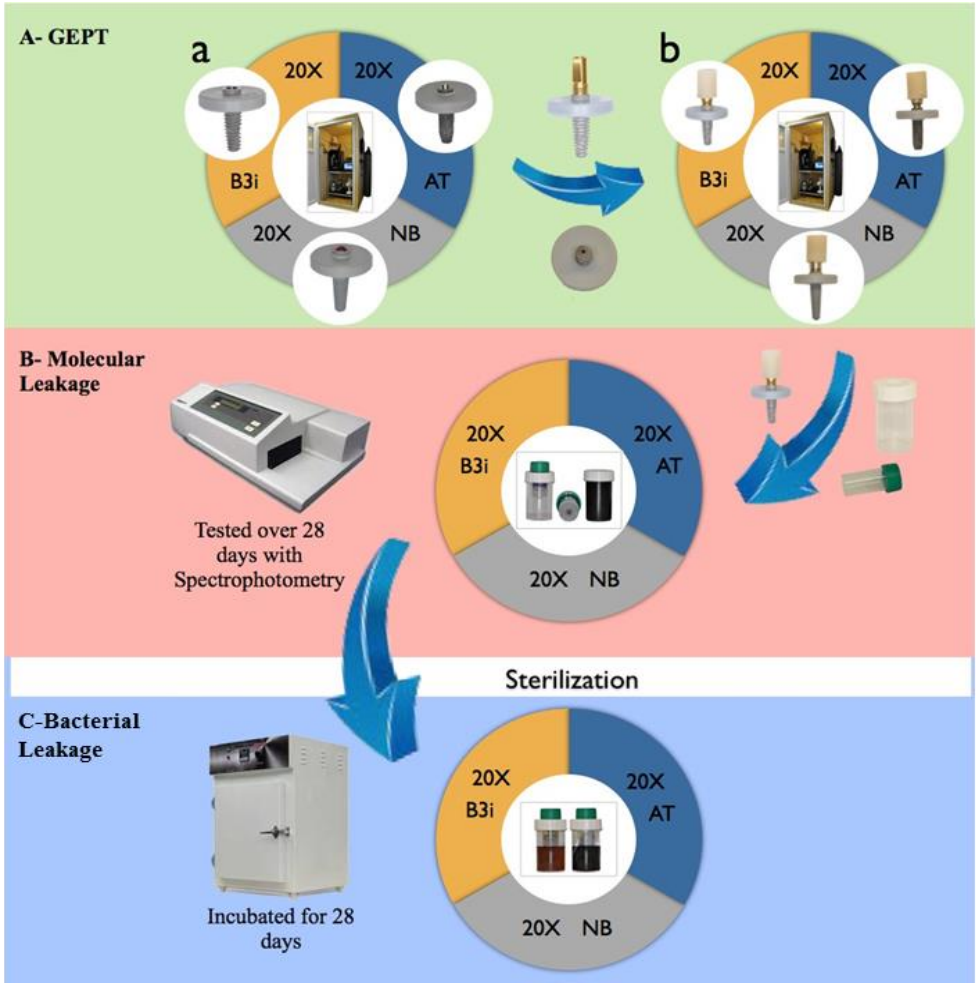


***Figure**: Testing flow chart*

***A**. GEPT*

***a**) Implants were mounted in discs and tested for their baseline leakage*

***b**) After hole drilling, the abutment was fixed and build up was made then the implants were retested to calculate for their absolute leakage*

***B**. Molecular Leakage*

*The same implants were further mounted in a two chambers system in which the upper chamber contained fluorescent molecules and the lower chamber was regularly tested for increasing fluorescent molecules content*

***C**. Bacterial Leakage*

*The same set up was used after washing and sterilization. The upper chamber contained E. fecalis strain which its leakage was indicated by turbidity of a selective media broth placed in the lower chamber*

### *4.4.3.1. Static molecular leakage in implants*

The same set of implants used in section 4.4.1.1 was used for both tests (4.4.3.1. molecular and 4.4.3.2 bacterial leakage tests).

Each implant was positioned in a shortened 15 ml centrifuge tube (Semadeni, Ostermundingen, Switzerland; Fig 13).

**Figure 13**

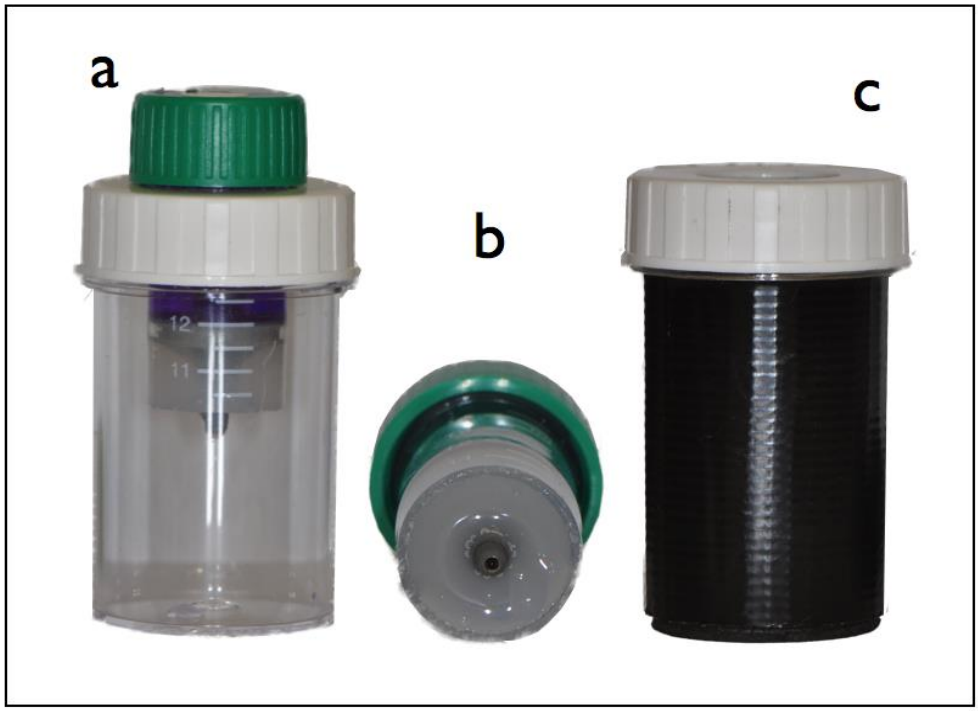


***Figure**: Molecular leakage set up*
***a**. Two chambers system. **b**. Implants further sealed from the apical side leaving the drilled hole free. **c**. Lower chamber taped to obtain light tight conditions.*

The tube was shortened by cutting-off the 8 cm from the tip side. The implant was positioned 1 cm from the established lower cut level. The created lower compartment below the embedding disc was additionally sealed with silicone

glue (Dow Corning 734, Dow Corning GmbH, Wiesband, Germany) leaving the drilled tip patent and free. The glue was allowed to dry for 24 h. A 30 ml transport tube (Semadeni, Ostermundingen, Switzerland) was used to create the counterpart of the two chambers system. It was drilled with a 15.5 mm drill to allow insertion of the first tube. The resulted custom-made two-chamber system allowed testing for permeation through all tested implants. The upper chamber was filled with three ml of 10'000 Dalton and 50% w/v Dextran Texas Red (Life Technologies Europe B.V., Zug, Switzerland), while 16 ml of deionized water were added to the lower ensuring the implant tip immersed in the water. The transport tube was then coated utilizing a black tape to make it lightproof. This was important to prevent potential fluorescent substrate degradation and loss during the storage. As an extra measure, the storage took place in a dark chamber. For regular calibration measures an extra tube holding 10 ml of the Dextran solution was used as a spectrophotometry contrast, out of which a dilution series were made at 600 nm wavelength at each testing day to establish a calibration curve and determine the detection limit. Evaluation of leakage took place utilizing a spectrophotometer (Spectramax M2, Bucher biotec AG, Basel, Switzerland). From the lower chamber of each sample, a 300 µl were pipetted and transferred to a 96 well plate and tested the presence of Dextran in the spectrophotometer. After each test, the pipetted 300 µl de-ionized water was substituted with an equal volume. Samples were tested on a daily basis in the first four days, then once every two days (for a total period of four days) and finally, once every four days until the 28 days testing period were completed. The sample was considered leaking if the spectrophotometry value was above the detection range one time and in all the subsequent measured time points. Time of leakage start was reported and considered to represent the leakage status of the implant.

#### *4.4.3.2. Static bacterial leakage in implants*

The same samples (section 4.4.3.1.) were further tested utilizing the same set-up described above. For this test, each sample with its mounting parts, were packed in a separate sealed sterilization bag. Subsequently, sterilization took place using ethylene oxide gas (3M AG, Rüschlikon, Switzerland) in a sterilizer (Sterivac 4XL, 3M AG, Rüschlikon, Switzerland) using the cold sterilization cycle at 37°C for 5,5 h. The seal for each pack was opened and the parts were re-assembled under a clean bench (EVZ 120, SKAN AG, Basel, Switzerland). Three ml of overnight culture, *E. fecalis* ATCC 29212 in fluid universal broth (FUM, Gmür and Gugenheim 1983), was filled in the upper chamber (Fig 14). The bacteria holding broth was previously adjusted to an optical density of 1.0 at 550 nm. To the lower chamber, 16 ml of enterococci-selective bile esculin azide broth (Enterococcosel Broth, Difco, Benton Dickinson & Co.,Sparks, MD, USA) were added. This medium has the ability to indicate bacterial leakage through color change. When *E. fecalis* hydrolysed the esculin the product produces turbidity and blackening of the broth. For optical contrast comparison an extra transport tube holding the same volume of 16 ml of selective media was used as a negative control. All the samples were then transported to an incubator in ambient air at 37°C. The samples were observed daily and assessed for leakage for 28 days. In case of leakage the day at which the sample showed a visible sign of leakage was reported and considered to present the leakage status of that implant. Bacterial viability was assessed at the end of the experiment by a bacterial swap, which was applied to the selective media in the lower chamber of the same corresponding sample and further incubated overnight. All samples assemblies had presented viable bacteria caused turbidity in the selective media broth.

**Figure 14**

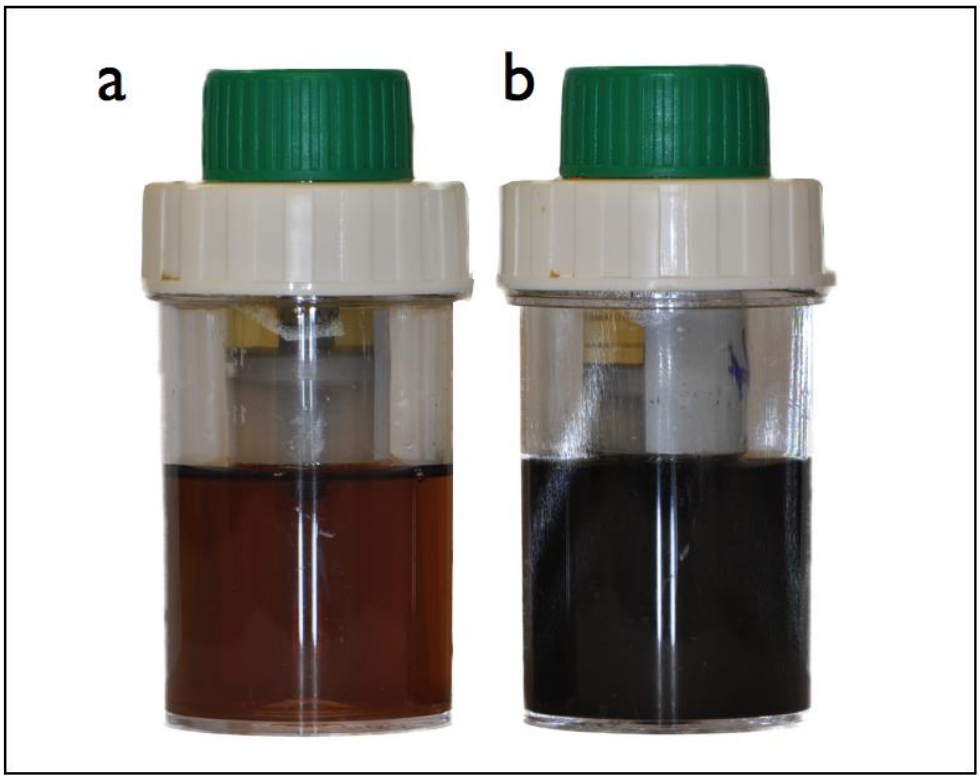


***Figure**: Bacterial Leakage*
***a**. Mounted set up; clear yellowish broth in lower chamber indicates no leakage. **b**. Darkening and turbidity of lower chamber broth indicating leakage.*

### 4.4.4. Implants leakage under thermo-mechanical loading

For this purpose 30 implants of implants systems described in Table 1 (n=8, controls= 2 each) were used. The principle of two separated chambers applied in endodontic root canal filling leakage testing under static conditions was adopted (Goldman *et al,* 1980) .The implant thermo-mechanical loading system consisted of two tightly separated chambers with the implant held in between (Fig 15). The lower chamber was based on two hard stainless steel parts and designed to be interlock with a screw system thus holding the mounted implant sample in

between two rubber washers (outer diameter 15 mm, inner diameter 10 mm and thickness 1 mm). The washers were placed on both sides of the mounting disc to ensure a hermetic seal. The upper chamber was created by an elastic, cylindrical and semi-transparent PVC lever, which was tightened on the lower holder and its opposing antagonistic disc with O-rings. The design allowed observing the colour change of detection media placed in the upper chamber. This is to happen when bacterial broth containing a bacterial strain (*E. fecalis* ATCC 29212 in fluid universal broth FUM (Gmür and Gugenheim 1983), placed in the lower chamber penetrated through the sample mounted in the middle. The colour of a detection media changed when the bacteria hydrolysed a certain component (esculin) resulting in turbidity and blackening of the broth in the visible compartment indicating their penetration from one compartment to another. Conceptually, if the embedding was tight, bacterial cells could only penetrate through the hole drilled at the implant apical tip to reach the IAI and then travel to the upper compartment. The antagonist was designed in such a way that it introduced a 30 degree angled surface in its contact surface, thereby allowing for exertion an additional luxation effect on the abutment. This was to simulate a more clinically relevant loading situation. The antagonist was designed to contain an inlet, through which the detection media could be applied prior to be tightly sealed with a rubber piece, to result in a hermetically sealed compartment.

**Figure 15**

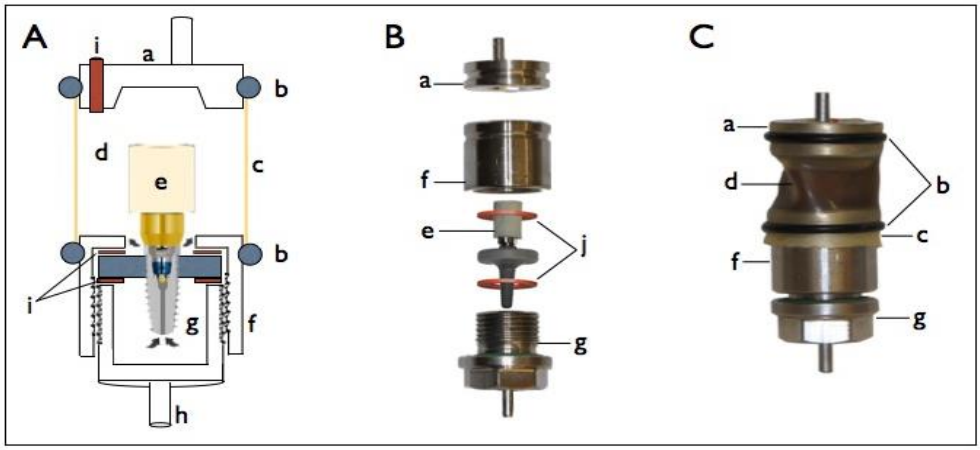


***Figure**: Schematic illustration of dynamic loading set up (**A**), photo of the different components prior to assembly (**B**) and fully assembled set-up (**C**).*
***a**. Antagonist, **b**. Tightening O-rings, **c**. Elastic semi-transparent lever, **d**. Upper compartment holding the indication medium, **e**. A mounted implant sample, **f**. Capping holder of lower chamber, **g**. Lower chamber compartment with screw third for tightening, **h**. Mounting holder for chewing machine cell, **i**. Indicating medium filling inlet, **j**. Sealing rubber washers*

All samples and assemblies to be configured into the test model were individually wrapped in autoclave sterilization bags. Gas sterilization took place utilizing ethylene oxide gas (3M AG, Rüschlikon, Switzerland) in an automatic sterilizer (Sterivac 4XL, 3M AG, Rüschlikon, Switzerland) using the cold sterilization cycle at 37°C for 5,5 h. This sterilization protocol has the benefit of administering a temperature, which is tolerable by all used materials. Thus no deterioration neither dimensional changes could theoretically happen. Under sterilized conditions in a clean bench (EVZ 120, SKAN AG, Basel, Switzerland), the packs were opened and the whole assembling process took place. A 1.5 ml of overnight culture of *E. fecalis* ATCC 29212 in fluid universal broth (FUM, Gmür and Gugenheim 1983) was added to the lower chamber. The bacterial culture was previously adjusted to 1.0 optical density at 550 nm. The two rubber washers were placed on the implant, which was then brought in position in the counterpart and

the whole assembly was then positioned on top. The two parts were then manually tightened together using pliers. The mounted part was brought in position again and held against the antagonist while maintaining a distance equivalent to the value established by the masticator chamber. The elastic semi-transparent lever taken out of finger cots (PVC medium size, 0.35 mm thick, MUCAMBO – GUMMI Matthias Jacoby, Altrip, Germany) was tightly mounted in its position and over the two parts with O-rings (outer diameter 22 mm , inner diameter 18 mm , thickness 2 mm; Fig.15, C). After assembling the parts fully together, the upper chamber was filled with a 3 ml of enterococci-selective bile esculin azide broth (Enterococcosel Broth, Difco, Benton Dickinson Co..Sparks, MD, USA). This allowed for bacterial leakage detection by inspecting the colour change (turbidity). Subsequently, the filling inlet was tightly sealed with a fitting cylindrical shaped rubber component (Fig 15, i). All the mounted specimens were placed in the computer-controlled masticator. The thermo-mechanical stress consisted of 1'200'000 loads under a stable water controlled temperature of 37°C. The samples were checked on a daily basis. Due to a slight change in the lever transparency, a light source (Laser class 3R, Intertronic, Interdiscount AG, Switzerland) was applied to confirm detection outcome. In the case of no leakage, the light could penetrate through the clear medium and resulted in a lamp glow appearance (Fig 16, A). In contrast, a reflected pointed light source on the outer surface was observed, when turbidity existed as a result of leakage. This observation reflected the shortage of light making through the medium (Fig 16, B).

The time by when an implant showed leakage was reported. At the end of the experimental period samples under aseptic conditions were obtained from both chambers and cultured overnight in blood agar plates (Colombia agar + 5% Sheep blood, bio Mérieux SA, Marcy l'Etoile, France) in an incubator (IL 115, INCU-Line, VWR, Dietikon, Switzerland) at 37°C to confirm the results and to ensure a single bacteria type involvement (i.e. no contamination from outside the system and the survival in the lower stock chamber in all cases).

**Figure 16**

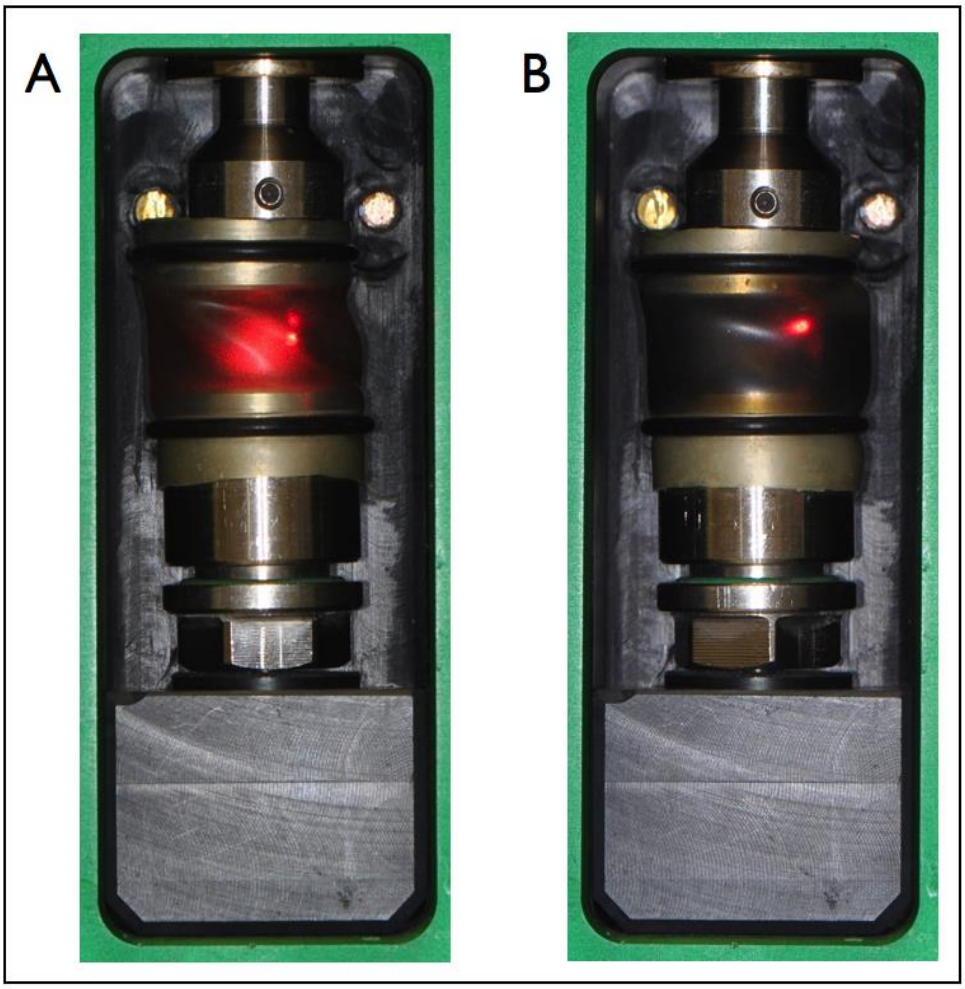


***Figure**: Visual comparison of bacterial leaking vs. tight implant.*
*(**A**) Tight implant and (**B**) leaking implant*

To ensure no leakage at the implant-disk interface after the thermo-mechanical loadings, the drilled apices of implants showed bacterial leakage during loading (AT n=4 and NB n= 6) were tightly sealed again, i.e. they were grit-blasted (50 µm aluminium oxide, Benzer-Dental AG, Zurich, Switzerland), further conditioned with Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein), and

adhesively treated (Clearfil SE Protect, Kuraray America Inc., USA) and finally filled with a resin build-up filling material (Luxa Core Automix, DMG, Hamburg, Germany). GEPT was measured again. The hypothesis was that the original leakage status (baseline) should be regained, provided that the marginal mounting was still tightly intact.

#### *4.4.4.1 SEM visual assessment of implant-abutment interface*

Implant systems from the thermo-mechanical loading investigation were embedded in epoxy resin (Stycast 1266, Emerson & Cuming, Henkel Eleotronlo Materials, Westerlo, Belgium) and left to set for 24 h. Thereafter, they were sectioned into halves utilizing a slow speed diamond saw (0.4 mm, Struers GmbH, Zweigniederlassung, Switzerland). The hardened resin blocks were mounted in SEM carriers (SCD 030, Balzer Union AG, Balzer-FL) and gold sputtered (Oerlikon Balzers Coating AG, Balzer, Liechtenstein). Sections were coated with a 90 nm gold layer under 0.08 mbar and current of 45 mA over a period of 3 min. Implants were observed under SEM (Zeiss Supra V50, Carl Zeiss, Oberkochen, Germany) at magnifications 50X, 500X and 5000X (Fig 17).

## 4.5. Statistical analyses

Due to the variability in measured data nature (leakage time points, percentages, numerical measured values, dichotomous values), different statistical tests were necessary to prove the correlation between different tests. The level of significance was set at 5% level ($p<0.05$) for all tests.

**Figure 17**

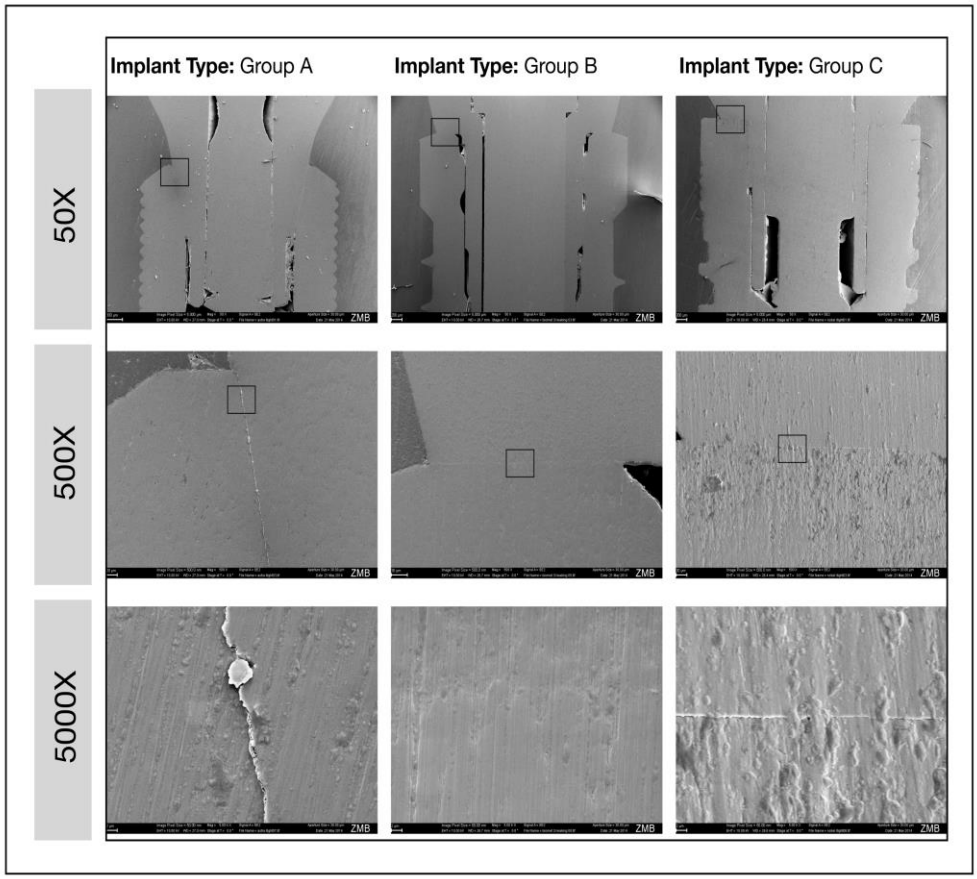


***Figure**: Representative samples of SEM images representing bacterially non-leaking samples with a GEPT score value of less than 0.090 hPa/min. The squared area determines the magnified section in each photo with higher magnification*

### 4.5.1. Validation of the GEPT system

To assess the repeatability of the individual pressure change difference measured for the same sample within the same treatment a linear mixed model was used. Thus, the GEPT measured permeability expressed as the slope in hPa/min as well the total permeated water volume expressed in ml, were calculated independently for each of the four conditions (baseline after embedding, CI I

preparation, full occlusal preparation and restoration). The results were presented as the range of data obtained in the individual measurements (original measurement and 7 repetitions).

To assess the detection limit, the measurement background noise in the test curves of the sound 6 teeth at fixed 9 time points with 2 min intervals was calculated mathematically. It was calculated by measuring the deviation from the ideal curve drawn between the two fixed time points to determine the leakage slope value independently. When the ideal slope value (hPa/min) and the time interval are known, it is possible to calculate the ideal measurement value at each point. The deviation from this was calculated, and average deviations were then pooled for each sample and used for further calculations (Gläser M *et al.* 2010).

To test whether the slope in the pressure change over time correlated with the collected saline solution (N = 6), the Pearson correlation coefficient was used ( Lorenz D *et al.* 2011)

### 4.5.2. Restorations leakage testing

The descriptive analysis is given separately for the three restorative treatments for the GEPT tests (before and after chewing machine), the SEM tests (before and after the thermo-mechanical loading) and the Fuchsin dye penetration test (only after thermo-mechanical loadin). For the negative control the GEPT test and the Fuchsin dye penetration test (after thermo-mechanical loading) were applied. The following tests were subsequently applied; Kolmogorov-Smirnov test, Wilcoxon signed rank test, Kruskal-Wallis tests, Mann-Whitney U tests and Spearman's rank correlation. All p-values are two-sided.

To evaluate the implants performance under thermo-mechanical loading, the GEPT measured data, mean values and standard deviations, were assessed prior to and following dynamic loading. An ANOVA was applied to test for

significance between systems at each stage of testing. Additionally, a Dunnett *post hoc* analysis was conducted to isolate the differences. While bacterial leakage was presented by means of days; exact test of Fisher was applied to compare between different implant systems.

### 4.5.3. Root canal filling leakage testing

Root canal fillings defects as well their GEPT performance, were presented as mean values and standard deviation for each tested group separately. To compare groups for the resulted filled canal volume as well for their performance under GEPT, t-test was applied. To test for correlation between the GEPT measurements to the root filling defective volume, as well the GEPT measurements to the permeated WV (ml), the Person correlation coefficient was applied for both situations.

### 4.5.4. Implants leakage under static conditions

To compare for implants performance with the GEPT, mean values and standard deviations were calculated. To test whether the slope in the pressure change over time correlated with the collected saline solution (N = 48), the Pearson correlation coefficient was used.

The time points for molecular and bacterial implants leakage under static conditions, were considered as absolute values representing the leakage status of samples individually. All testing values were presented in a table trying to detect leakage sequence pattern.

### 4.5.5. Implants leakage under thermo-mechanical loading

To evaluate the implants performance under thermo-mechanical loading, the GEPT measured data, mean values and standard deviations, were assessed prior to and following thermo-mechanical loading. An ANOVA was applied to test

for significance between systems at each stage of testing. Additionally, a Dunnett *post hoc* analysis was conducted to isolate the differences. While bacterial leakage was presented by means of days; exact test of Fisher was applied to compare between different implant systems.

# 5. RESULTS

## 5.1. Validation of the GEPT system

The baseline mean slope values (Table 2) determined with GEPT, i.e. the measurements of the sample permeability status before treatment ranged between 0.014 -0.034 hPa/min. This indicated the embedding protocol to be tight and providing a proper seal of the specimens. The low variation after repeated measurements of a sample did not exceed the 0.01 hPa/min thus, presented a high linearity (Table 2), which indicated a consistent measured results when the same specimen was measured multiple times.

**Table 2:** Slopes of regression lines according to respective specimen

| Specimen | Initial hPa/min | Class 1 preparation hPa/min | Occlusal Full preparation hPa/min | After restoration hPa/min |
|---|---|---|---|---|
| Metal disc | 0.014 (0.013, 0.014) | - | - | - |
| Resin composite disc 1 | 0.016 (0.016, 0.017) | - | - | - |
| Resin composite disc 2 | 0.032 (0.031, 0.034) | - | - | - |
| Resin composite disc 3 | 0.025 (0.024, 0.026) | - | - | - |
| Third molar 1 | 0.020 (0.019, 0.021) | 0.193 (0.192, 0.194) | 0.355 (0.353, 0.356) | 0.024 (0.023, 0.026) |
| Third molar 2 | 0.016 (0.015, 0.017) | 0.216 (0.214, 0.218) | 0.417 (0.415, 0.418) | 0.018 (0.017, 0.019) |
| Third molar 3 | 0.029 (0.028, 0.030) | 0.235 (0.233, 0.237) | 0.478 (0.477, 0.481) | 0.034 (0.033, 0.036) |

Values indicate means and ranges (in parentheses) of 8 individual experiments.

The detection limit i.e. the minimal measured value with accuracy was calculated to be 0.043 hPa. This value correlated to a slope value of 0.002 hPa/min and corresponding fluid infiltration to 0.023 µl/min. The Pearson coefficient testing for the pressure difference- infiltrated to fluid volume correlation was set to the confidence interval set at ($p=0.05$). It showed the point estimate of 0.99785 with standard deviation of 0.0002387463 ($R^2 =0.996$) (Table 3). This result, confirmed the high correlation between the measured pressure difference change and the permeated fluid volume (Fig 18).

**Table 3:** correlation of permeation slope values and total water volume for the purposes of system validation

| Variant | Initial | Class 1 preparation | Occlusal full preparation | After restoration |
|---|---|---|---|---|
| Slope | 0.9863832 | 0.9964485 | 0.999631 | 0.9814998 |
| Fluid Volume | 1 | 0.9907555 | 0.9992009 | 1 |

Pearson correlation coefficient values for permeation slope values and total water volume for repeated measurements after different treatments

**Figure 18**

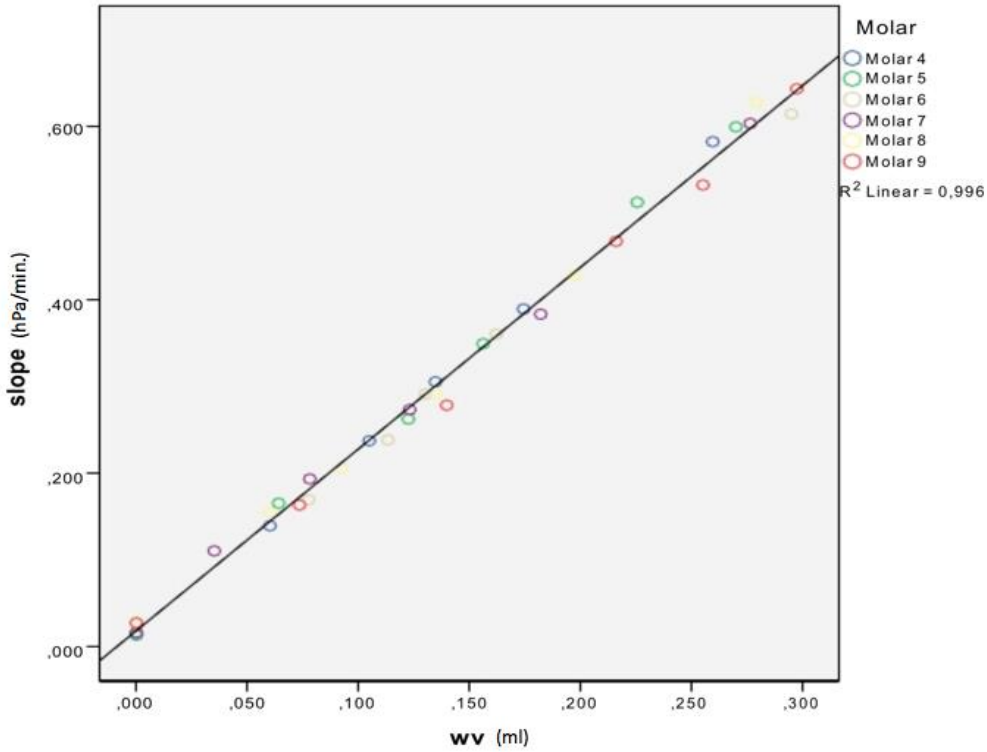


***Figure:*** *Plotted linear regression line, showing the correlation between the slope value (y-axis, denoted as Slope, measurement unit: hPa/min), and the permeated saline volume (x-axis, denoted by wv, measurement unit: ml*

## 5.2. Restorations leakage testing

The different tested groups performance was presented as mean values ± standard deviation (Table 4). The assumption of a normal distribution for the GEPT results was rejected for all groups before the thermo-mechanical loading (p-values 0.003, 0.027, 0.013 for groups A1, A2 and B respectively) and nearly to all tested groups after thermo-mechanical loading (p-values 0.009, 0.200, 0.000, 0.026 for groups A1, A2 and B and negative control respectively) once tested by Kolmogorov-Smirnov test. For the SEM results, normality was not rejected. However, the groups sizes were small, thus only nonparametric tests were applied.

The values comparison for before and after thermo-mechanical loading (separately within each group) led to the result that only the GEPT results in group A1 changed significantly (Wilcoxon signed rank test, p-value 0.016) and were unexpectedly improved after thermo-mechanical loading. In all other groups measured GEPT values (A2, p-value 0.084 and B, p-value 0.129) as well as for the SEM performance (A1 0.114, A2 0.139, B 0.169), no significant changes could be observed (Figures 19 and 20). The negative control group, was not set for this test, as the comparison was meaningless.

Furthermore, examination reviled GEPT measurements and SEM values (before and after thermo-mechanical loading), between the groups A1, A2 and B (and negative control for GEPT after thermo-mechanical loading) to differ significantly (Kruskal-Wallis tests). To test for differences in between the groups, pairwise Mann-Whitney U group comparisons were applied. Under these circumstances, group A1 and group A2 showed a significant difference their GEPT performance at before and after loading and in their SEM values only after thermo-mechanical loading. Comparing group A1 and B, tests showed significant different outcomes at all testing time points. On the counterpart, groups A2 and B, showed only significant differences after thermo-mechanical loading in their SEM values.

The Fuchsin test presented significant difference between all groups (Fisher's exact test, p-value $< 0.0001$). This finding was mainly because of the performance of group A1, where a high number sample showed a high leakage score (8 out of 10).

**Table 4:** Results of the different test methods with regard to the respective treatment groups

| | Before Thermo-mechanical Loading | | After Thermo-mechanical Loading | | |
|---|---|---|---|---|---|
| Group | GEPT hPa/min | SEM marginal defect analysis (%) | GEPT hPa/min | SEM marginal defect analysis (%) | Fuchsin (% of samples with dye reaching pulp chamber) |
| Group A1 (Resin composite restoration without bonding) | 0.431 ± 0.449 $^{A}$ | 18.9 ± 9.2 $^{a}$ | 0.131± 0.076 $^{A*}$ | 26.7 ± 11.0 $^{a}$ | 80.0 $^{A}$ |
| Group A2 (Resin composite restoration with bonding) | 0.074 ± 0.020 $^{B}$ | 15.2 ± 9.8 $^{ab}$ | 0.065 ± 0.014 $^{B}$ | 11.2 ± 6.5 $^{b}$ | 10.0 $^{B}$ |
| Group B (Ceramic indirect restoration) | 0.065 ± 0.010 $^{B}$ | 3.6 ± 4.3 $^{b}$ | 0.060 ± 0.008 $^{B}$ | 5.7 ± 4.4 $^{c}$ | 0.0 $^{C}$ |
| Group C (Negative Control) | 0.062 ± 0.005 $^{B}$ | - | 0.064 ± 0.005 $^{B}$ | - | 0.0 $^{C}$ |

Test results are presented as mean values and standard deviations when applicable.
Different superscript capitals represent statistically significant differences in GEPT measurement/Fuchsin dye penetration, between the different treatment groups ($p < 0.05$; read vertically). Different superscript lower case letters represent statistically significant differences in SEM assessment between the different treatment groups ($p < 0.05$; read vertically). Astaricks indicate statistically significant change in the measured after thermodynamic loading value compared to the before thermodynamic loading measured value of a respective treatment group ($p < 0.05$; read horizontally).

Finally, the correlation between tests was assessed (global over all groups). Spearman's rank correlation was used for correlating the different test results. For GEPT and SEM before thermo-mechanical loading, the tests outcomes correlated only moderately (0.359) but not significantly (p-value 0.051). However, this correlation was significant after thermo-mechanical loading (0.662, p-value < 0.0001). Though, the correlation of the Fuchsin test with GEPT and SEM (after

loading) was significant (0.777 and 0.534, p-values <0.0001 and 0.002 respectively), the highest level of significance was in the favour of GEPT evaluation technique.

**Figure 19**

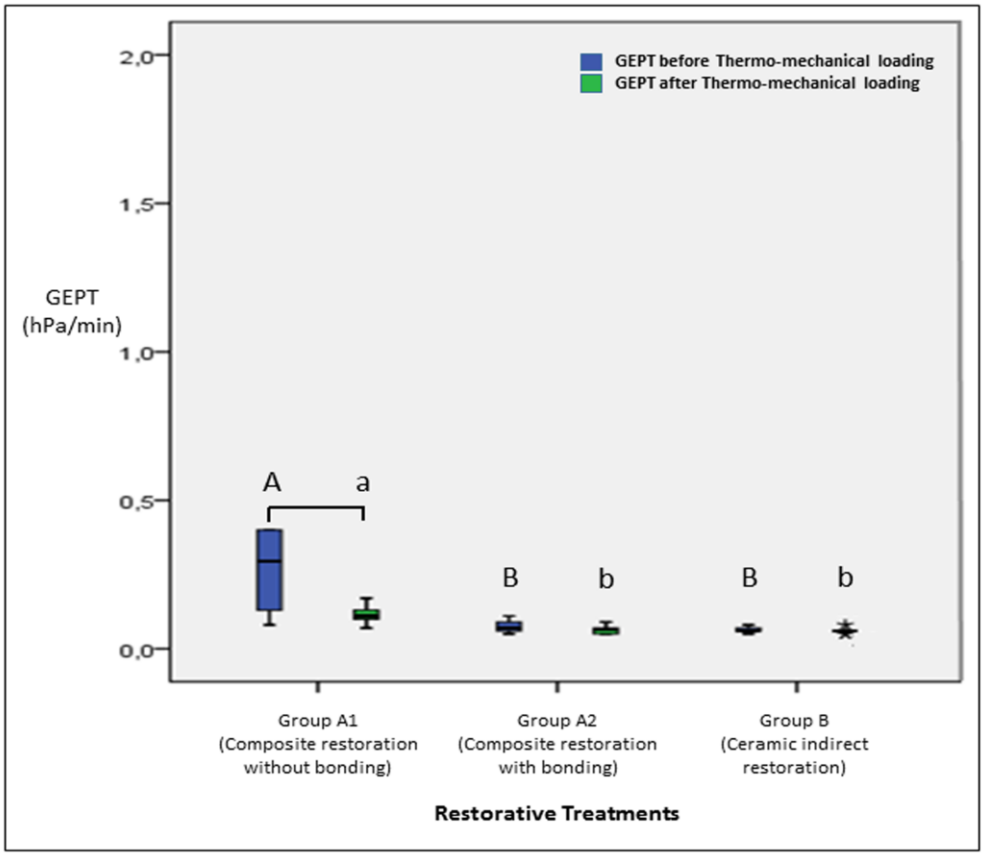


***Figure:*** *Results of the GEPT evaluation using a box-plot illustration for the three groups before and after thermodynamic loading. Statistically significant differences (p-values < 0.05) are indicated with a bar for before and after thermodynamic loading changes. Different capital letters indicated statistical difference between groups before thermodynamic loading. Different small letters indicated statistical difference between groups after thermodynamic loading.*

**Figure 20**

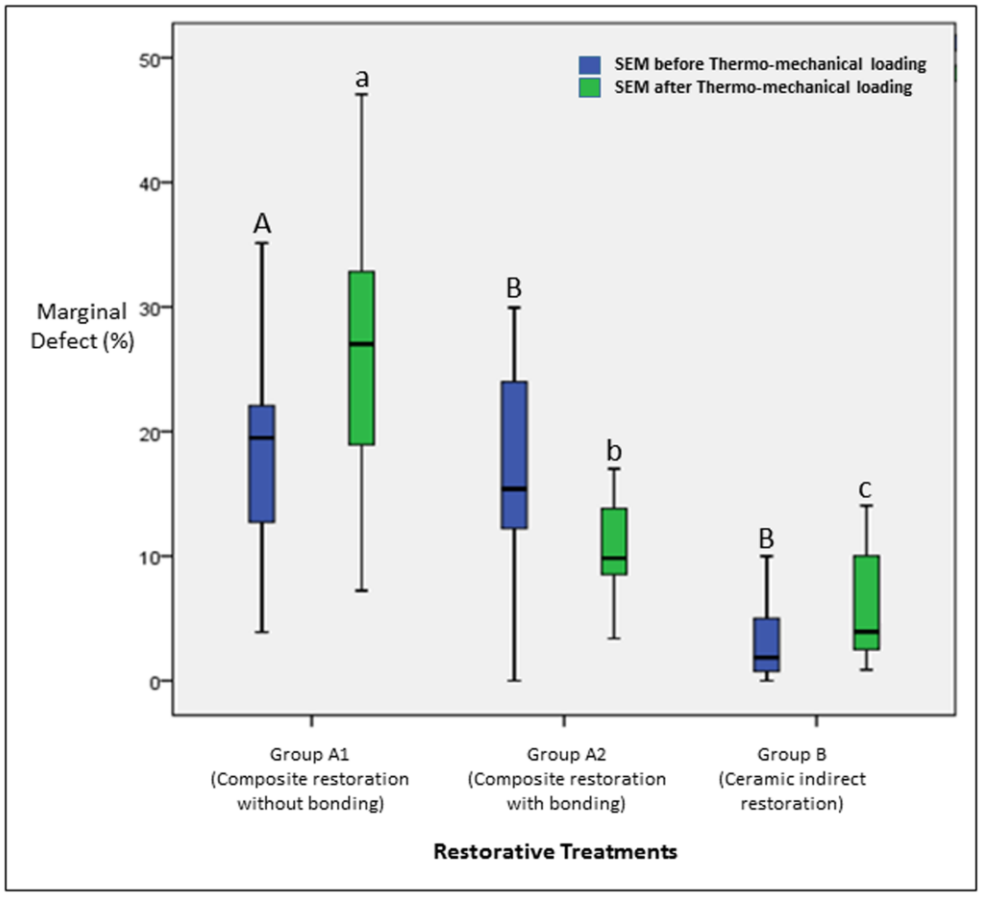


***Figure:*** *Results of the SEM surface marginal analysis using a box-plot illustration for the three groups before and after thermodynamic loading. Statistically significant differences (p-values < 0.05) between groups are indicated with different capital letters for before thermodynamic loading. Different small letters indicated statistical difference between groups after thermodynamic loading*

## 5.3. Root canal filling leakage testing

For this test, one sample from group MRLM was excluded after detecting a crack in the outer root wall. The void volumes were presented as a percentage of the whole root canal volume, they showed equal and normal distribution for both test groups UCI and MRLM (Table 6). t-test showed no significant difference in

the void volume between both groups. On the other hand, it showed more leakage to happen in the MRLM group, which resulted in a significant difference once comparing the GEPT outcomes for both test groups (Table 5). For the correlation of the void volume to the GEPT outcome within the groups, Pearson coefficient test was applied with the confidence interval set at (p=0.05). The MRLM group showed a high correlation between the void volume and the corresponding measured GEPT values ($R^2$ = 0.981, P< 0.001), while in the UCI group, the correlation detected was low ($R^2$ = 0.467, P= 0.126) (Figure 21). The correlation between the measured pressure difference and the infiltrated water volume was high ($R^2$ = 989, P< 0.001). All the samples accepted leakage values close to their base line values once the species sealed with differences ranged between 0.00-0.01 hPa/min.

**Table 5:** Root fillings group performance

| Group | Root filling defect (%) | Effective GEPT (hPa/min) | Effective fluid infiltration (ml) |
|---|---|---|---|
| UCI | 13.74 ± 6.23 | 0.102 ± 0.072 $^{A}$ | 0.049 ± 0.036 $^{A}$ |
| MRLM | 14.17 ± 6.83 | 0.321 ± 0.154 $^{B}$ | 0.144 ± 0.073 $^{B}$ |

Results presented as mean values ± SD . Capital letters indicates significance. Different letters indicate statistically significant difference (read vertical)

**Table 6:** Detailed root fillings performance under all tests.

| Upper Central Incisor (UCI) | | | | Mesial Roots Lower Molar (MRLM) | | | |
|---|---|---|---|---|---|---|---|
| Sample No. | Defect Volume (%) | Effective GEPT (hPa/min) | Effective Fluid infiltration (ml) | Sample No. | Defect Volume (%) | Effective GEPT (hPa/min) | Effective Fluid infiltration (ml) |
| UCI 1 | 17.43 | 0.22 | 0.13 | MRLM 1 | 25.95 | 0.61 | 0.286 |
| UCI 2 | 13.03 | 0.19 | 0.08 | MRLM 2 | 10.49 | 0.23 | 0.102 |
| UCI 3 | 6.13 | 0.04 | 0.025 | MRLM 3 | 8.73 | 0.2 | 0.097 |
| UCI 4 | 10.65 | 0.182 | 0.083 | MRLM 4 | 7.76 | 0.19 | 0.076 |
| UCI 5 | 18.99 | 0.18 | 0.072 | MRLM 5 | 12.73 | 0.32 | 0.132 |
| UCI 6 | 11.99 | 0.04 | 0.015 | MRLM 6 | 22.43 | 0.48 | 0.235 |
| UCI 7 | 24.22 | 0.11 | 0.053 | MRLM 7 | 4.45 | 0.13 | 0.051 |
| UCI 8 | 13.18 | 0.05 | 0.033 | MRLM 8 | 12.66 | 0.28 | 0.121 |
| UCI 9 | 5.14 | 0.02 | 0.007 | MRLM 9 | 11.03 | 0.21 | 0.11 |
| UCI 10 | 20.96 | 0.09 | 0.041 | MRLM 10 | 18.71 | 0.37 | 0.157 |
| UCI 11 | 17.43 | 0.072 | 0.027 | MRLM 11 | Excluded | Excluded | Excluded |
| UCI 12 | 17.43 | 0.03 | 0.02 | MRLM 12 | 20.94 | 0.37 | 0.215 |

Test results presented in terms of the root filling defect volume, GEPT leakage and the infiltrated fluid volume for each sample separately

**Figure 21**

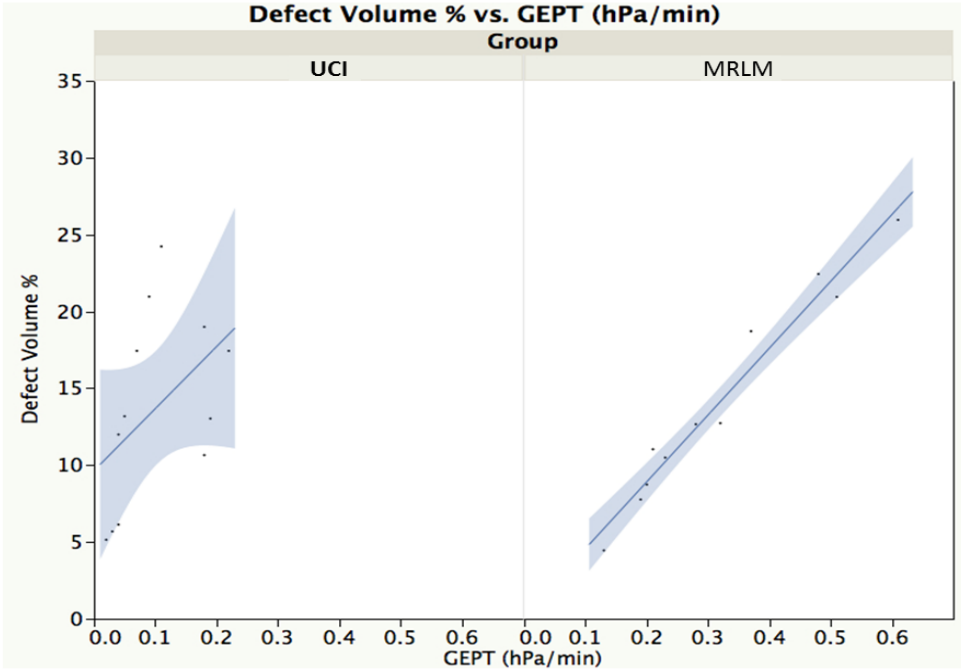


***Figure:*** *Correlation of the root filling defect to the measured leakage (hPa/min). The blue area represents the distribution of 90% of samples. The MRLM shows a higher correlation ($R^2$ = 0.981, P< 0.001). In contrast the UCI group, showed a lower correlation ($R^2$ = 0.467, P= 0.126).*

## 5.4. The implant-abutment interface

### 5.4.1. Implants leakage under static conditions

All negative controls presented at tight seal against leakage at all stages under all testing conditions. The implants test groups performed significantly different under the current test protocols i.e. under the GEPT testing conditions, molecular and bacterial leakage (table 7). In the AT implants, Twenty-five percent of the investigated implants, failed to withstand the test (did not last the whole testing time), while 12.5% of the NB failed to withstand the initial phase. B3i presented the lowest slope leakage values with a mean of 0.01±0.01, followed by

NB 0.23±0.03 and the AT implants with the highest leakage performance 0.85±0.71 (Table 8). The infiltrated saline volume through the IAI, which accounted for 0.56±0.50 ml (AT), 0.12±0.20 ml (NB) and 0±0 ml (B3i), respectively. The performance comparison between the different implant types was statistically significantly different ($p < 0.05$). Person correlation test of leakage slopes measured with GEPT, to the corresponding permeated fluid volumes show an almost perfect correlation ($R^2 = 0.965$).

When different tests were compared to their measured values, both GEPT and bacterial leakage showed well matching patterns by means of leakage status of the individual implants (Fig. 22), whereas the molecular leakage evaluation varied by its testing outcomes as compared the other two applied test methods, mainly in terms of the time point, when the first leakage was detected (Fig. 23).

**Table 7:** Detailed implants performance for all carried out tests

| Astra Tech | | | | Nobel Biocare | | | | Biomet 3i | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) | Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) | Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) |
| 20 | 0.002 | - | - | 18 | 0.000 | - | - | 4 | 0.000 | - | - |
| 19 | 0.001 | - | - | 17 | 0.001 | - | - | 16 | 0.000 | - | - |
| 18 | 0.000 | - | - | 20 | 0.003 | - | - | 18 | 0.001 | - | - |
| 17 | 0.001 | - | - | 1 | 0.004 | - | - | 8 | 0.001 | - | - |
| 3 | 0.089 | - | - | 6 | 0.006 | - | - | 12 | 0.002 | - | - |
| 1 | 0.125 | - | 12 | 19 | 0.007 | - | - | 2 | 0.002 | - | - |
| 12 | 0.227 | - | 11 | 5 | 0.015 | - | - | 6 | 0.004 | - | - |
| 6 | 0.249 | - | 9 | 14 | 0.043 | - | - | 5 | 0.005 | - | - |
| 4 | 0.488 | 1 | 5 | 15 | 0.049 | - | - | 1 | 0.008 | - | - |
| 8 | 0.559 | - | 6 | 4 | 0.065 | - | - | 7 | 0.009 | - | - |
| 9 | 0.744 | - | 5 | 13 | 0.075 | - | - | 9 | 0.009 | - | - |
| 10 | 0.921 | 20 | 4 | 8 | 0.080 | - | - | 11 | 0.009 | - | - |
| 15 | 1.278 | 20 | 2 | 7 | 0.173 | - | 12 | 10 | 0.012 | - | - |
| 11 | 1.286 | 2 | 1 | 11 | 0.252 | - | 10 | 17 | 0.013 | - | - |
| 2 | 2.008 | 2 | 1 | 16 | 0.253 | - | 10 | 19 | 0.013 | - | - |
| 5 | 2.171 | 8 | 2 | 10 | 0.346 | - | 8 | 13 | 0.014 | - | - |
| 7 | Failed | 12 | 1 | 3 | 0.846 | 24 | 5 | 3 | 0.015 | - | - |
| 13 | Failed | 8 | 1 | 9 | 0.940 | 16 | 5 | 20 | 0.016 | - | - |
| 14 | Failed | 1 | 1 | 2 | Failed | 12 | 1 | 14 | 0.025 | - | - |
| 16 | Failed | 3 | 1 | 12 | Failed | 6 | 1 | 15 | 0.029 | - | - |

Different tests results comparison. Implants were aligned in an ascending manner according to the GEPT leakage status

**Table 8:** Infiltrated saline volumes with the corresponding slope values detected in implants tested under thermo-mechanical loading

| Implant Type | Mean Slope Value (hPa/min) | Infiltrated Saline Volume (ml) |
|---|---|---|
| Astra Tech | | |
| mean ± SD | 0.85 ± 0.71 [A] | 0.56 ± 0.50 |
| median (IQR) | 0.65 (1.05) | 0.43 (0.95) |
| Nobel Biocare | | |
| mean ± SD | 0.23 ± 0.30 [B] | 0.12 ± 0.20 |
| median (IQR) | 0.08 (0.27) | 0.10 (0.14) |
| Biomet 3i | | |
| mean ± SD | 0.01 ± 0.01 [C] | 0.00 ± 0.00 |
| median (IQR) | 0.01 (0.01) | 0.00 (0.00) |

Mean and median values with respective standard deviations and interquartile ranges (IQR) for detected leakage. Statistically significant differences are marked with superscript capitals (read vertically)

## Figure 22

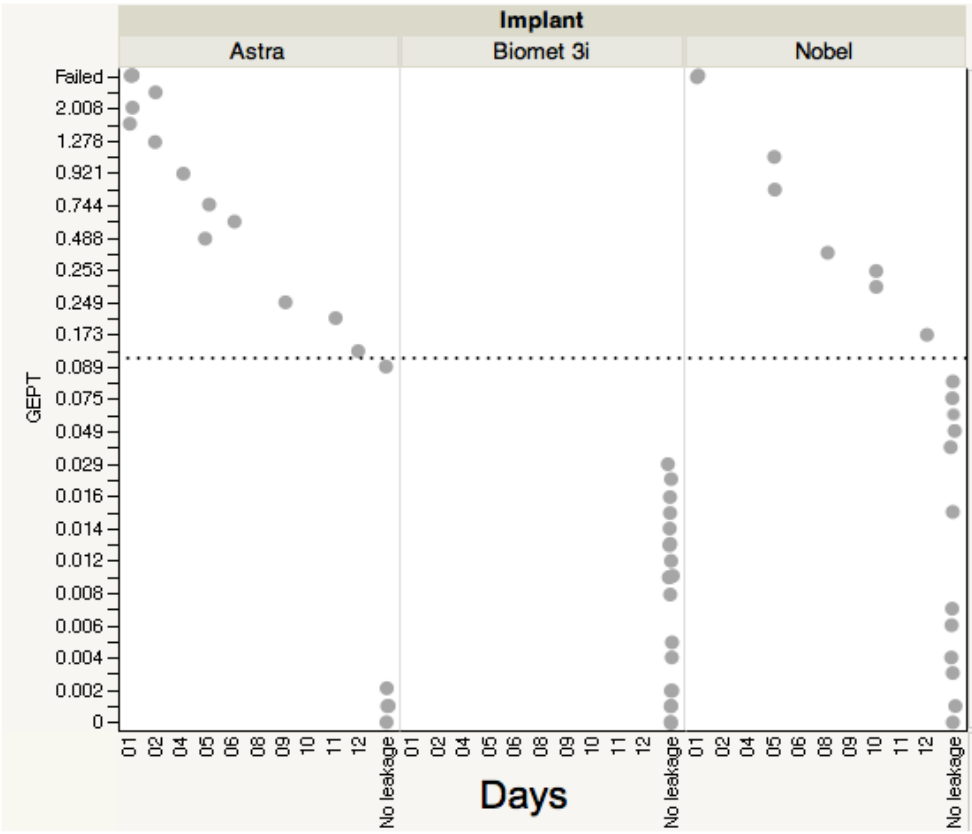


***Figure:*** *GEPT correlated to Bacterial:*
*The high correlation between the two tests indicated by the bacterial leakage time point to the slope leakage value with the minimal bacterial tight implant slope value set at 0.089 hPa/min.*

**Figure 23**

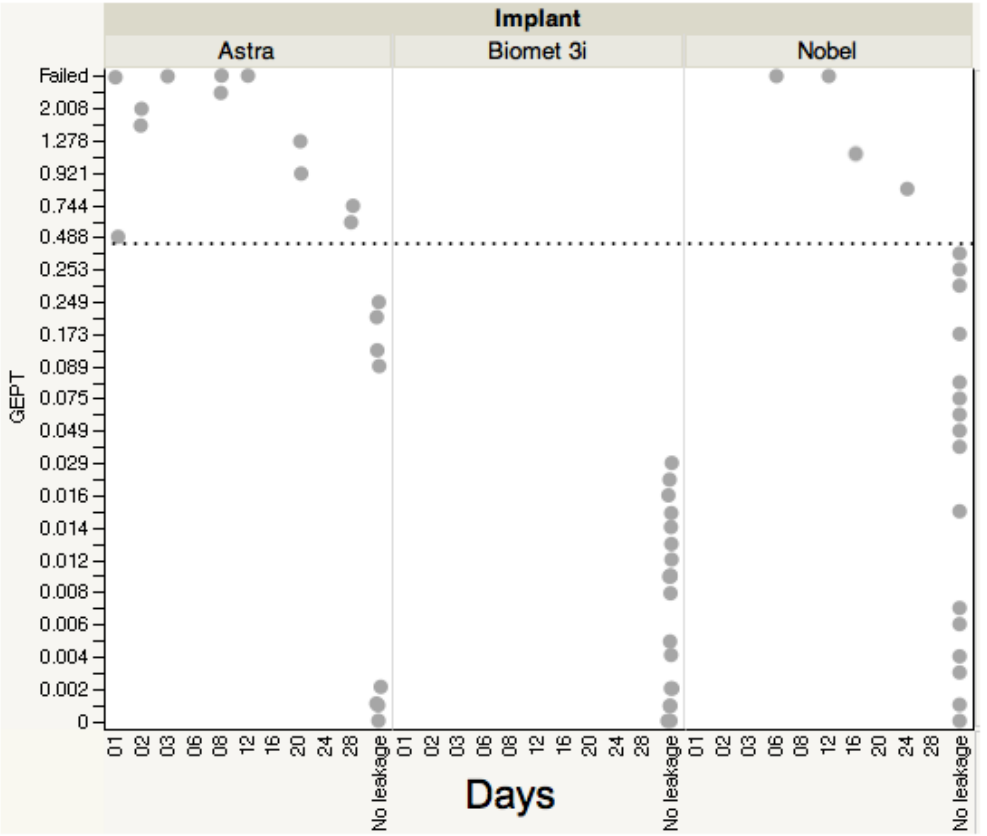


***Figure:*** *GEPT correlated to Molecular leakage:*
*The graph shows no correlation by means of time points by which the leakage was first detected with less sensitivity in molecular leakage detecting limit.*

### 5.4.2. Implants leakage under thermo-mechanical loading

Before the thermo-mechanical loading, the effective leakage measured with the GEPT of the three implant systems was (mean ± SD) (Table 9). It was found to be 2.104 ± 2.831 for group AT, 0.012 ± 0.007 for group B3i, and 1.456 ± 2.516 for group NB. After thermo-mechanical loading the values were; group AT 0.826 ± 1.921, group B3i 0.049 ± 0.017 and 2.814 ± 2.925 for group NB (Figure 24). Applying an ANOVA test, showed an overall significant difference to be

manifested only after dynamic loading (p-value 0.034). AT implants compared with Dunnett *post hoc* analysis with group NB set as a control group, showed the significant difference to be mainly due to the significant lower average leakage values of group B3i compared to NB group (p-value 0.023).

**Table 9:** Implants leakage before and after thermo-mechanical loading

| Implant Type | Mean Slope Value (hPa/min) (Before Thermo-mechanical loading) | Mean Slope Value (hPa/min) (After Thermo-mechanical loading) |
|---|---|---|
| Astra Tech | 2.104 ± 2.831 | 0.826 ± 1.921 $^{A}$ |
| Nobel Biocare | 1.456 ± 2.516 | 2.814 ± 2.925 $^{A}$ |
| Biomet 3i | 0.012 ± 0.007 | 0.049 ± 0.017 $^{B}$ |

Mean values with respective standard deviations for detected leakage. Statistically significant differences are marked with superscript capitals (read vertically)

Bacterial leakage was not detected in any of the B3i implants, while 4 implants of the AT group presented leakage after 1 day. Also, 4 implants of the NB implants were detected to have early leakage after 1 day and another 2 implants showed after 2 days. The exact Fisher test once applied to the corresponding 3 by 3 contingency table of leakage (no leakage, leakage after 1 day, leakage after 2 days) with the three-implant systems performance reported. It showed a better performance of the B3i implants (p-value was 0.009).

**Figure 24**

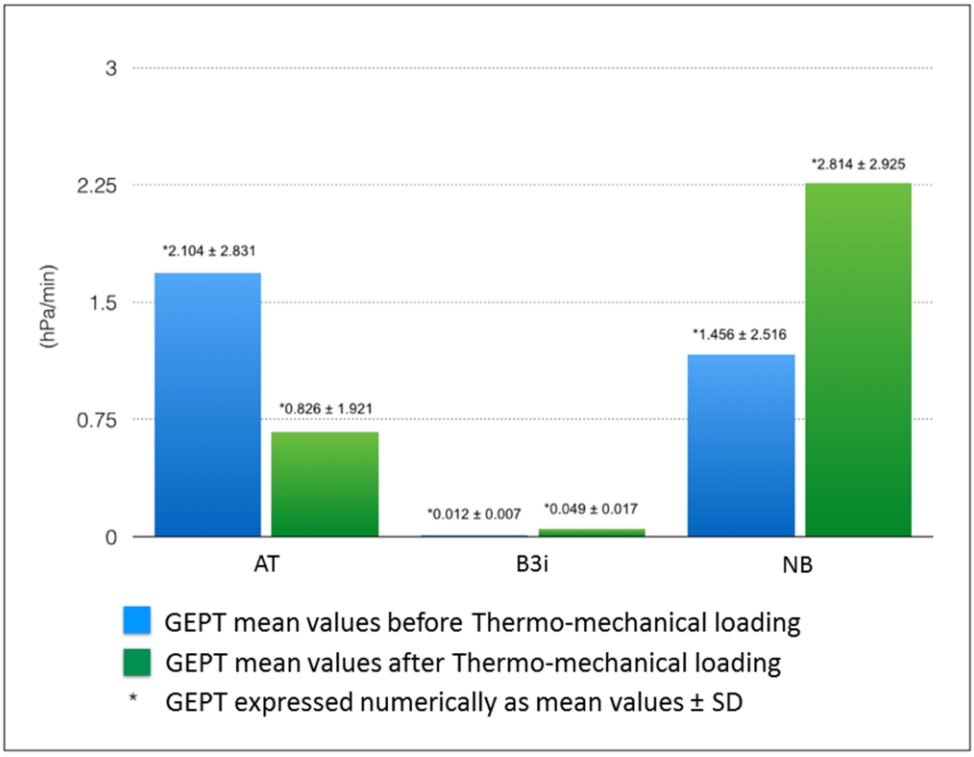


***Figure:*** *A graph showing a comparison of implants performance before and after dynamic loading presented as mean values ± SD.*

# 6. DISCUSSION

## 6.1. The GEPT test system

Varying permeation test methods have been used over the last decades (Raskin et al. 2001). They present many modifications in the sample embedding and mounting, penetrated substrates and the detection method, as well the conditions under which the testing procedure were carried out. Variance in methodology, unfortunately, does not allow interpretation and comparison of results between different studies (Da Silva-Neto *et al.* 2012). Therefore, leakage and permeability testing is not unanimously accepted in some scientific journals due to the fact that it cannot be ensured that leakage or permeation measured is necessarily related to actual tested treatment status (Rechenber *et al* 2011).

This current series of studies was aimed to establish a one-to-all permeation/leakage testing system based on previous leakage testing methods by combining their advantages and overcoming the shortcomings. This set-up proved its applicability to all clinically relevant dental fields, where it is important to maintain a tight seal. With just slight modifications in the embedding process, the same system could be used to assess different dental materials as well treatments needed in the restoration process. The method showed accuracy, repeatability, and presented a conservative method to test for leakage and permeability. The established embedding and mounting allowed for testing at different time points, after different treatments as well to assess with different test protocols utilizing the same samples. This variation in possibilities, allowed for a better understanding of the leakage mechanisms and the factors which might enhance its propagation.

During system development, some problems were encountered. Main two problems were temperature variation and embedding material and protocol.

The first stage in the system development took place in developing the testing split chamber. Its special design fulfilled the aim of tightly allowing multiple mounting of the same sample. It also allowed for simultaneous measurement of both variables; the pressure difference change over time as well as the penetrated fluid volume. However, in the early development phase of the system, pressure difference change in repeated multiple measurements was found to vary greatly between different days but not for the infiltrated fluid. The split chamber was kept in an open room, where temperature changed over time considerably. Change in temperature led to immediate changes in the gas pressure values. To solve the problem, the split cell was installed in a chamber with constant temperature of 35°C. This upgrade reduced the temperature effect, but still suffered from temperature change during the time of sample mounting. To mount a sample the door of the isolation chamber needed to be open for about two min, which resulted in dramatic temperature change. Step wise temperature control was then established and presented. The whole system was transferred to a bigger experimental box in which the temperature was stabilized at 31°C. With this set up the temperature could be controlled at all testing stages and times.

The second problem was the embedding process. Waxes, acryl, epoxy glue, silicone and resin composite were tested for their ability to seal around samples and to perform steadily over the whole testing period. Waxes did not seal properly, while acryl showed a high shrinkage in its volume and did not have adhesive binding to the mounted sample i.e. it only hold the sample mechanically in position. The epoxy glues performed perfectly in case the samples to be preserved under dry conditions. If the samples were kept under moist conditions, the epoxy material showed signs of imbibition and turned to be leathery in consistency. Silicone glue materials could not provide a tight seal when applied in thin sections and furthermore could not tolerate the pressure exerted to perform the test. While resin composite materials are too expensive and it is one of the materials under investigation, an alternative material was searched for. Finally, a clear, semi

flowable gel material, commercially sold to build up nails, was tested and found to present a tight seal of embedding and no signs of deterioration once stored under dry or moisture conditions. Once light cured, the material presented a hard consistency build-up with a proper adherence to all mounted parts with property to withstand the applied pressures.

It was shown the embedding procedure to provide a perfect separation of the two chambers. Up to very minute variation in repeated measurements of the evaluated samples after treatments was found ($p=0.05$) (Figure 7, A). Unlike previous testing methods, embedding status for each sample can be assessed independently. This allows for an establishment of a baseline status to be considered once the effective permeability is calculated for. In previous tests, embedding seal and accuracy was dependant on the researcher skills and the material used. Technically, this does not play a major role if individually assessed for each sample, as in the current set-up, where it will be compensated for. Although the embedding base line measurement varied slightly among the samples, any possible false positive error was overcome by subtracting the base line slope value from the subsequent test measurements. Thus, the absolute permeation valued could be calculated.

To test permeability and leakage under standardized temperature is mostly an ignored aspect (Outhwaite *et al.* 1976). In most studies room temperature was considered for testing (Pashley *et al.* 1996). The current system presents a technique, which allows leakage assessment mimicking oral conditions. The conditions were simulated by applying a net effective pressure 1030 hPa close to atmospheric air pressure and a standardized moisture simulated mouth under temperature of 35°C (Moore *et al.* 1999). The need for testing at a constant temperature is important, as it has been demonstrated that permeability increases with higher temperatures (Outhwaite *et al.* 1976 and Pashley *et al.* 1983). Unlike pure gas testing units and porometers, the device tests under moisturized

conditions and thus prevents sample dehydration. This procedure prolongs the sample survival and allows for further testing of the same samples without affecting their physical properties.

The purpose to simultaneously apply pressure and vacuum is to eliminate any bubble entrapment which might interfere with the permeability testing in the passive permeation testing (Wu *et al.* 1994).

One of the advantages of the new system is the new chamber design. It allowed for an easy re-mount of specimens for consecutive testing series. In addition, the simple small carrier system opened the door for multiple steps and interventional studies using the same samples under different conditions, which produced comparative data out of which proper conclusions could be made.

The idea of collecting the fluid volume infiltrated through the sample overcomes the air bubble calibration problem (to bring the bubble in position), which is a problem encountered in the original fluid infiltration method (Brannstrom *et al.* 1967).

In comparison to the current method, contrast/substance permeation methods outcome depends on the permeated substrate molecular size, osmolarity and possible capability of entrapment or reacting with other substrates within the tested samples. As an end effect, this may result in under estimation of the real permeability status of the specimen. The current set-up overcomes this shortcoming by using a physiologic saline solution, which does not have any interaction or interference with the permeation process.

A high correlation was found between the saline and the gas pressure changes ($R^2$=0.996, $p<0.0001$). The method had also presented a low detection limit (0.002 hPa/min for the pressure difference slope and 0.023 μl/min for the fluid infiltration volume). A slight deviation from zero was observed in correlating

the pressure difference measurement to the collected infiltrated fluid, however, the correlation was still high. This may be due to either the difficulty in collecting some entrapped fluids within the sample, or due to possible evaporation under the applied low pressurized conditions.

The validation of the new method revealed an evidence of accuracy and repeatability in the measured leakage for biological and artificial samples. Therefore, this method appears to suit dental longitudinal *in vitro* studies, where repeated measurements applicable. The ease of embedding process and subsequently the sample mounting, reduces the effort to set up the samples during the testing procedure.

GEPT is a non-destructive testing method i.e. samples are not sacrificed at the end to assess results. This characteristic gives it an advantage over the dye penetration method, where the samples must be sectioned to study the paths of leakage. Once combined with μCT radiography, the method prove to be the ultimate method to study leakage together with its paths more precisely.

To conclude and after intensive systematic investigation, the developed GEPT method appears to be a fast, reliable, and exact tool to assess permeability non-destructively and repeatedly.

## 6.2. Restorations leakage testing

Leakage of dental restorations were studied with the GEPT method in comparison with the traditional surface SEM analysis and the subsurface dye penetration test. The outcomes of all tests were compared and could be corroborated to support the hypothesis that leaking samples are expected to display poor marginal adaptation and an increased dye penetration profile. A significant

correlation of the dye test - a gold standard displaying the liquid penetration leakage pathways - with both, the GEPT and the SEM tests was found (0.777 and 0.534, p-values <0.0001 and 0.002, respectively). Although the correlation was statistically significant, the SEM marginal analysis do not necessarily represented the true performance of the filling especially before the thermo-mechanical loading. This is in line with an other study where the evaluation with the SEM method showed limited relevance to the clinical situation (Heintze *et al.* 2011). The SEM method seems to rather lead to false negative conclusions than to true ones. This is due to the limited nature of the test which assists the superficial surface of the sample only.

The results utilizing the SEM analysis to assess the restoration marginal quality, corresponded well to previously published results from studies assessing the marginal quality after loading utilizing comparable evaluation techniques, i.e. SEM and microleakage (Schmidlin *et al.* 2008 and Zaruba *et al.* 2013)

Although the dye penetration test has a high detection limit, it allows only for one test time per sample. It allowed just for testing at the end of testing while it requires sectioning of samples, thus could not compare the real effect of thermo-mechanical loading.

Within this context, the GEPT test showed a high detection sensitivity for leakage without destroying the sample. This was confirmed by the high significant correlation in the favour of GEPT method to the dye penetration test. Unlike previous testing setups where the dye and fluids were applied in retrograde direction (Derkson *et al.* 1986, Fiasconaro *et al.* 1952 and Pickard *et al.* 1965), the current method assessed the normal possible direction of leakage, thus, simulates the clinical situation in a more realistic way where leakage occurs from the oral cavity towards the pulp.

When analysing the performance of the different restorative treatments, the unbonded restorations showed the highest GEPT values at all test time points compared to the bonded restorations and the adhesively placed ceramic restorations. However, as a surprise the GEPT values dropped after the thermo-mechanical loading. This could be partly referred to some occlusion of the dentinal tubules due to the frictional smear layer production during the thermo-mechanical loading. Also, it could have resulted from hygroscopic effects after fluid uptake (Alrahlah *et al.* 2014).

## 6.3. Root canal filling leakage testing

Root fillings leakage is a controversial issue in the endodontic field. Clinical studies suggest different opinions with weighing the importance of the coronal seal, the root filling tightness or both. A previous study highlighted the importance of the root canal treatment quality as the determinant factor for success (Ricucci *et al.* 2000). Another study (Kirkevang *et al.* 2000) observed a better success rate once coronal seal is achieved regardless of the quality of the root filling. Recently, retrospective clinical studies emphasized the important of both seals to be established to ensure the best outcome of the root canal treatment (Song *et al.* 2014 and Archana *et al.* 2015). However, it is generally agreed that microleakage is the most effective risk factor responsible for apical periodontitis (Muliyar *et al.* 2014).

In the present study, the aim was to establish a correlation or a cause and effect between the root filling quality and the leakage value measured. The defect of root filling was found to be relatively high in both groups the UCI and the MRLM (13.74% ± 6.23% and 14.17% ± 6.83% respectively). This finding is comparable to the observations of a previous study where the root filling defect was assessed (Rechenberg *et al.* 2013). The high defect volume can be referred mainly to the limited experience of the operator who did the root fillings. On the

other hand, this event resulted in equal and normal distribution of root filling voids between the test groups.

Another observation was the higher leakage in the MRLM group which was significantly different compared to UCI group. The leakage in the MRLM group was highly correlating to the detected void volume and unlike in the UCI group where no correlation could be established. This indicate that voids do not necessarily correlate with leakage, unless they are through-and through. In teeth with complex anatomy like in mesial roots of lower molars, the voids most likely occupy the area in recesses and hard to reach by the root filling, and hence they maintain their continuity along the whole root length (Fig. 11). While in a simple root canal anatomy like in the UCI group, the void exists but a seal could be established at any level within the root canal i.e. the void is entrapped within the root filling. (Fig. 11)

Under the current investigation conditions MRLM showed higher leakage values which furthermore highly correlated to the root canal filling quality. This corroborates with a clinical finding where apical periodontitis was significantly more detected in molars with shorter root fillings than the anatomical root apex (Zhao and Xu 2014). Di Filippo and co-workers established in their retrospective study a high correlation between poor root canal filling quality and the presence of periapical periodontitis. They emphasised the importance of establishing a high root canal filling quality to ensure a higher success rate of the root canal treatment (Di Filippo *et al.* 2014).

The tested samples were found to acquire their initial leakage status once the apices were properly sealed. This suggests that the detected leakage correlated only to the bath through the canal. The combination of the current set up to the μCT quantitative analysis seems to present a promising approach to have more understanding and to confirm the paths through which leakage is happening under different morphological tooth variations.

## 6.4. The implant-abutment interface

### 6.4.1. Implants leakage under static conditions

To the best of our knowledge the present study represents the first study to compare three different leakage models for implants utilizing the same set of implants. In previous studies different testing models were combined to compare different implants systems but not the methods (Piattelli et al. 2001). In these studies, different implants were used. Another study used a set-up aimed to correlate the gap at the IAI to the leakage status (Jansen et al. 1997), however no correlation was found. This finding can be due to the fact that detected gap is not being necessary continuous (Dias et al. 2012).

Previous studies, where bacterial implants leakage was investigated under static conditions, presented variation in leakage ranging from 20-80% for internal hex IAI design and 10-60% for taper lock IAI designs (Assenza et al. 2012 and Aloise et al. 2010 and D'Ercole et al. 2011). The considerable variations within the same IAI design can be referred to the studies with different testing designs.

In the present study implants were found to perform significantly differently based on their IAI design. The system with a flat-flat with an internal hexagonal mate (B3i) showed almost no leakage, followed by the flat-flat with a trilobed mate IAI (NB), while the taper lock with internal hexagonal mate IAI (AT), showed the most leakage. In addition to the design, another possible factor which may control leakage and implant performance is the manufacturing tolerance of error i.e. the precision of the assembly interfaces.

When findings of leakage of the individual implants of different tests were compared, both the GEPT and bacterial leakage showed well matching patterns (Fig. 22). On the other hand, the molecular leakage outcomes varied compared to

the other two applied test methods. This was mainly in terms of the time point when the first leakage was detected (Fig. 23).

Under the current investigation conditions, a reliable comparison could be carried out between the different tests. As the results suggest, the GEPT system allowed for quantitative and a sensitive detection of leakage even for small values as compared to the bacterial and molecular leakage testing. The variations among tests might be enhanced by some limitations related to the size of gaps, perforating substance and the physical nature of the diffusion process. The diffusion in the GEPT, of an ionized fluid facilitated by pressure, had overcome the limitations of the leakage process, regardless of the gap size.

The mechanism of bacterial leakage is different. The active bacterial growth phenomena "pushed" cells in available empty spaces, given open pathways, thus, not only passive diffusion takes place in this case. In contrast to the molecular leakage, only the physical passive diffusion plays a role given the available gaps allow for substrate diffusion. Based on that, air entrapment might if occurred, be able to retard the leakage process (Wu and Wesselink 1993). This may explain the varying results in implants performance shown by this test compared to the other tests.

Comparing the testing methods for the time required, the GEPT needs two testing times each of 40 min., to verify the leakage status of an implant. On the other hand, both bacterial and molecular leakage, a period of 28 days minimum is required to perform the same test. Another observed advantages of the GEPT over other testing methods is its quantitative assessment implant leakage even in small volumes.

The drilling of implants had negligible measured changes on the embedding applied, consequently, on the overall implant leakage status. This was confirmed by the minor to no change in the negative controls leakage status when

tested before and after their incomplete drilling. The negative control implants in all testing groups showed no leakage under the other two testing conditions (molecular and bacterial leakage), indicating a prober implants embedding protocol.

It was a mandatory pre-request, as the implants parts are made of different alloys composition (Bordin et al. 2015). Therefore, temperature changes are expected to have an influence on the implants parts dimensions (Gaal and Gaal 2009), it might also have an influence on the fluid viscosities as well (Wu and Wesselink 1993).

Although the leakage testing conditions were set close to oral conditions, it is uncertain how well the results correlate to clinical implant performance, since the study was carried out under static conditions.

### 6.4.2. Implants leakage under thermo-mechanical loading

In previous studies concerning implants leakage under thermo-mechanical loading, the implants were tested in a masticator in a surrounding medium cultivated with bacteria. At the end of testing period, abutments were dissembled and a sample was collected from the inner implant cavity to test for bacterial presence. A positive bacterial culture indicated leakage at the IAI (Steinebrunner *et al.* 2005, Koutouzis *et al.* 2011). Compared to the current study, it was not necessary to dissemble the abutments from their implants and leakage could be directly detected through the change of selective medium colour in the semi-transparent chamber.

As discussed above, proper leakage study of implants needs to be carried out in conditions mimicking oral moisture, temperature and pressure. Furthermore, dynamic loading is essential to expose the implants to forces simulating the oral masticatory forces.

Therefore, leakage testing under thermo-mechanical loading was important. In addition, it was important to correlate the implants bacterial leakage performance under thermo-mechanical loading to the leakage as assessed using the GEPT system. The hypothesis was that a tight IAI under static conditions would also present a tight seal under thermo-mechanical loading condition. The hypothesis was accepted since implants with initially tight IAI also showed a better sealing against bacterial leakage under thermo-mechanical loading. Despite the fact that no statistical significance could be established between the different used implant designs before loading, statistically significant difference was observed after the thermo-mechanical loading between the B3i and NB implant groups (p-value 0.034). The findings under static / preloading conditions are in contrast to the previous investigation, where significant differences between the groups could be detected (study II). However, similar trends were obtained in both investigations. The lack of statistical difference observed is attributable to the lower sample size of the present investigation, where in each group only 8 test implants were investigated compared to 16 test implants in each group for the previous study.

The thermo-mechanical loading revealed group NB to exhibit the highest number of leaking implants, followed by group AT (6 and 4 of test implants respectively), while B3i implants showed no leakage. The group AT with taper lock and internal hexagonal mate IAI design was the only implant system to show some improvement in tightness tested with GEPT after thermo-mechanical loading. However, the detected improvement was not statistically significant. The findings could be indirectly correlated to findings of previous study assessed tapered lock IAI design under thermo-mechanical loading. In Koutouzis *et al.* study (2011) the torque to loosen the mounting screw after different stresses was measured. Their findings showed an increase in the screw loosening torque value after thermo-mechanical loading for this design category. On the other hand, group NB showed

an overall increase in leakage after the thermo-mechanical loading as compared to the group B3i in the same category of flat-to-flat design.

The differences in tightness corresponded to the different mating surface designs, which can be explained by the degree of micro-motions at the IAI (Saidin *et al.* 2012). Higher micro-motions at the polygonal region in a trilobed IAI design as compared to the internal hexagonal design could be observed in a finite element analysis (Saidin *et al.* 2012). The authors suggested that this instability may lead to more bacterial penetration i.e. leakage, which was supported by the findings of the current investigation.

The present SEM gap analysis of implants was not quantitatively performed. Instead, it was used to have a view of the situation in implants with GEPT values close to a value of 0.9 hPa/min. This value was defined in the previous study, under static conditions (study II), to be the cut-off value below which no bacterial leakage was detected. Observations of the SEM revealed the presence of a small split gap at the IAI only in implants which showed bacterial leakage. This finding confirms again the high sensitivity of GEPT measurements in detecting implant leakage. If spatial analysis is to be carried out, a 3D μCT analysis as a precise tool can be suggested.

The current study design, was the first in which the non-loaded performance of an implant was correlated to its performance under thermo-mechanical loading conditions. With this set-up it was possible to provide a continuous analysis of implants testing before, during and after thermo-mechanic loading. The bacterial leakage model (turbidity detection) has been in use for leakage evaluation for both conventional dentistry (Torabinejad *et al.* 1995) and implant dentistry (Duarte *et al.* 2006, Dias *et al.* 2012) but only under static conditions. This study presents the first time by which the turbidity detection method is applied in a thermo-mechanical loading model. The challenge in detecting bacterial leakage directly during the thermo-mechanical loading lies in the

continuous dynamic movement, which interferes with the sampling process. To overcome this obstacle, an isolating split chamber system design was established. In one of the chambers, a visual turbidity detection through the clear or semi-clear wall was possible. Once the bacteria penetrates through the available gaps at the IAI from the chamber they positioned at, to the other chamber containing the selective medium, the bacteria will hydrolyse the esculin and cause a detectable blackening of the selective media. The wall of the turbidity detection chamber was made of an elastic material which does not interfere with the thermo-mechanical loading process.

Cell cultures were taken at the end of the thermo-mechanical loading from both sides of the split chamber system. This was aimed to confirm the leakage (turbidity) observed is related to the used bacterial strain (*E-fecalis*) only and not due to an external contamination during the assembly process neither through unwanted routes during the thermo-dynamic loading. In addition, it provided a proof that bacteria could survive the thermo-mechanical loading conditions during the whole testing period.

To assure the quality of the mounting after all experimental steps, samples were re-sealed at their apices and were re-tested. Theoretically, the baseline values should be re-gained resulting in a slope difference of zero. Indeed, only very small differences were found ranging from -0.010 to +0.009 hPa/min, such differences are neglectable and reflect an intact implant embedding and sealing quality at all times.

Survival/failure of implants due to prosthetic assembly fixed on top depends on the following considerations: mechanical factors related to the applied load (Naert *et al.* 2012), abutment retention type (cemented vs. screw retained) (Piatteli *et al.* 2001), and prosthesis retention (cemented vs. screw retained) (Cicciu *et al.* 2014). Furthermore, bacterial inhabitation in uncleansable niches is an important factor (Quirynen *et al.* 2002).

Studies have detected a correlation between implant failures and the presence of gaps and their size at the IAI (Piattelli *et al.* 2003, Hermann *et al.* 2001 and King *et al.* 2002). The leakage phenomenon provides an indirect indication of gaps at the implant-abutment interface. It can therefore be considered as a quantitative assessing parameter to present the quality of the connection at the IAI (Passos *et al.* 2013). The long-term survival of dental implants has been linked to the precision of assembly of the implant parts, which in turn is considered to be a determinant factor in the preservation of the surrounding bone level (Rangert *et al.* 1989 and McCartney J. 1991). Nakazato and co-workers have elaborated bacterial colonization at the IAI after 4 h exposure to the oral environment. The colonization took place at the IAI with gaps which allowed fluid and bacteria shifts in the screw thread compartment. Thus, the presence of gaps at the IAI can be classified as a risk factor which may jeopardize the prognosis of an implant (van Winkelhoff *et al.* 2000). Histological studies have confirmed the importance of gap levels in relation to the bone crest: the closer the gap to the bone crest the higher the risk of peri-implantitis (Hermann *et al.* 1997, van Winkelhoff *et al.* 2000, Quirynen *et al.* 2001, King *et al.* 2002). Quirynen and co-workers 2002 have furthermore shown that persistent bacterial inoculation at the IAI is associated with a chronic inflammatory response at the bone crest.

## 6.5. Limitations of the method and future perspectives

The GEPT method showed a high sensitivity in detecting leakage, seem very sophisticated and therefore the technological and methodological principles seem - at first sight - not to fully reflect the clinical conditions in the oral environment. However, the association of pressure change, defective restorations and pathological changes in teeth is of clinical relevance in the so called field of barodontalgia and aerodontalgia (Zadik 2009). Barometric changes during flights,

diving or climbing which result in pressure changes of 1 bar can cause such a physiologic or pathologic phenomena, which may induce a transient pain. These conditions were observed to be associated with carious teeth, defective restorations (including insufficient root canal fillings) and teeth suffering pulpal pathological changes. But after all, this physiological interrelationship provides evidence for the applied method using pressure difference to the clinical settings. It also highlights its importance in detecting the tightness or leakage of applied dental materials whenever providing definitive and preferably tight restorations is necessary.

GEPT should not be misconceived to be used as the sole technique to detect leakage phenomena, but rather complement existing methods and established tests, i.e. SEM, μ-CT or other methods in order to better understand individual leakage processes and the influencing factors. Noteworthy in this context, GEPT can measure the total possible leakage in a sample, but never determine the route of leakage within the sample like in dye penetration tests. In addition, this method is not able to measure the space size responsible for the respective leakage. It is also obvious that GEPT takes place as only one evaluation step in a whole series of possible preclinical performance and validation steps when dental materials are evaluated in order to understand/screen pathological processes and avoid them under clinical conditions whenever possible.

The current series of investigations was able to cover some of the main aspects of assessing leakage of dental materials. Future studies should include comparisons between bonding methods and agents, different direct fillings materials and their application techniques, precision of CAD/CAM restorations, differences among root canal filling techniques and different materials as well as testing the efficiency of dentine desensitizers in reducing tooth sensitivity. The promising findings encourage continuing further exploring the test method and its applications.

# 7. CONCLUSIONS

## 7.1. Validation of the GEPT system

- The presented GEPT method presents a suitable method to non-destructively study the leakage/permeation behaviour in dentine samples, restorations, root canal fillings and implants, while still allowing for multiple tests and treatments utilizing the same set of samples.

- The detection limit to assess permeation is low.

- The repeated measurement of identical samples results in reproducible results.

- The embedding causes no false-positive measurments and the chamber model *per se* is tightly sealed.

- The liquid collected during the permeation test correlates to the gas pressure differences.

## 7.2. Restoration leakage testing

- The GEPT system, was able sensitively to detect leakage/permeation in a comparable manner to the standard well-known dye leakage test, fluid infiltration test and the bacterial leakage test

- SEM may be a suitable method to judge surface adaptation but does not necessarily display trans-dental leakage.

## 7.3. Root canal filling leakage testing

- Under the current investigation conditions, mesial roots of lower molars (presented complicated anatomy) showed the highest leakage values which correlated to the root filling quality.

## 7.4. Implant leakage under static conditions

- The measured GEPT values correlated highly to the bacterial leakage time point but not to the molecular leakage once applied to the same individual implants.

- Under the current testing conditions, completely different leakage patterns could be shown. They were influenced by the IAI design. The flat-flat with internal hexagonal mate presented in the B3i implant group showed better performance than the relatively equivalent design (flat-flat with internal triloped mate) presented in the NB implants system and the taper lock design with an internal hexagonal mate presented by the AT implant system.

## 7.5. Implants leakage under thermo-mechanical loading

- Investigation of the implants leakage under static conditions utilizing the GEPT system, correlated highly to their performance under thermo-mechanical loading.

- It could be proofed that tight implants under static conditions will provide better sealing characteristics under dynamic conditions, which in turn highlights the importance and relevance of *in-vitro* implant system leakage testing under static conditions

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ORIGINAL ARTICLE

# Laboratory validation of a new gas-enhanced dentine liquid permeation evaluation system

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**Abstract**
*Aim* To validate a new automated dentine permeability testing platform based on pressure change measurements.
*Methodology* A split chamber was designed allowing for concomitant measurement of fluid permeation and pressure difference. In a first test, system reliability was assessed by interposing a solid metal disk, embedded composite resin disks, or teeth by consecutively measuring eight times under standardized conditions. Secondly, the repeatability and applicability of the method was tested in a dentine wound model by using intact third molars: Class I (2×5 mm) and a full occlusal preparation as well a ceramic restoration were consecutively performed and repeatedly measured eight times each. In the last test, the system detection limit as well correlation between gas pressure difference and liquid permeation were evaluated: Again, third molars were used and occlusal preparations of increasing size (2×5, 3×5, 4×5, and 5×5 mm and full occlusal preparations, respectively) were made. Data was analyzed for the linearity of measurement, and $R^2$ values were calculated.
*Results* The embedding procedure allowed for perfect separation of the two chambers, and no significant variation in repeated measurements of evaluated samples for the respective treatments ($p$=0.05) was found. The detection was 0.002 hPa/min for the pressure slope and 0.0225 μl/min for the fluid infiltration, respectively. The saline volume was highly correlating to the gas pressure changes ($R^2$=0.996, $p$<0.0001).
*Conclusions* The presented method is a reliable and exact tool to assess dentine permeability by nondestructive and repeatable measurements.
*Clinical relevance* This method is suitable for measurements and comparison of the effectiveness of dentine wounds sealing materials.

**Keywords** Dentine · Sealability · Permeability · Restoration leakage

## Introduction

The unique tubular structure of dentine is mainly related to evolutionary functional adaptation to enable mastication by transducing bite pressures into tensile forces in the collagen matrix [1]. In addition, fluid-filled dentinal tubules allow for transducing stimuli to the underlying pulp [2]. This results in a sophisticated functional and sensitive organ. On the other hand, exposed dentinal tubules can lead to dentine hypersensitivity or—if adjacent to infectious processes—reflect pathological conditions like caries [3]. Effective protection of dentinal tubules has therefore a pivotal role in clinical dentistry.

After the observation that fluids could permeate through dentinal tubules of extracted teeth [2, 4], various in vitro models were established to study dentine wounds and were modified later to test for leakage in restorations and root canal fillings. The versatile split-chamber model to test infiltration of isotopes was revolutionary in that field [5]. It had a simple design to hold and test small dentinal disk specimens. A decade later, Derkson and coworkers introduced—inspired by the fluid shift model of Brännstrom—their pressurized

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fluid transport model, which aimed to test the seal around restorative fillings [6]. The same setup was adapted to test the seal of root canal fillings [7]. The fluid shift model was later digitized to measure the infiltrated fluid volume in real time [8]. In 2008, Romieu and coworkers [9] introduced a new dimension in leakage measurements using a testing system with two pressurized chambers. By continuously recording the air pressure difference between the two differently pressurized chambers, the ratio of pressure change provided an indirect value of air leakage. However, this evaluation was performed under dry conditions, which may be considered a significant shortcoming of this method and potentially results in dehydrated test specimens and an unrealistic simulation with regard to the originally intended oral cavity conditions to be tested.

Since the hydrodynamic theory is widely accepted to explain dentine sensitivity [10], the fluid infiltration method may still be considered as the gold standard in permeability/leakage testing and it can be adopted to many types of leakage testing. However, most of these currently available testing models exhibit some disadvantages. Among them, the long testing time, the difficulty of establishing a repeatable setup, the lack of internal control and possible entrapment or reaction of perfusing substances with the sample are worth mentioning. Another potential bias, which was underestimated for a long time was the permanent fixation in adhesive materials (epoxy resins, waxes, etc.) without adequate testing before and after treatment, which resulted in a lack of an internal quality control. Therefore—not surprisingly—it has been shown that these embedding processes can also lead to potential overestimation in permeation testing [11]. Another disadvantage of most setups, namely to test only at a single time, additionally limits the possibility to compare between different treatments or even the same treatment at different stages using the same specimen.

Due to these limitations, a new testing platform was designed aiming to reliably measure sealability based on a combination of previously mentioned models, namely a split-chamber model measuring fluid permeation and the resulting gas pressure difference simultaneously. The aim of this study was to validate the accuracy as well the leakage-free embedding of samples. Reproducibility of repeated measurements was assessed. The working hypotheses and requirements were as follows:

1. The embedding causes no false-positive measurements.
2. The repeated measurements of identical samples result in reproducible results.
3. The detection limit to assess permeation is low.
4. The liquid collected during the permeation test correlates to the gas pressure differences.

## Materials and methods

### Setup of the leakage/permeability measuring device

The split testing chamber model consisted of two custom-made plexiglass parts, which were tightened together using three solid screws (Fig. 1). The embedded specimens were fixed between the two parts using a rubber O-ring with an outer diameter of 22 mm, an inner diameter of 15 mm, and a thickness of 3.5 mm, thus forming two fully separated chambers with the sample fixated in between. The lower chamber was opened at its lower terminal with an adapter fixed to the outside allowing the placement of an Eppendorf tube to collect the permeating liquid. The two chambers were connected to two valves to stabilize their pressure during testing once they were closed.

The temperature was controlled as follows: The permeability/leakage unit (Fig. 2a) was installed in an isolation chamber (Fig. 2b), in which the temperature was constantly held at 35 °C. This chamber was situated in a second larger experimental box (Fig. 2c), in which the temperature was always kept at 31 °C. The room temperature was stable at 25 °C.

### Pressure difference measurements

A pressure difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) was connected by its two inlets to the tubes connected to the upper and lower chambers just before the valves, which allowed for real-time measurement. The measuring device was connected to a computer unit running a proprietary program (V 4.2 SP2, Testo AG, Germany). The O-ring was lubricated with a silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) to improve the sealability between the two chambers. The specimen was then positioned in the ring, and 2.5 ml of a pre-pressurized ($N_2$ gas 860 hPa) 0.9 % NaCl solution was added on top in the upper chamber. The cover was repositioned and the three screws were tightened using a torque-controlled screwdriver. The upper chamber was then pressurized with $N_2$ gas to 860 hPa. The lower chamber was negatively pressurized down to minus 170 hPa. This resulted in an effective pressure difference of 1,030 hPa between the two chambers. Given the hypothesis that there is a connection between the two chambers, i.e., leakage through the sample, this would affect the pressure difference. The pressure difference would change and become smaller by penetration of the NaCl solution from the positive pressure chamber to the low pressurized chamber causing a pressure drop in the positive side and a pressure increase in the

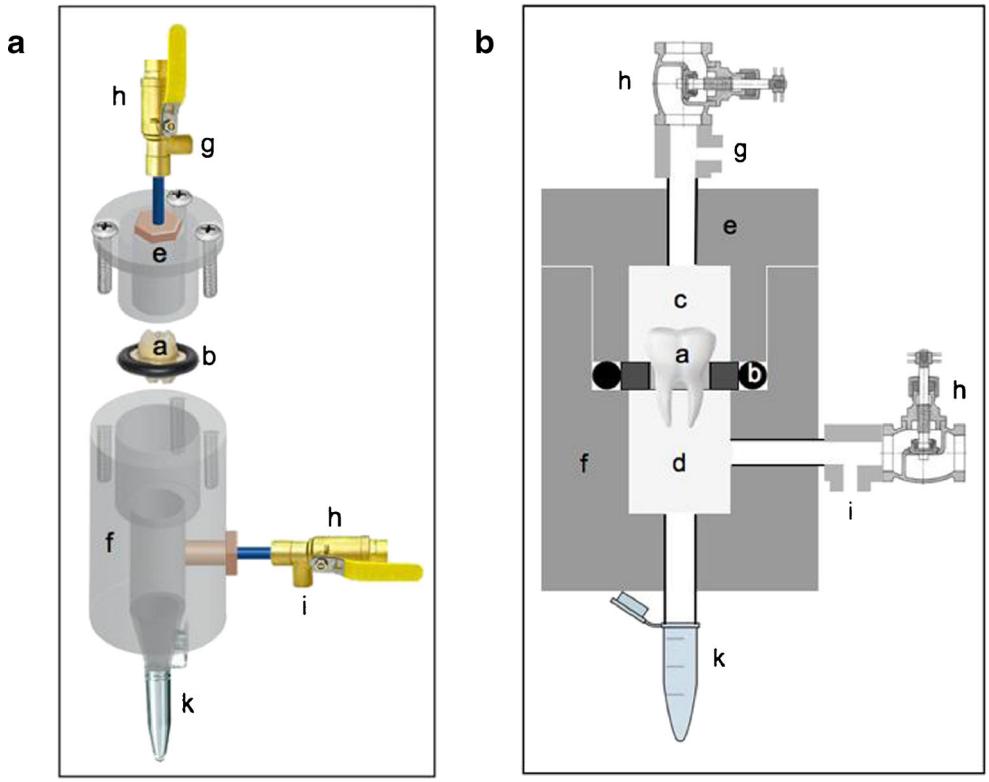


**Fig. 1** Split chamber with the two valves connected to control pressure on both sides. **a** 3D graph; **b** enhanced schematic drawing showing the position of the mounted tooth in testing. The parts are matched in both drawings. (*a*) A tooth sample mounted in a disk carrier. (*b*) O-Ring. (*c*) Positive pressurized chamber. (*d*) Low pressurized chamber. (*e*) Split-chamber cover. (*f*) Split-chamber body. (*g*) Positive outlet attached to the pressure difference measuring device. (*h*) Securing valves. (*i*) Negative outlet attached to the pressure difference measuring device. (*k*) Eppendorf tube to collect permeating fluid

negative side, until the pressure is equalized in both chambers and the difference reaches 0 hPa. The pressure difference measurements were started and continued for 40 min at a rate of 1 measurement/s. The reading resulted in a data set and a curve representing the rate of pressure change expressed as a

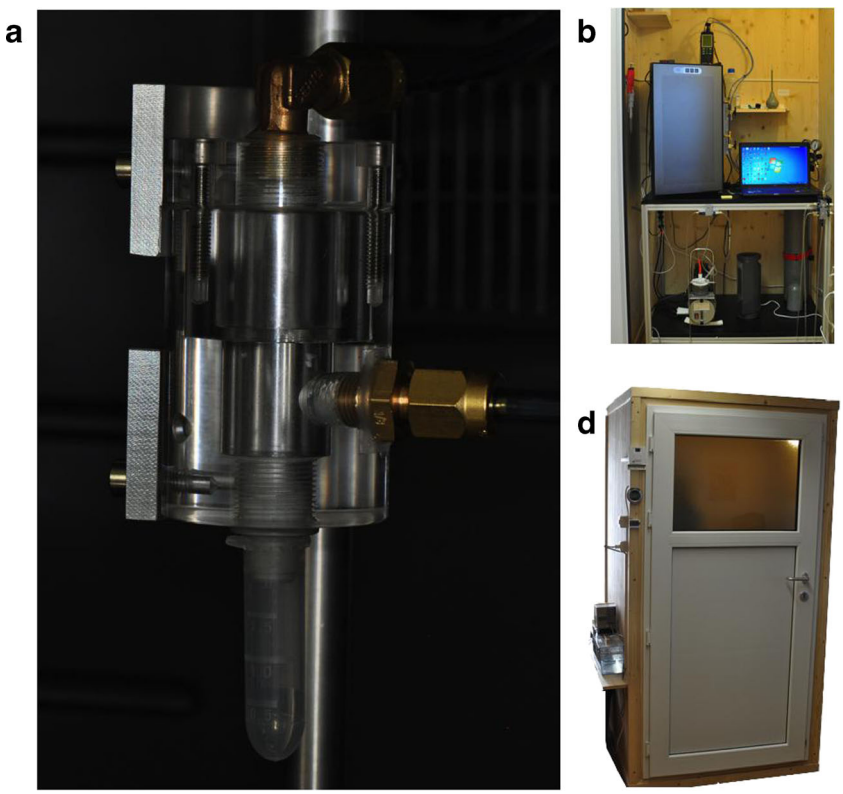


**Fig. 2** Stepwise temperature control; **a** Split chamber mounted in the testing inner isolation room. **b** Inner Isolation chamber. **c** Outer Isolation room

drop in pressure difference over time. The pressure value at two fixed time points (1,200 and 2,400 s) were defined to calculate the slope in between:

$$\text{Slope} = \frac{P2-P1}{T2-T1}\text{hPa/min.}$$

All results were expressed as positive values for the statistical analysis for the ease of understanding, as we aimed to show a positive correlation with the infiltrated fluid volume.

These optimal time points to detect the slope were found by preliminary observations on repeated measurements of the same sample to be reproducible (data not shown). In addition, the infiltrated physiological saline solution was collected and weighed to calculate the volume that permeated the specimen (see "System detection limit and correlation between pressure difference and fluid permeation" below).

## Specimen preparation

To test the tightness/sealability, repeatability, detection limit, correlation between the measured outcomes, and the capability of the embedding procedures in maintaining a tight seal after multiple measurements with no or minimal changes, a solid metal disk, embedded composite disks, and third molars were interposed. The solid metal disk (3 mm thick and had a diameter of 15 mm) was chosen as gold standard for tightness, as no embedding procedure was involved, and thus no additional interfaces were created. The solid metal disk had the exact thickness and outer dimensions of the embedding brass rings used in the setup (Fig. 5, Exp. A). It was used to measure the internal system leakage at all joints and connections. Therefore—hypothetically—this test should result in no leakage and served as an internal system tightness control.

The round composite disks had a diameter of 7 mm and a thickness of 3 mm and were fabricated using a Teflon mold and composed of dual cure composite buildup material (Luxa Core Automix, DMG, Hamburg, Germany). This allowed for the formation of a nonporous solid biomaterial/tooth surrogate sample given the hypothesis that no leakage should occur given an adequate sealing around it. Accordingly, third molars were selected as natural products from the department's pool of extracted teeth. They were extracted for reasons not related to the current study from patients aged 18–20 years. All teeth were free of caries and restorations. The roots were not fully developed ensuring proper pass to the pulp chamber and allowing for retrograde pulp extirpation. Samples were stored in 0.2 % thymol at a temperature of 5 °C for no longer than 1 year. Both, composite disks and teeth, were embedded in custom-made brass rings with an outer diameter of 15 mm, an inner counterpart of 10 mm, and a thickness of 3 mm. The rings were sandblasted on their inner surface using 50-μm aluminum oxide (Benzer-Dental AG, Zurich, Switzerland), and the specimens were embedded using a light-curing nail build-up material kit (Sina, Shenzhen Cyber Technology Ltd, Mainland, China). This material consisted of a primer, a gel, and a glaze material. The teeth as well the rings were primed and light-cured for 2 min in a light-cure chamber (Spectramat, Ivoclar Vivadent, Schaan, Liechtenstein). Subsequently, the parts were fixed in position using a rubber carrier made of a putty material (Optosil, Heraeus Kulzer GmbH, Hanau, Germany) (Fig. 3). The gel was applied in one increment to fill the space between the ring and sample. Care was taken not to allow excess material formation on the two upper or lower surfaces of the metal ring. The buildup was then light-cured for 4 min. Finally, the glaze layer was applied to the surface to eliminate any imperfections in the embedding gel buildup, which was finally light-cured for another 4 min. This embedding method was used for all repeatability and correlation samples tested as described in this study.

## Sealing accuracy and repeatability evaluation

The metal and the composite disks as well three intact third molars were prepared as described above, and pressure difference measurements were repeated eight times each (Fig. 4a) to establish an initial reference baseline.

In addition, three third molar teeth were measured for permeability after creation of dentine wounds (class I preparations; 2×5 mm and a depth of 2 mm from the fissure level) and a subsequent full occlusal surface preparation, which completely removed the occlusal enamel until the CI preparation floor. All preparations were made using a tapered diamond bur (Number 8117, Intensiv SA, Montagnola, Switzerland) attached to a parallel drill holder (Cendres & Metaux SA, Biel, Switzerland). To ensure no effect of the repeated measurements on the embedding, the teeth then were restored after conditioning (Clearfil SE Protect, Kuraray America Inc., USA) according to the manufacturer's instructions using CAD/CAM onlays (Sirona Cerec Blocs, VITA Zahnfabric, Bad Säckingen, Germany) cemented with Multilink (Ivoclar Vivadent AG, Liechtenstein). Again, all samples were tested eight times at each step (Fig. 5, Exp. A and B). The different measurements for each sample for the respective treatments were carried out on different days to assess potential influence of storage on the embedding and permeability. For the interim, samples were kept in physiologic saline at room temperature.

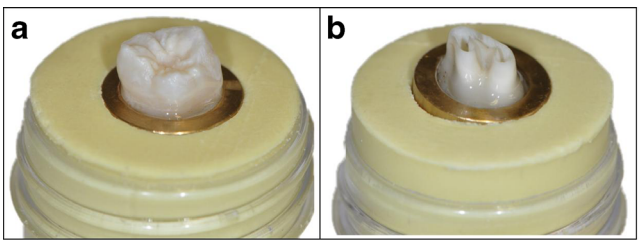


**Fig. 3** **a** Embedding from coronal side; **b** Embedding from apical side

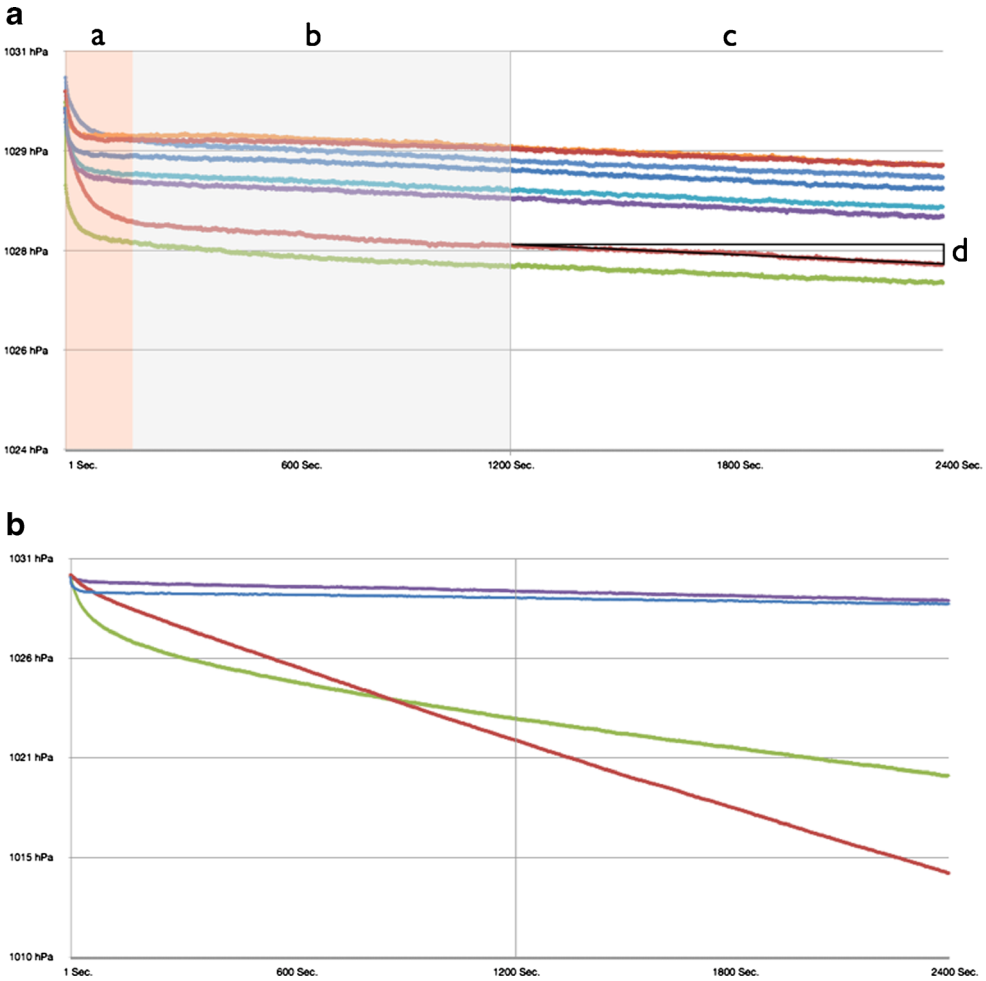


**Fig. 4** **a** A representative graph of a tested sample with eight repeated measurements for its baseline permeability (hectopascal per minute): (*a*) The gas compensation curve (each pressurized gas will behave unstable for a period of time). (*b*) System stabilization curve, which is related to temperature compensation. (*c*) The permeability curve which is related to the sample permeability status. (*d*) The permeability slope. **b** A representative graph showing the permeability curves of a sample tested for multiple treatments. Baseline curve (*blue*). After CI I preparation (*green*). After full occlusal preparation (*red*). After Cerec onlay restoration (*purple*).

System detection limit and correlation between pressure difference and fluid permeation

To assess the correlation between the two quantitative primary outcome parameters of the device, i.e., gas pressure difference change and liquid permeation, six additional third molar teeth from the department's collection of extracted teeth were used (molars 4–9). They were tested after embedding and before preparation to assess the baseline performance, i.e., tightness. The measured curves were used to determine the method detection limit, i.e., the minimum measured permeability value that could be observed in a sample with confidence. Subsequently, consecutive preparations were performed in all specimens with increasing invasiveness and dimensions (2×5, 3×5, 4×5, and 5×5 mm and a depth of 2 mm from the fissure level) and finally a full occlusal trimming was performed as described under "Sealing accuracy and repeatability evaluation" (Fig. 5, Exp. C). After each step, the pressure difference change was measured as described above (Fig. 4b). In addition, the saline that permeated each specimen was collected in the tube that was attached to the apparatus. The volume of liquid was measured by calculating the weight difference of the tube before and after the experiment using a precision scale (Mettler AT261 Delta Range, Greifensee, Switzerland).

Data presentation and analysis

Repeatability of the individual pressure change difference within the same sample for the same treatment was assessed using a linear mixed model.

Permeability expressed as the slope in hectopascal per minute and the total permeating water volume were calculated separately for each of the four conditions (baseline after embedding, CI I preparation, full occlusal preparation, and restoration) and results were presented as the range of data obtained in the individual measurements (original measurement and seven repetitions).

To assess the detection limit, the measurement background noise in the test curves of the sound 6 teeth at fixed 9 time points with 120-s intervals was calculated mathematically. It was calculated by measuring the deviation from the ideal curve drawn between the two fixed time points to determine the leakage slope value independently. When the ideal slope value (hectopascal per minute) and the time interval are

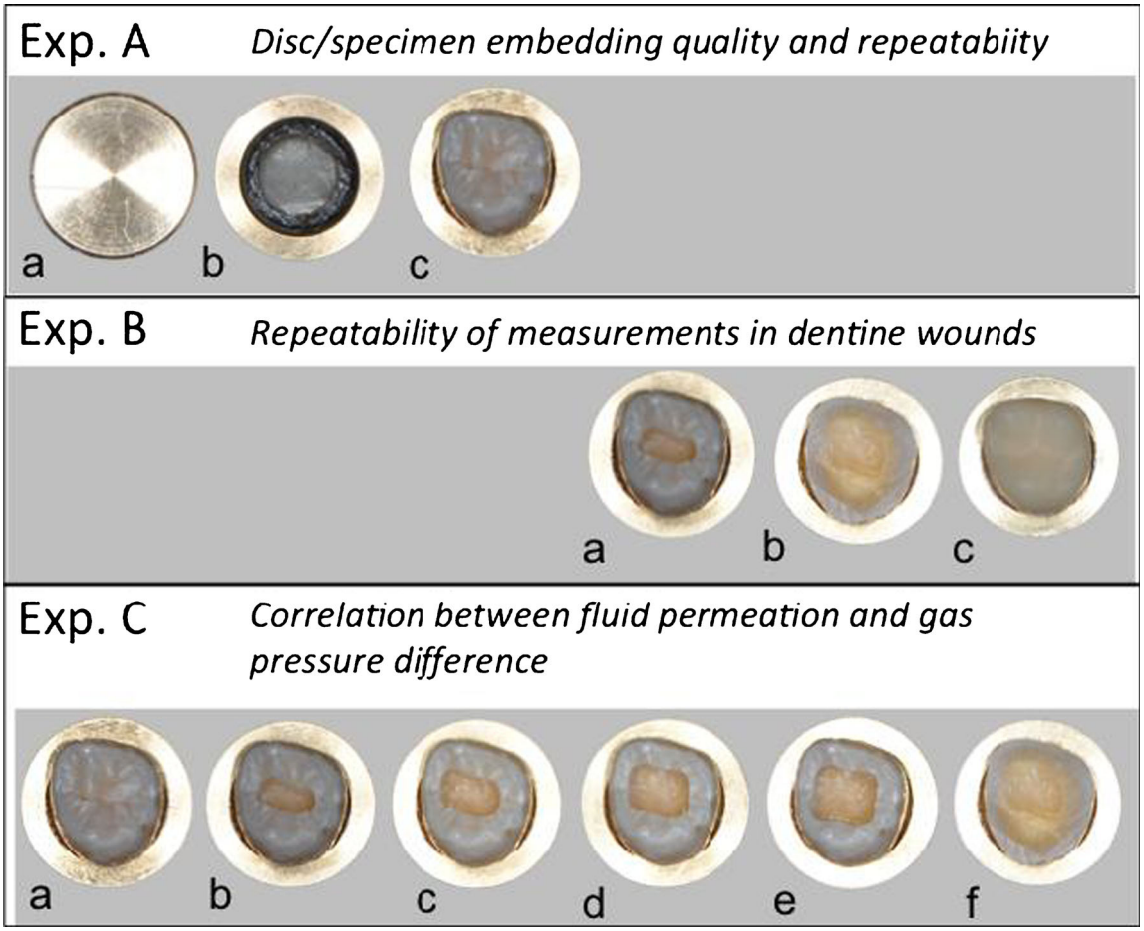


**Fig. 5** **a** Disk/specimen embedding quality and repeatability; one full metal disk (*a*,no embedding), three embedded composite disks (*b*), and three embedded third molars (*c*); eight consecutive measurements in each sample. **b** Repeatability of measurements in dentine wounds; three molars (of **a**) with 2×5 mm (*a*) and full occlusal preparation (*b*) as well as consecutive restoration (*c*); eight consecutive measurements in each sample. **c** Correlation between fluid permeation and gas pressure difference; six third molars (*a*) with stepwise increasing preparation size of 2×5, 3×5, 4×5, and 5×5 mm (*b*–*e*) and full preparation (*f*); one measurement per sample

known, it is possible to calculate the ideal measurement value at each point. The deviation from this was calculated, and average deviations were then pooled for each sample and used for further calculations [12].

To test whether the slope in the pressure change over time correlated with the collected saline solution ($N$=6), the Pearson correlation coefficient was used [13] (Fig. 6).

## Results

The mean slope values (Table 1) for the baseline measurements, i.e., the measurements of the sample permeability status before treatment, ranged between 0.01 and 0.03 hPa/min, indicating proper embedding seal of the specimens. The range of variation after repeated measurements of a sample did not exceed the 0.01 hPa/min. Testing for repeatability, a high linearity was shown (Table 2), indicating consistent results obtained with specimens that were measured multiple times.

The detection limit of 0.043 hPa for the pressure difference was calculated, which correlated to a slope value of 0.002 hPa/min and a fluid infiltration of 0.0225 μl/min. Testing for the pressure difference–infiltrated fluid volume correlation using the Pearson coefficient with the confidence interval set at ($p$=0.05) showed the point estimate of 0.99785 with standard deviation of 0.0002387463 ($R^2$=0.996). This confirmed the high correlation between pressure change and fluid filtration (Fig. 6).

Consequently, all four working hypotheses were accepted.

## Discussion

Permeation testing methods varied over the last years with many modifications; however, most models focussed on fluid infiltration [2]. The variance in methodology, unfortunately, still makes it difficult to interpret and compare results. Therefore, leakage testing is not any more unambiguously accepted in some scientific journals due to the fact that it cannot be ensured that leakage measured is related to actual treatment

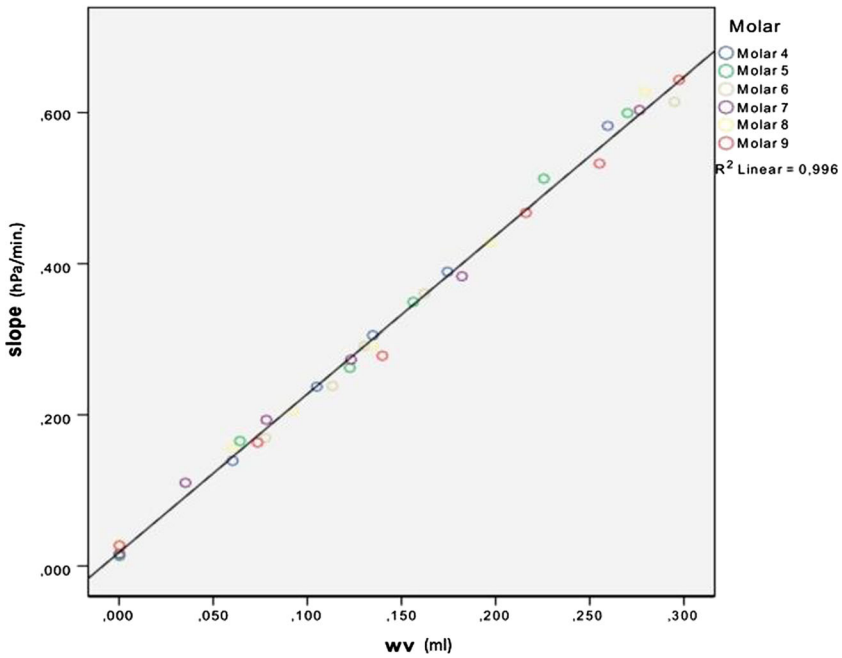


**Fig. 6** Plotted linear regression line, showing the correlation between the slope value (*y*-axis, denoted as *Slope*, measurement unit: hectopascal per minute), and the permeated saline volume (*x*-axis, denoted by *wv*, measurement unit: milliliters)

status only [14]. Permeation might also occur through other niches leading to false-positive results. This study therefore tried to establish and validate a novel device to test dentin permeability more reliably under standardized conditions. The focus of this study was basically to assess the accuracy of the combined determination of fluid permeation and pressure changes over time as well as the leakage-free embedding of samples and the reproducibility of their repeated measurements, which altogether build the basis for any kind of evaluation using this device in the future.

The presented setup is a nondestructive technique allowing testing under environmental conditions because of the use of a net effective pressure 1,030 hPa close to atmospheric air pressure under a standardized simulated mouth temperature of 35° [15]. The repeatability and accuracy can be related to the standardized conditions.

This study showed that the embedding procedure allowed for perfect separation of the two chambers and that no to only a very minute variation in repeated measurements of the evaluated samples for all treatments was found. In addition, the saline volume was highly correlating to gas pressure changes with a low detection limit. Therefore, the presented method appears to be a fast, reliable, and exact tool to assess permeability allowing for nondestructive and repeatable leakage measurements.

Unlike previous methods, the embedding procedure for each sample was tested independently. This allowed for the baseline status to be considered once the effective permeability is calculated for. Under normal conditions, embedding seal depends on the researcher skills and the material used. Technically, this does not play a major role in the current setup, as it will be compensated for. The embedding material used was chosen after long trials with other materials. Waxes proved not to be sealing properly, especially with teeth. Epoxy glue resins were also screened: Although they were initially tight, they could not withstand storage conditions in liquids for more than 24 hours, while composite resin materials had problems to stick to brass and silicon and were not adequately sealing in

**Table 1** Slopes of regression lines according to respective specimen

| Specimen | Initial (hPa/min) | Class 1 preparation (hPa/min) | Occlusal full preparation (hPa/min) | After restoration (hPa/min) |
|---|---|---|---|---|
| Metal disk | 0.01 (0.01, 0.01) | – | – | – |
| Composite disk 1 | 0.02 (0.02, 0.02) | – | – | – |
| Composite disk 2 | 0.03 (0.03, 0.03) | – | – | – |
| Composite disk 3 | 0.02 (0.02, 0.03) | – | – | – |
| Third molar 1 | 0.02 (0.02, 0.02) | 0.19 (0.19, 0.19) | 0.36 (0.36, 0.36) | 0.02 (0.02, 0.03) |
| Third molar 2 | 0.02 (0.02, 0.02) | 0.21 (0.21, 0.22) | 0.42 (0.42, 0.42) | 0.02 (0.02, 0.02) |
| Third molar 3 | 0.03 (0.03, 0.03) | 0.23 (0.22, 0.23) | 0.48 (0.48, 0.48) | 0.03 (0.03, 0.04) |

Values indicate means and ranges (in parentheses) of eight individual experiments

**Table 2** Pearson correlation coefficient values for permeation slope values and total water volume, for repeated measurements after different treatments

| Variable | Initial | Class 1 preparation | Occlusal full preparation | After restoration |
|---|---|---|---|---|
| Slope | 0.9863832 | 0.9964485 | 0.999631 | 0.9814998 |
| Fluid volume | 1 | 0.9907555 | 0.9992009 | 1 |

thin sections. The only material found to last after long storage in liquids and multiple measurements with a proper adherence to all mounted parts was a simple nail buildup gel as presented. Although the embedding baseline measurement varied slightly among the samples, the possible false-positive error was overcome by subtracting the baseline slope value from the subsequent measurements to calculate for the absolute permeation value.

The testing under standardized temperature is usually also an ignored aspect [16]. In many studies, testing was done at room temperature [10]. The system allowed testing under moisturized conditions and a temperature of 35°, which is the average temperature in the oral cavity [15]. The need for testing at this constant temperature is important, as it was demonstrated that dentine permeability increases with higher temperatures [16, 17]. Unlike pure gas testing units and porometers, the device prevents sample dehydration, which allows for further testing of the same samples without affecting their physical properties. Another reason to simultaneously apply pressure and vacuum is the wish to eliminate any bubble entrapment which might interfere with the permeability testing as it is the case in passive permeation testing [18].

In addition, the new chamber design and embedding makes it easy to remount specimens for consecutive testing, which overcomes the problem of calibrating the air bubble in position, a problem encountered in the latter method. In addition, the simple small carrier system opens the door for multiple steps and interventional studies using the same sample in different conditions to produce comparative data for proper conclusions. This contrasts with substance permeation methods, in which the results depend on the permeated substrate molecular size, osmolarity, and possible capability of entrapment or reacting with other substrates in the tested samples, and as an end effect might result of under estimation of the real permeability status of the specimen. The current setup overcomes these shortcomings by using a physiologic saline solution, which does not have any interaction or interference with the permeation process.

While validating the new method, a strong evidence of accuracy and repeatability in correlation to the permeated fluid volume for both biological as well artificial samples was found. Therefore, this method appears suitable for longitudinal in vitro studies with repeated measurements in the dental field. Although there was a slight deviation from 0, the correlation was high. This can be explained either by the difficulty in collecting some entrapped fluids in the sample, or possible evaporation under low-pressurized conditions. The ease of embedding process and mounting of samples reduced the effort during the testing procedure; the samples pretesting before treatments ensured the compensation for the error related to the baseline status of the sample.

## Conclusion

The embedding causes no false-positive measurements and the chamber model per se is tightly sealed as evidenced by the following:

1. The repeated measurement of identical samples results in reproducible results.
2. The detection limit to assess permeation is low.
3. The liquid collected during the permeation test correlates to the gas pressure differences.

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**Conflicts of interest** The authors declare that they have no conflict of interest.

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CLINICAL ORAL IMPLANTS RESEARCH

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# Comparison of three *in vitro* implant leakage testing methods

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**Key words:** abutment, implants, *in vitro*, leakage, sealability

**Abstract**

**Aims:** To assess the accuracy and sensitivity in detecting implants leakage with a gas-enhanced permeation test (GEPT) and to compare with a molecular- and a bacterial-based leakage tests.

**Materials and methods:** Three implants systems were tested ($n$ = 20 per group): Nobel Biocare (NB), Astra Tech (AT) and Biomet 3i (B3i). Implants were mounted in PVC disks and were first tested for gas pressure change and infiltrated saline volume over 40 min. The same implants were then subjected to a molecular leakage evaluation using fluorescent Dextran for 28 days. After cleaning and sterilization, bacterial permeation (*E. faecalis*) was evaluated by selective media turbidity for another 28 days. Slopes in the pressure change and the perfused saline rate were used as a measure of leakage in the GEPT model and the times of positive events, that is, color change, after molecular and bacterial tests were recorded. Data were analyzed using Kolmogorov–Smirnov/Shapiro–Wilk, Kruskal–Wallis H and Spearman's Rho tests ($P < 0.05$).

**Results:** The gas and saline (ml) leakage values accounted for 0.85 ± 0.71 and 0.56 ± 0.50 ml (AT), 0.23 ± 0.030 and 0.12 ± 0.20 ml (NB) and 0.01 ± 0.01 and 0 ± 0 ml (B3i), respectively, and were significantly different from each other ($P < 0.001$). Slope in the pressure change over time showed a significant positive correlation with the collected saline solution ($r = 0.91$; $P < 0.001$). Molecular and bacterial leakage was positive at the same implants, which also showed increased leakage values in the GEPT setup. The development of positive events in the timeline of the bacterial leakage evaluation corresponded well to the GEPT leakage model.

**Conclusion:** The GEPT proved to be a reliable method to quantify leakage. The B3i showed the best sealing among the tested systems.

Implants show high success and excellent survival rates (Bazrafshan & Darby 2013). However, implants still may encounter some biological, technical and prosthetic problems in the long term. With regard to biological goals, the establishment of stable hard and soft tissue integration is of outmost importance. A complexity of bacteria, however, may hamper this goal (Mombelli et al. 1987), as plaque retentive niches like the implant–abutment interface bare the potential to accumulate bacteria and their bi-products and result in moderate bone remodeling or even pathologic bone resorption (Broggini et al. 2006). Favorably, implants should therefore be fabricated with perfect seal to prevent or limit any biofilm accumulation inducing inflammatory reactions at the adjacent tissues. As a consequence, the interest in studying the implant interfaces and related complications and failures remains an important research focus. It is nowadays strongly believed that the quality and the position of the interface are an important factor of bone loss around implants (Piattelli et al. 2003). *In vitro,* models have been proposed to study leakage phenomena, but still with indirect correlation to the disease initiation and progression. However, given the hypothesis that leakage may harbor the risk of developing bacterial-based alterations, the evaluation of a tight seal is important in assessing and comparing different implant systems and the accuracy of fabrication.

The history of leakage testing is long, and the first leakage study was conducted in 1961 (Swartz & Phillips 1961). Based on the principles of leakage testing in other dental fields, leakage test methods were adopted and modified to test for implants leakage as well. Evaluation of the gap at the interface level with radiographs, SEM or other optical means is a simple method to detect inadequacies in the connection (Meleo et al. 2012). However, this testing seems to be less reliable and does not necessarily reflect the real situation as the

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continuity and depth of the gap cannot be evaluated. Other accepted testing techniques are microbial leakage or bacterial seal testing (Assenza et al. 2012), endotoxin or other molecular microleakage tests (Harder et al. 2010), spectrophotometric determination of dye penetration (Park et al. 2012) and the gas permeability test (Torres et al. 2011). The latter method not only provides information whether the implant leaks or not, but rather provides the leakage amount over time (speed of leakage) allowing for quantitative comparison between different implants systems.

Due to the great clinical implication of leakage phenomena and its control on preventing peri-implant remodeling and inflammatory bone loss, these tests need to be further developed, investigated and validated to overcome their limitations in order to reliably determine the leakage status of implants. Therefore, this study aimed to compare two commonly used implant leakage methods, namely the microbial and molecular ones with a newly modified gas-enhanced permeation testing device, the so-called GEPT test, under standardized conditions. We hypothesized – given the microbial test as being the so-called gold standard – that the GEPT is at least as effective in detecting leaking implants and that the time sequence of leakage development is comparable, that is, implants with high GEPT values show positive leakage at an earlier stage.

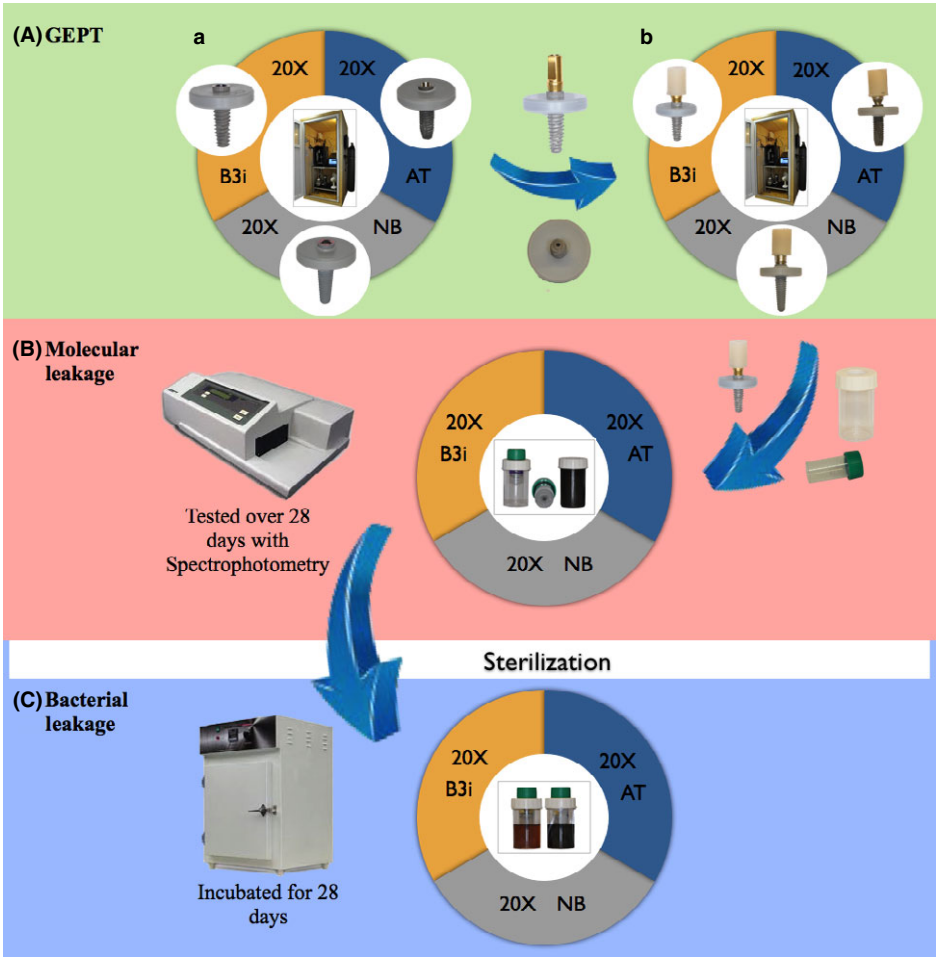


*Fig. 1.* Testing flow chart (A) GEPT (a) Implants were mounted in disks and tested for their baseline leakage (b) After hole drilling, the abutment was fixed and build up was made then the implants were retested to calculate for their absolute leakage. (B). Molecular Leakage. The same implants were further mounted in a two chambers system in which the upper chamber contained fluorescent molecules and the lower chamber was regularly tested for increasing fluorescent molecules content. (C). Bacterial leakage. The same setup was used after washing and sterilization. The upper chamber contained *E. fecalis* strain which its leakage was indicated by turbidity of a selective media broth placed in the lower chamber.

## Material and methods

The same set of implants was used in all the three tests (Fig. 1) allowing for a comparison of performance end event occurrence in identical samples.

### Gas-Enhanced Permeation Test (GEPT)

The first test setup was based on the gas leakage testing (Torres et al. 2011). It was modified by adding a split-chamber design (Fig. 2), which allowed for easy and multiple mounting at different times intervals, ensuring identical repositioning and experimental conditions all times. In addition, the split chamber allowed not only for gas testing but also for simultaneous fluid permeation testing by the collection of permeated fluid in an Eppendorf tube, which was mounted to the lower chamber terminal. The split chamber was installed in an isolation chamber (Fig. 3) maintaining a constant temperature of 35°C (Moore et al. 1999), and the whole device was mounted again in a testing room ensuring a constant temperature of 31.5°C. The outside room temperature was kept at 25°C.

Three different implants brands were chosen (Table 1). Two had a platform design (Biomet 3i, Palm Beach Gardens, FL, USA & Nobel Biocare, Göteborg, Sweden) and one had a taper lock design (Astra Tech, Mölndal, Sweden). Twenty implants of each type were used. They were mounted in PVC disks with a diameter of 15 mm and a thickness of 3 mm (Fig. 4). The diameter at each type was measured at the level of 1 mm from the implant–abutment interface. A drill with a reduced diameter of 0.2 mm from the measured implant diameter was performed in the corresponding disks using a parallelometer. The dimensions amounted to 3.3 mm (Biomet 3i), 4.0 mm (Nobel Biocare) and 3.8 mm (Astra Tech). The implants were then screwed in position displaying a final position, which was 1 mm of the implant–abutment interface. To ensure perfect sealing around the disk–implant interface, the latter was sandblasted with 50 μm aluminum oxide from the apical side (Benzer-Dental AG, Zurich, Switzerland) and then conditioned and sealed using a commercially available nail buildup gel material (Sina, Shenzhen Cyber Technology Ltd, Mainland, China), which proved a much better performance than any dental adhesive material in pretests (unpublished data).

All implants were then tested for their baseline seal to exclude any leakage related to the embedding procedure before starting the experiment; the mounted implants were positioned in the split chamber surrounded by an O-ring lubricated with silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany), and 2.5 ml physiologic saline was added on top. The 2 parts of the split chamber were tightened together with

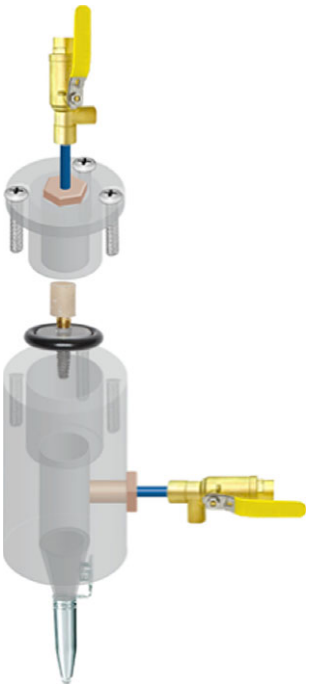

*Fig. 2.* Split chamber allowed for multiple repeated measurement.

***Table 1.*** **Implants systems in test. Parts used and their codes**

| | Astra Tech | Nobel Biocare | Biomet 3i |
|---|---|---|---|
| Description | Astra Tech™ OsseoSpeed™ TX/S | Nobel Replace® Tapered Platform Switch | OSSEOTITE® Tapered Certain® PREVAIL® |
| Size | 4.0 × 15 mm | 4.3 × 16 mm | 4.0 × 15 mm |
| Item No. | 24944 | 36895 | XIITP4315 |
| Abutment | TiDesign 3.5/4.0–1.5 mm | Esthetic Abutment NP - 3 mm | GingiHue® - 2 mm |
| Abutment item No. | 24285 | 36824 | IMAP32G |
| Screw | Uncoated Screw | Uncoated Screw | Gold Coated Gold-Tite® Screw |
| Screw item No. | Included with Abutment | Included with Abutment | IUNIHG |

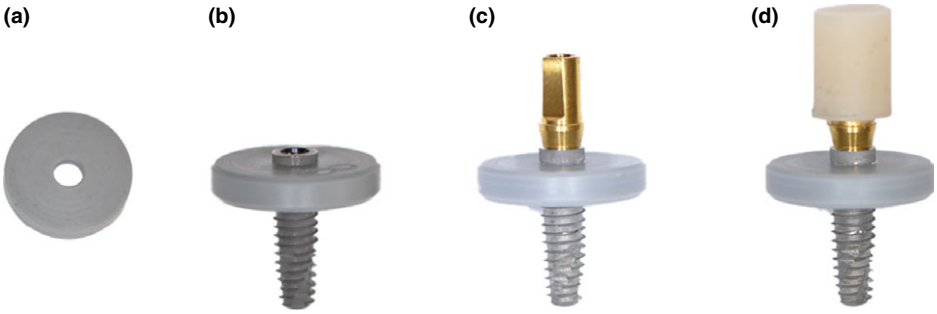


*Fig. 4.* Samples embedding for GEPT test. (a). Drilled disks. (b). Blank implant screwed in the disks. (c). Abutments fixed. (d). Standard core build up.

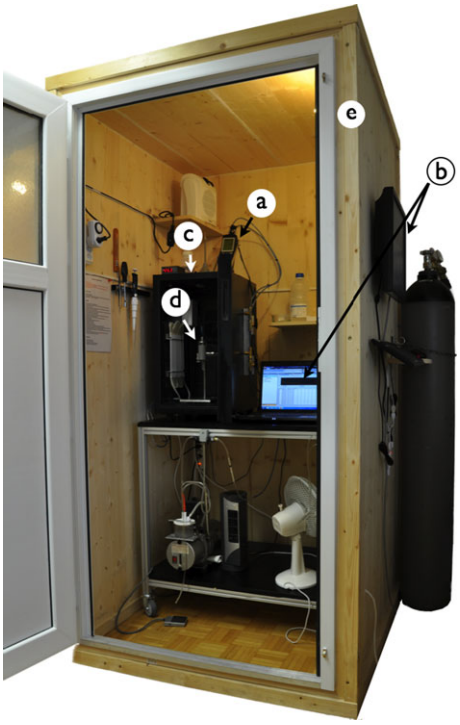


*Fig. 3.* Temperature controlled testing unit (a). Pressure difference measuring device. (b). Inner and outer monitoring and control units. (c). Inner temperature controlled isolation chamber. (d). Split-chamber unit. (e). Outer temperature controlled isolation room.

three screws using a torque-controlled screwdriver. The upper chamber was positively pressurized to 860 hPa, and the lower chamber was negatively pressurized to –175 hPa, resulting in a net effective pressure difference of 1035 hPa, which was monitored continuously over 40 min (1 measurement/second) using a pressure difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany). Data were plotted in a curve using a customized computer program (V 4.2 SP2, Testo AG, Germany). The slope between the measurements at 1200 and 2400 s was considered to present the leakage status of implant assessing individual slope values as follows: Slope = $\frac{P1-P2}{T2-T1}$.

The leaked fluid volume was measured then additionally measured by weighing the Eppendorf tube before and after collecting the fluid, and the weight value was converted to the respective volumes in milliliters.

### Leakage measurement

The implants were then held in an inverted position in the parallelometer, and an inside–outside connection was created by drilling a hole from the apical direction right to the internal fixation using a 1 mm hard metal drill at a speed of 1100 rpm under extensive continuous water cooling. Special attention was paid not to harm the internal threads. In the negative control group, the drilling was performed without getting access to the screw to study the potential deleterious effect of drilling on the integrity of the previously assessed embedding procedure. The implant was held in a straight Kelly hemostat (Hu-Friedy Mfg. Co., Chicago, IL, USA), and the abutment was then attached to the implant using the respective screws and the manufacturer recommended torque. The abutments were then sandblasted with 50 μm aluminum oxide while protecting the platform with a punched metal matrices. The screw channel was filled and protected with Teflon strip and tightly packed: Then, a standardized composite build up (6 mm diameter and 10 mm height) (Luxa Core Automix, DMG, Hamburg, Germany), extending to the abutment restoration finish line, was made in a Teflon mold after conditioning with Monobond Plus (ivoclar vivadent AG, Schaan, Liechtenstein) and an adhesive material (Clearfil SE Protect, Kuraray America Inc., New York, NY, USA). The implant was tested again as described in the previous chapter, and this baseline slope was subtracted from the slope after build up to achieve the absolute leakage slope. The saline flow was recorded again.

### Molecular leakage testing

The same set of implants was used for both upcoming tests, that is, the molecular and the bacterial leakage tests.

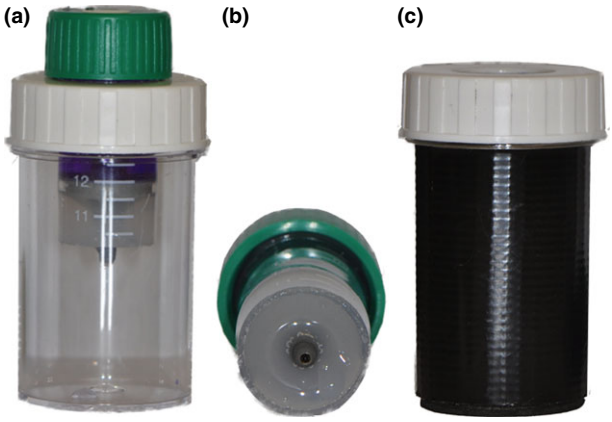


*Fig. 5.* Molecular leakage setup (a). Two chambers system. (b). Implants further sealed from the apical side leaving the drilled hole free. (c). Lower chamber taped to obtain light tight conditions.

Each implant was positioned in a 15 mm centrifuge tube (Semadeni, Ostermundingen, Switzerland; Fig. 5), which was shortened by cutting-off 8 cm from the tip and was positioned 1 cm from this lower cut level. The lower part below the disk was additionally sealed with silicon glue (Dow Corning 734, Dow Corning GmbH, Wiesband, Germany) leaving the drilled tip free and was allowed to dry for 24 h. A 30-ml transport tube (Semadeni, Ostermundingen, Switzerland) was then prepared accordingly with a 15.5 mm drill to allow insertion of the first tube. This created a custom-made two-chamber system to test for permeation through all tested implants. Three ml of 10'000 Dalton and 50% w/v Dextran–Texas Red (Life Technologies Europe B.V., Zug, Switzerland) was then placed in the upper chamber, while the lower chamber was filled with 16 ml of de-ionized water in such a way that the implant tip was constantly immersed in the water. The tube was then coated with a black tape to make it lightproof to avoid any potential fluorescent substrate loss during the storage, which also took place in a dark chamber. An extra tube holding 10 ml of the Dextran was used for spectrophotometry contrast at each testing day. To test for leakage, a spectrophotometer was used (Spectramax M2, Bucher biotec AG, Basel, Switzerland), and dilution series were made at a wavelength of 600 nm at each day of measurement to establish a calibration curve and determine the detection limit. From each sample, 300 µl from the lower chamber was pipetted in a 96-well plate and tested for leakage. After each test, another 300 µl de-ionized water was added to substitute the taken sample. Samples were tested on a daily basis in the first 4 days, then once every 2 days and finally, once every 4 days until 28 days were completed. The sample was considered leaking if the spectrophotometry value was above the detection range one time and in all the subsequent measured time points. Time of leakage starts was reported and considered to represent the leakage status of the implant.

#### Bacterial leakage testing

The same samples were mounted according to the setup described above. But this time, the mounting parts for each sample were packed in a sealed sterilization bag, which was sterilized using ethylene oxide gas (3M AG, Rüschlikon, Switzerland) in a sterilizer (Sterivac 4XL, 3M AG, Rüschlikon, Switzerland) using the cold sterilization cycle at 37°C for 5.5 h. Sterile packs were opened, and the parts were remounted under a clean bench (EVZ 120, SKAN AG, Basel, Switzerland). Three ml of overnight culture of *E. fecalis* ATCC 29212 in fluid universal broth (FUM, Gmür and Gugenheim 1983), which was previously adjusted to an optical density of 1.0 at 550 nm, was pipetted in the upper chamber (Fig 6). In the lower chamber, 16 ml of enterococci-selective bile esculin azide broth (Enterococcosel Broth, Difco, Benton Dickinson Co.,Sparks, MD, USA) was used to detect bacterial leakage by inspecting color change. The hydrolysis of the esculin by *E. fecalis* resulted in turbidity and blackening of the broth. An extra transport tube holding 16 ml of selective media was used as a negative contrast medium for the color change assessment. All samples were incubated in ambient air at 37°C. The samples were monitored on a daily basis for 28 days. The day at which the samples visibly leaked was reported and considered to present the initial leakage status of the implant. At the end of the experiment and to check for the bacterial viability, a swap of the bacteria was made and applied to the selective media in the lower chamber of the same sample and further incubated overnight to prove the viability of the bacteria.

#### Statistical analysis

Data were recorded in Microsoft® Excel® spreadsheets (Microsoft® Corporation Microsoft Corporation, Redmond, WA, USA) and analyzed in SPSS Version 20 (IBM®; SPSS Inc., Chicago, IL, USA). Descriptive statistics such as mean, standard deviation, median and interquartile ranges were computed. Normality of the data distribution was checked

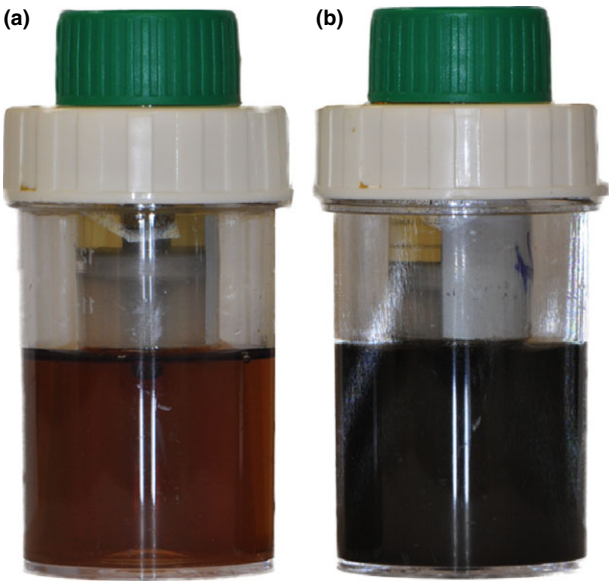


*Fig. 6.* Bacterial Leakage (a). Mounted set up; clear yellowish broth in lower chamber indicates no leakage. (b). Darkening and turbidity of lower chamber broth indicating leakage.

*Table 2.* **Comparison of different tests results. Implants were aligned in an ascending manner according to the GEPT leakage status**

| Astra Tech | | | | Nobel Biocare | | | | Biomet 3i | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) | Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) | Imp. No. | GEPT hPa/min | Mol. Leak (Days) | Bac. Leak (Days) |
| 18 | 0.000 | – | – | 18 | 0.000 | – | – | 4 | 0.000 | – | – |
| 19 | 0.001 | – | – | 17 | 0.001 | – | – | 16 | 0.000 | – | – |
| 17 | 0.001 | – | – | 20 | 0.003 | – | – | 18 | 0.001 | – | – |
| 20 | 0.002 | – | – | 1 | 0.004 | – | – | 8 | 0.001 | – | – |
| 3 | 0.089 | – | – | 6 | 0.006 | – | – | 12 | 0.002 | – | – |
| 1 | 0.125 | – | 12 | 19 | 0.007 | – | – | 2 | 0.002 | – | – |
| 12 | 0.227 | – | 11 | 5 | 0.015 | – | – | 6 | 0.004 | – | – |
| 6 | 0.249 | – | 9 | 14 | 0.043 | – | – | 5 | 0.005 | – | – |
| 4 | 0.488 | 1 | 5 | 15 | 0.049 | – | – | 1 | 0.008 | – | – |
| 8 | 0.559 | – | 6 | 4 | 0.065 | – | – | 7 | 0.009 | – | – |
| 9 | 0.744 | – | 5 | 13 | 0.075 | – | – | 9 | 0.009 | – | – |
| 10 | 0.921 | 20 | 4 | 8 | 0.080 | – | – | 11 | 0.009 | – | – |
| 15 | 1.278 | 20 | 2 | 7 | 0.173 | – | 12 | 10 | 0.012 | – | – |
| 11 | 1.286 | 2 | 1 | 11 | 0.252 | – | 10 | 17 | 0.013 | – | – |
| 2 | 2.008 | 2 | 1 | 16 | 0.253 | – | 10 | 19 | 0.013 | – | – |
| 5 | 2.171 | 8 | 2 | 10 | 0.346 | – | 8 | 13 | 0.014 | – | – |
| 7 | Failed | 12 | 1 | 3 | 0.846 | 24 | 5 | 3 | 0.015 | – | – |
| 13 | Failed | 8 | 1 | 9 | 0.940 | 16 | 5 | 20 | 0.016 | – | – |
| 14 | Failed | 1 | 1 | 2 | Failed | 12 | 1 | 14 | 0.025 | – | – |
| 16 | Failed | 3 | 1 | 12 | Failed | 6 | 1 | 15 | 0.029 | – | – |

using Kolmogorov–Smirnov and Shapiro–Wilk tests. To compare for implants performance with the GEPT, results were analyzed using the nonparametric Kruskal–Wallis H test. The level of significance was set at 5% ($P < 0.05$). To test whether the slope in the pressure change over time correlated with the collected saline solution, the Spearman's rho correlation coefficient was used.

The times for molecular and bacterial leakage were considered as absolute values representing the leakage status of samples individually. Results of all tests were presented in a table to provide a leakage sequence pattern end elucidate the interrelationship.

*Table 3.* **Mean and median values with respective standard deviations and interquartile ranges (IQR) for detected leakage. Statistically significant differences are marked with superscript capitals (read vertically)**

| Implant type | Mean slope value (hPa/min) | Infiltrated saline volume (ml) |
|---|---|---|
| Astra Tech | | |
| Mean ± SD | 0.85 ± 0.71 | 0.56 ± 0.50 |
| Median (IQR) | 0.65 (1.05)$^{A}$ | 0.43 (0.95) |
| Nobel Biocare | | |
| Mean ± SD | 0.23 ± 0.30 | 0.12 ± 0.20 |
| Median (IQR) | 0.08 (0.27)$^{B}$ | 0.10 (0.14) |
| Biomet 3i | | |
| Mean ± SD | 0.01 ± 0.01 | 0.00 ± 0.00 |
| Median (IQR) | 0.01 (0.01)$^{C}$ | 0.00 (0.00) |

## Results

None of the negative controls showed any signs of leakage.

The implants showed different performance under the GEPT testing conditions according to Table 3. Twenty-five percent of the Astra tech and 12.5% of the Nobel Biocare implants failed to withstand the initial phase of the test, that is, could not last the whole testing time. The Biomet 3i implants showed the least slope leakage values with a mean of 0.01 ± 0.01, followed by Nobel Biocare 0.23 ± 0.03 and the Astra tech implants 0.85 ± 0.71. The saline infiltration through the implant–abutment interface accounted for 0.56 ± 0.50 ml (Astra Tech), 0.12 ± 0.20 ml (Nobel Biocare) and 0 ± 0 ml (Biomet 3i), respectively. The results between the different implant types were statistically significantly different ($P < 0.001$). The nonparametric Spearman's rho correlation test of leakage slopes to permeated fluid volumes shows a significant positive correlation ($r = 0.91$; $P < 0.001$).

When comparing the different tests (Table 2), both GEPT and bacterial leakage showed well-matching patterns in terms of leakage status of the individual implants (Fig. 7), whereas the molecular leakage evaluation showed some varying testing results as compared the other two test methods, especially in terms of the time point, when the first leakage was observed (Fig. 8).

## Discussion

This was – according to the author's knowledge – the first study comparing these different leakage models for implants. Other studies combined different testing models to compare different implants systems (Piattelli et al. 2001), while others tried to correlate gaps at the implant–abutment interface to the leakage status (Jansen et al. 1997). The latter study showed no correlation as the continuity of gap could not be ensured (Dias et al. 2012).

In the current study, it was managed to use the identical implants in all tests, which allowed a more reliable comparison between different tests. As being shown, the GEPT allowed for quantification and a sensitive detection revealing even small values in leakage as compared to the bacterial and molecular leakage testing. The variations among tests might be due to their limitations by the size of gaps, perforating substance and the nature of diffusion process. In the GEPT, the infiltration of an ionized fluid under pressure eliminated limitations of the leakage regardless of the gap size and also provided two parameters: The gas pressure difference and behaved independently of both, the gap and substrate size. The

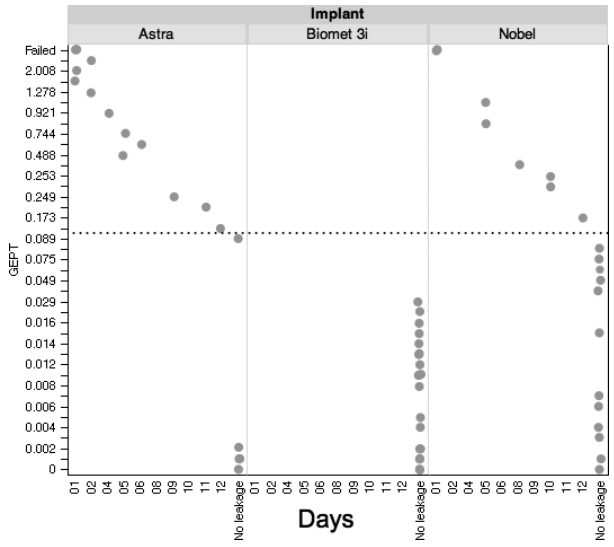


*Fig. 7.* Correlation of GEPT with bacterial leakage event timeline: The high correlation between the two tests indicated by the bacterial leakage time point to the slope leakage value with the minimal bacterial tight implant slope value set at 0.089 hPa/min.

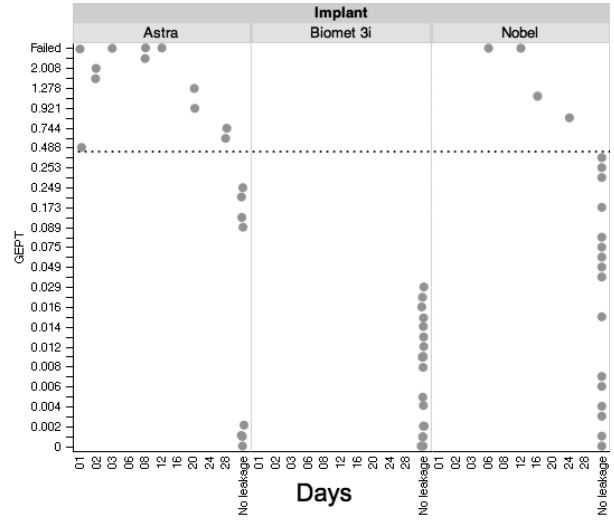


*Fig. 8.* Correlation of GEPT with molecular leakage event timeline : The graph shows no correlation by means of time points by which the leakage was first detected with less sensitivity in molecular leakage detecting limit.

vacuum exerted in the lower chamber eliminated any air entrapment, which might have retarded the leakage process. Bacterial leakage depended more on the gap and not only passive diffusion but also active bacterial growth phenomena "pushed" cells in available empty spaces, given open pathways and spaces. In contrast, the molecular leakage depended more on the substrate and gap size and on physical passive diffusion rules. In addition, air entrapment might have occurred (Wu & Wesselink 1993), which may explain the varying results shown by this test in terms occurring leakage times.

In the current study, the molecular testing utilized endotoxin like molecules detected by spectrophotometry, this allowed validation of both the controlled molecule size leakage as well the detection method. Once comparing the testing time, GEPT required 2 testing times each of 40 min to verify the leakage status of the implant, while in both bacterial and molecular leakage, it needed 28 days minimum testing time.

Some of the advantage of the GEPT over other testing systems is as follows: The disk embedding system of the GEPT allowed for the ease of precision mounting for multiple testing time points, which could allow for non-destructive comparison of implant performance under different conditions, for example, before and after dynamic loading. It also may allow for compensation of possible mounting error, which might result in a false positive measurement (Rechenberg et al. 2011), by individually subtracting the initial status slope from the respective "after treatment" slope, which allows to calculate the absolute leakage slope value. The drilling of implants showed negligible changes on the embedding and the overall leakage status as well. This can be confirmed by the minor changes in the negative controls when tested before and after incomplete drilling. In addition, the GEPT also allowed for testing under environmental conditions (liquid, oral temperature, and atmospheric pressure), which is mandatory, as the parts of an implant are composed of different alloys. Therefore, temperature changes might have a great influence on the implants part dimensions and the fluid viscosities as well.

## Conclusion

This study also showed that the method could determine leakage performance of different implant systems and may be used as a valuable screening method. Under the current testing conditions, completely different leakage patterns could be shown. The Biomet 3i with a platform showed better performance than the equivalent design of Nobel Biocare the taper lock design by Astra tech.

## Acknowledgement

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## Conflict of interest

The study design was conceived and executed independently; however, the presenter's PhD fellowship is supported – in part - by Biomet 3i.

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# Impact of Dynamic Loading on the Implant-abutment Interface Using a Gas-enhanced Permeation Test *In Vitro*

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**Abstract:** *Purpose*: To assess implant leakage under static conditions as well as during and after dynamic loading. *Materials and methods*: Implants (Astra Tech (A), Biomet 3i (B) and Nobel Biocare (C)) were evaluated for leakage (n=8/group). Testing to assess the gas pressure change over time (hPa/min) and infiltrated fluid volume, was performed in a Gas Enhanced Permeation Test (GEPT) to qualify embedding. Implant apexes were then drilled, abutments were mounted and resin build-ups were fabricated. GEPT was reassessed. Samples were afterward mounted in a computer-controlled masticator while tested to bacterial leakage, they were daily observed for turbidity. Samples were then reassessed using GEPT. Dunnett's and Fisher's exact tests were utilized to compare implant and to analyze bacterial leakage. *Results*: Significant differences in GEPT values were shown after loading (p=0.034). Leakage resistance was best for B when compared to C (p=0.023). Samples with higher GEPT values demonstrated earlier bacterial leakage, occurring after 1 or 2 days (A=4, B=0, C=6) and showing favorability for implant system B (p=0.009). *Conclusion*: Implants leaking under static conditions had increased potential for bacterial leakage under dynamic conditions. As strongly correlating to sophisticated analytical methods, GEPT is a promising technique for assessing the overall implant system leakage resistance.

**Keywords:** Dynamic loading, implants leakage, static implants leakage.

## 1. INTRODUCTION

Significant emphasis has been placed on the research and development of implant-abutment interfaces, as well as the corresponding test methods because of the potential for bacterial harborage within the implant should this interface leak. Due to its - in most cases - submucosal location and configuration, the implant-abutment interface is difficult to clean or disinfect and may be regarded as a potent source for continuous infection [1], which may lead to mucositis and even peri-implantitis [2, 3]. Even implant failure was correlated to this bacterial inhabitation [4].

Several models have been employed to test implants for the implant-abutment interface integrity and numerous designs have been proposed by manufacturers to increase and enhance its tightness: Molecular, bacterial and fluid penetration, were the most investigated models under static conditions for testing implants leakage. A recent study [5] showed a high correlation between bacterial leakage and fluid permeation utilizing a gas-enhanced permeation test (GEPT) with a high sensitivity for fluid permeation in detecting leakage in implants.

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Other studies investigated leakage under dynamic conditions, as it is more relevant to the clinical situation [6, 7]. It was suggested that implant systems are more susceptible to leakage under dynamic conditions due to the so-called pumping effect [7]. Specific connection designs such as the taper lock were suspected to be tighter after dynamic loading due to the relative displacement over time at the implant abutment interface, which might reduce the assembly movements due to the theoretical gap reduction between the interfacial surfaces [7]. Based on this knowledge, it is more clinically relevant to study leakage under dynamic conditions as the implant-abutment assembly is experiencing different functional adaptations, which might lead to deterioration or perhaps even improvement of the implant abutment interface in terms of leakage. However, most studies concentrated on implant leakage during dynamic loading only regardless of the preloading status and quality of the Implant-abutment interface, mainly because de-assembling the abutment was required to reach and then sample the inner implant chamber [6]. A non-destructive protocol allowing for a correlation between static status and implant performance during and after dynamic conditions is still warranted. It is because existing protocols interfere with the integrity of implant-abutment interface due to the methods required to disruption of the implant-abutment interface and repeated screw tightening [8]. Ideally, the evaluation of implant leakage should therefore be performed under static and thermo-mechanical dynamic loading conditions in one set of identical implants without being re-assembled.

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This study represents one of a series of studies, which aimed to analyse the leakage of different implant systems *in vitro*. Whereas a previous study assessed these implant systems only with regard to their leakage status under static conditions [5], this follow-up study aimed to test the same three implant system designs, but now under dynamic conditions with correlation to their static preloading status. It was hypothesized that a tight implant under static conditions would stay tight under loading conditions and vice versa. In addition, it was suggested that the implant design does influence the implant performance and stability under dynamic conditions.

## 2. MATERIALS AND METHODS

Three implant systems were selected (Table **1**): one with a taper lock and internal hexagonal mating surface design (Astra Tech (A)). a second system with a flat-to-flat interface design and internal hexagonal mating surface (Biomet 3i (B)) and one system, with a flat-to-flat and a trilobe mating surface (Nobel Biocare (C)). For each system, eight implants were assigned for leakage testing. Two additional implants were used as negative controls without drilling a connection between the two chambers, thus serving to control the adequate embedding set-up. All implants were mounted and static GEPT values were assessed according to a previously published and validated protocol [5, 9].

### 2.1. Mounting of the Implants

All implants were mounted in custom made PVC discs with a diameter of 15 mm and a thickness of 3 mm. First, a drill corresponding to the implant diameter but with a reduction of 0.2 mm, measured at a distance 1 mm from the implant-abutment interface, was made in corresponding discs utilizing a parallelometer. The respective diameters were 3.8 mm (A), 3.3 mm (B) and 4.0 mm (C). Implants were then mounted to allow for exposure of 1mm of the implant-abutment interface. To promote robust sealing at the implant-disc interface, the disc was sandblasted from its lower side (50 μm aluminum oxide, Benzer-Dental AG, Zurich, Switzerland) and further conditioned and sealed using a light cured nail build-up gel system (Sina, Shenzhen Cyber Technology Ltd, Mainland, China).

### 2.2. Gas-enhanced Permeation test (GEPT)

All implant systems were tested for their baseline leakage value to ensure that the mounting procedure was perfectly sealed. These baseline values were used later as a reference to calculate the absolute implant leakage value [5]. The whole sample was mounted with an O-ring, which was lubricated with silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) at the middle of a split chamber set-up, thereby forming two completely isolated chambers. The upper compartment contained 2.5 ml physiologic saline solution and was positively pressurized, while a negative pressure was applied to the lower compartment. A total effective pressure difference of 1030 hPa was created between the two chambers and the drop in pressure difference was monitored utilizing a pressure difference-measuring device (Testo 526, Testo AG, Lenzkirch, Germany) over 40 min at a rate of 1 measurement/sec. The slope of the pressure drop at two fixed points of testing (1200 sec and 2400 sec) was used to quantify the pressure difference drop for each test system:

$$\text{Slope} = \frac{P1 - P2}{T2 - T1}$$

The permeated fluid volume was calculated by collecting it from the lower chamber and weighing it to determine its volume in milliliters.

After this baseline reading, implants were mounted inverted in a parallelometer and a connection was created by drilling from the apical direction towards the internal fixture using a 1 mm hard metal drill at a speed of 1100 rpm while undergoing continuous water-cooling. The implants were assessed to ensure that the internal threads were not damaged. For the negative control, two implants received the same treatment but without penetrating into the internal thread compartment as to test for possible deleterious effects of drilling on the integrity of previously assessed embedding procedure.

Implants were then held in a straight Kelly hemostat (Hu-Friedy Mfg. Co., Chicago, USA) and the abutments were positioned and attached to the implant using the respective screws according to the manufacturer's instructions with the recommended torques (Group A 20 Ncm, Group B 20 Ncm and Group C 35 Ncm). The abutments were then sandblasted (50 μm aluminum oxide) while the platform was protected

**Table 1. Implants and specifications of parts used in the study.**

| | Group A | Group B | Group C |
|---|---|---|---|
| Description | Astra Tech™<br>OsseoSpeed™ TX/S<br>4.0x15 mm | OSSEOTITE®<br>Tapered Certain®<br>PREVAIL®<br>4.0x15 mm | Nobel Replace®<br>Tapered Platform<br>Switch<br>4.3x16 mm |
| Abutment | TiDesign<br>3.5/4.0-1.5 mm | GingiHue® -<br>2 mm | Esthetic Abutment<br>NP - 3mm |
| Screw | Uncoated Screw | Gold Coated<br>Gold-Tite® Screw | Uncoated Screw |

Table 1: Parts used in each group assembly

with a punched rubber matrice. The screw channel was filled with a Teflon strip (Pink Waterline PTFE Tape, Oatey, Cleveland, Ohio, USA) and was then pre-treated with Monobond Plus (ivoclar vivadent AG, Schaan, Liechtenstein). Afterwards, an adhesive material (Clearfil SE Protect, Kuraray America Inc., USA) was applied and a standardized composite build-up (6mm diameter and 10 mm height) (Luxa Core Automix, DMG, Hamburg, Germany) was fabricated, which extended to the abutment restoration finish line without interfering with the implant-abutment interface.

The implants were tested again as described above and the baseline slope was subtracted from the test slope after build-up to determine the absolute leakage slope under static conditions and again after the thermodynamic loading to assess the effect after loading. The saline flow was recorded again (Table **2**).

A maximum value of 5.55 hPa/min was allotted for implants deemed incapable of withstanding the initial testing period, which represents the highest slope corresponding to 2.5 ml fluid penetration over the whole testing period.

### 2.3. Bacterial Testing Dynamic Model

The loading system consisted of two tightly separated chambers as with the prior experiment (Fig. **1**). The lower chamber was based on two hard stainless steel parts designed to be interlocked with a screw system and holding the mounted implant sample in between two rubber washers (outer diameter 15mm, inner diameter 10mm and thickness 1mm), which were located on both sides of the mounting disc. The upper chamber was created by an elastic, semi-transparent PVC lever, which was tightened on the lower holder and on the opposing antagonistic side with O-rings. This design allowed for placement of bacterial broth containing a bacterial strain (in the lower chamber), which can change the color of a detection media by hydrolyzing a certain component resulting in turbidity and blackening of the broth in the visible compartment. Conceptually, the bacterial cells can only penetrate through the drilled hole at the apical tip of the implant to reach the implant-abutment interface and then travel to the upper compartment. The antagonist was designed so that it forms a 30 degree angled surface, thereby allowing for exertion a luxation effect on the abutment and simulating a more the clinically relevant situation. The antagonist also contained a drilled hole through which the detection media can be filled prior to being sealed with a rubber piece to form a sealed compartment.

### 2.4. Mounting the Samples for the Dynamic Loading

All samples and parts to be mounted into the test model were individually wrapped in autoclave sterilization bags. Gas sterilization took place utilizing ethylene oxide gas (3M AG, Rüschlikon, Switzerland) in a sterilizer (Sterivac 4XL, 3M AG, Rüschlikon, Switzerland) using the cold sterilization cycle at 37°C for 5,5 hours. All the packs were opened and the assemblies were then made under a clean bench (EVZ 120, SKAN AG, Basel, Switzerland). The lower chamber was initially filled with 1.5 ml of overnight culture of *E. fecalis* ATCC 29212 in fluid universal broth (FUM, Gmür and Gugenheim 1983). The culture was previously adjusted to 1.0 optical density at 550 nm. The implant and the two rubber washers were placed in position in the counterpart and were positioned on top. The two parts were then manually tightened together using pliers. The assembled part was held in a holder against the antagonist with a separating distance equivalent to the value established in the chewing chamber. The elastic semi-transparent lever taken out of finger Cots (PVC medium size, 0.35 mm thick, MUCAMBO – GUMMI Matthias Jacoby, Altrip, Germany) was mounted in position and tightened over the two sides with the O-rings (outer diameter 22 mm, inner diameter 18 mm, thickness 2mm; Fig. **1**, **C**). After completion of this assembly, the upper chamber was filled with a 3 ml of enterococci-selective bile esculin azide broth (Enterococcosel Broth, Difco, Benton Dickinson Co..Sparks, MD, USA) to detect bacterial leakage by inspecting color change. The filling inlet was then sealed with a tight fitting cylindrical shaped rubber component (Fig. **1**, **i**). The assemblies were mounted in the chewing machine and subjected to a computer-controlled mastication; 1'200'000 loadings under a stable water controlled temperature of 37°C. The samples were observed on a daily basis. Due to the slight change in the lever transparency, a light source (Laser class 3R, Intertronic, Interdiscount AG, Switzerland) was applied to improve detection. In the case of no leakage the light penetration through the clear medium resulted in a lamp glow appearance (Fig. **2**, **A**). Whereas when leakage occurred, the pointed light source was reflected on the outer surface and could not penetrate through the darkened turbid medium (Fig. **2**, **B**). At the end of the observation period, aseptic samples were obtained from both chambers (upper and lower) and cultured overnight in bloodagar plates (Colombia agar + 5% Sheep blood, bio Mérieux SA, Marcy l'Etoile, France) in an incubator (IL 115, INCU-Line, VWR, Dietikon, Switzerland) at 37°C to confirm the results and to ensure the involvement of a single bacteria type (i.e. no contamination and the survival in the lower stock chamber in all cases). To exclude any leakage after the thermo-mechanical challenge at the implant-disk interface, the drilled apices of all implants, which showed bacterial leakage under dynamic loading (A n=4 and B n= 6) were sealed again, i.e. they were sandblasted (50 µm aluminum oxide, Benzer-Dental AG, Zurich, Switzerland), further conditioned with Monobond Plus (ivoclar vivadent AG, Schaan, Liechtenstein), adhesivly treated (Clearfil SE Protect, Kuraray America Inc., USA) and closed with a resin build-up (Luxa Core Automix, DMG, Hamburg, Germany). GEPT was determined again given the hypotheses that the original leakage status (baseline) should be achieved again provided that the marginal mounting was still perfectly intact.

### 2.5. SEM Visual Assessment of Implants

Implant systems were assembled and then embedded in epoxy resin (Stycast 1266, Emerson & Cuming, Henkel Eleotronlo Materials, Westerlo, Belguim) and left to set for 24 hours. Afterward, they were sectioned into halves utilizing a slow speed diamond saw (0.4 mm, Strures GmbH, Zweigniederlassung, Switzerland). The hardened resin blocks were mounted in SEM carriers (SCD 030, Balzer Union AG, Balzer-FL) and gold sputtered (Oerlikon Balzers Coating AG, Balzer, Liechtenstein): Sections were coated with a 90 nm gold layer under 0.08 mbar and current of 45 mA over a period of 3 minutes. Implants were observed under SEM (Zeiss Supra V50, Carl Zeiss, Oberkochen, Germany) at magnifications 50X, 500X and 5000X (Fig. **3**).

**Table 2. Implants performance under static and dynamic loading conditions.**

| | Imp. No. | Effective leakage (hPa/min) | Water Volume before (ml) | Time of Bac. Leakage (day) | Influence on leakage After Dyn. Loading (hPa/min) | Water Volume after (ml) |
|---|---|---|---|---|---|---|
| Group A | 1 | 0.004 | 0.000 | No leakage | -0.017 | 0.000 |
| | 2 | 0.002 | 0.000 | No leakage | 0.001 | 0.000 |
| | 3 | 0.068 | 0.042 | No leakage | -0.066 | 0.000 |
| | 4 | 0.004 | 0.000 | No leakage | 0.003 | 0.000 |
| | 5 | 5.531 | 2.500 | 1 | -4.900 | 0.258 |
| | 6 | 5.53 | 2.500 | 1 | 0.000 | 2.500 |
| | 7 | 0.189 | 0.096 | 1 | -0.030 | 0.000 |
| | 8 | 5.503 | 2.500 | 1 | -5.325 | 0.089 |
| | *9 | 0.025 | 0.000 | No leakage | 0.002 | 0.000 |
| | *10 | 0.027 | 0.000 | No leakage | -0.002 | 0.000 |
| Group B | 1 | 0.008 | 0.000 | No leakage | 0.010 | 0.000 |
| | 2 | 0.009 | 0.000 | No leakage | 0.016 | 0.000 |
| | 3 | 0.026 | 0.000 | No leakage | 0.014 | 0.000 |
| | 4 | 0.008 | 0.000 | No leakage | 0.008 | 0.000 |
| | 5 | 0.020 | 0.000 | No leakage | 0.010 | 0.000 |
| | 6 | 0.014 | 0.000 | No leakage | 0.030 | 0.000 |
| | 7 | 0.004 | 0.000 | No leakage | -0.005 | 0.000 |
| | 8 | 0.008 | 0.000 | No leakage | -0.007 | 0.000 |
| | *9 | 0.002 | 0.000 | No leakage | 0.001 | 0.000 |
| | *10 | 0.016 | 0.000 | No leakage | -0.003 | 0.000 |
| Group C | 1 | 0.048 | 0.036 | 1 | 5.463 | 2.500 |
| | 2 | 0.007 | 0.000 | No leakage | 0.025 | 0.000 |
| | 3 | 0.338 | 0.161 | 2 | -0.300 | 0.000 |
| | 4 | 0.005 | 0.000 | 2 | 5.509 | 2.500 |
| | 5 | 5.531 | 2.500 | 1 | 0.000 | 2.500 |
| | 6 | 0.042 | 0.026 | No leakage | -0.018 | 0.000 |
| | 7 | 0.151 | 0.076 | 1 | 0.014 | 0.073 |
| | 8 | | 2.500 | 1 | 0.000 | 2.500 |
| | *9 | 0.013 | 0.000 | No leakage | -0.002 | 0.000 |
| | *10 | 0.001 | 0.000 | No leakage | -0.001 | 0.000 |

Table 2: Detailed implant test performance. * Implants served as negative controls

### 2.6. Statistical Analysis

GEPT performance data, mean values and standard deviations, were assessed prior to and following dynamic loading. An ANOVA was applied to test for significance between systems at each stage of testing. Additionally, a Dunnett post-hoc analysis was conducted to isolate the differences. While bacterial leakage was presented by means of days; exact test of Fisher was applied to compare between different implant systems.

## 3. RESULTS

Before dynamic loading the effective leakage of the three implant systems was (mean ± sd) 2.104 ± 2.831 for group A, 0.012 ± 0.007 for group B, and 1.456 ± 2.516 for group C. After dynamic loading the values were; group A 0.826 ± 1.921, group B 0.049 ± 0.017 and 2.814 ± 2.925 for group C (Fig. **4**). An ANOVA resulted in an overall difference only after dynamic loading (p-value 0.034). A Dunnett post-hoc analysis with group C as a control group shows that the

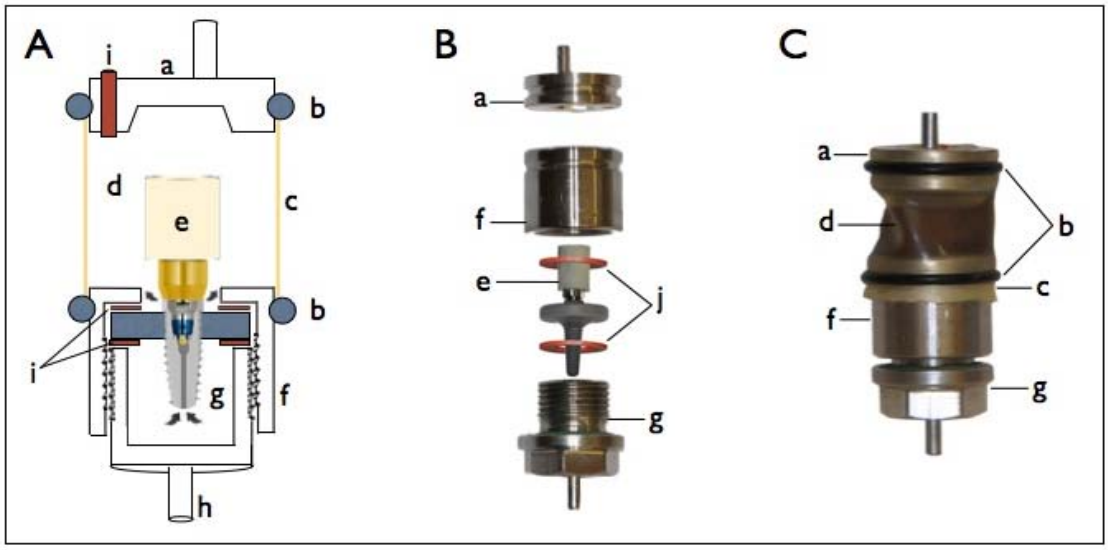


**Fig. (1).** Schematic illustration of dynamic loading set up (**A**), photo of the different components prior to assembly (**B**) and fully assembled set-up (**C**). **a**. Antagonist, **b**. Tightening O-rings, **c**. Elastic semi-transparent lever, **d**. Upper compartment holding the indication medium, **e**. A mounted implant sample, **f**. Capping holder of lower chamber, **g**. Lower chamber compartment with screw third for tightening, **h**. Mounting holder for chewing machine cell, **i**. Indicating medium filling inlet, **j**. Sealing rubber washers.

difference is mainly due to the significant lower average leakage value of group B compared to group C (p-value 0.023).

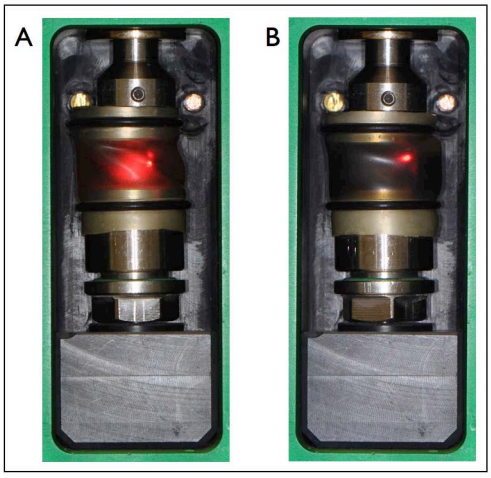


**Fig. (2).** Visual comparison of bacterial leaking vs. tight implant. (A) Tight implant and (B) leaking implant.

Bacterial leakage did not occur for any of group B implants, while 4 of the group A implants showed leakage after 1 day. Also, 4 of the group C implants showed early leakage after 1 day and 2 of the implants leaked after 2 days. The exact test of Fisher was applied to the corresponding 3 by 3 contingency table of leakage (no leakage, leakage after 1 day, leakage after 2 days) with the three-implant systems. The p-value was 0.009 in favor of group B.

## 4. DISCUSSION

The current study aimed to establish a protocol for testing implants under dynamic conditions using a validated gas-enhanced permeation testing method [5]. The protocol included the pre-evaluation of the implant seal status under static conditions before loading, which served as a baseline value. It was hypothesized that a tight implant under static conditions would also show a tight seal under dynamic conditions. This was corroborated by the findings of the present study where initially tight implants also showed a better sealing behavior under dynamic loading. Although no statistical significance could be found between different implant system designs before loading, there was a statistically significant difference observed after loading between group B and group C (p-value 0.034). The findings under static conditions are in contrast to a previous study, which showed significant differences between the groups [5]. However, the trends obtained in the present study are the same and the fact that no statistical difference could be found is attributable to the lower sample size of the present study, where only 8 implants were used as compared to 16 samples for each group in the previous study. Previous studies showed varying results regarding bacterial leakage under static conditions which ranged from 20-80% for internal hex and 20-60% for taper lock designs [10, 11]. These considerable variations may be related to the differences in study designs. However, once again during the dynamic loading, group C exhibited the highest number of leaking implants, followed by group A (6 and 4 of test implants respectively), while group B showed no leakage. The taper lock design (group A) was the only design which showed some improvement in tightness after dynamic loading (Fig. **4**), but this was not statistically significant. This finding indirectly correlates to a previous study assessing tapered lock implants under dynamic loading, where an increase in the loosening torque after loading was observed [6]. On the other hand, group C, showed an overall increase in leakage as compared to the equivalent flat-to-flat design in group B. The difference in tightness corresponds to the difference in mating surface design. A finite element method to study micro-motions at the implant-abutment interface at different mating surfaces [12] showed higher micro-motions at the polygonal region in a trilobe

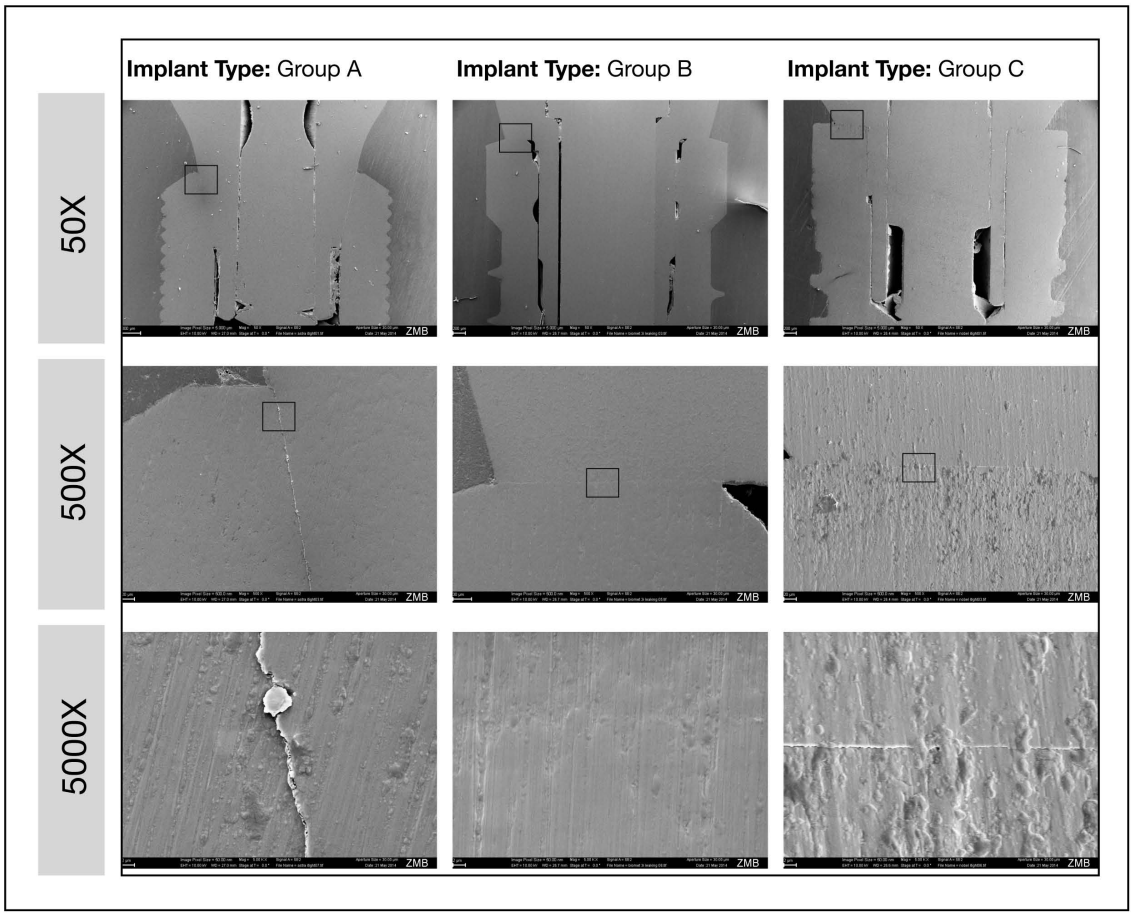


**Fig. (3).** Representative samples of SEM images representing bacterially non-leaking samples with a GEPT score value of less than 0.090 hPa/min. The squared area determines the magnified section in each photo with higher magnification.

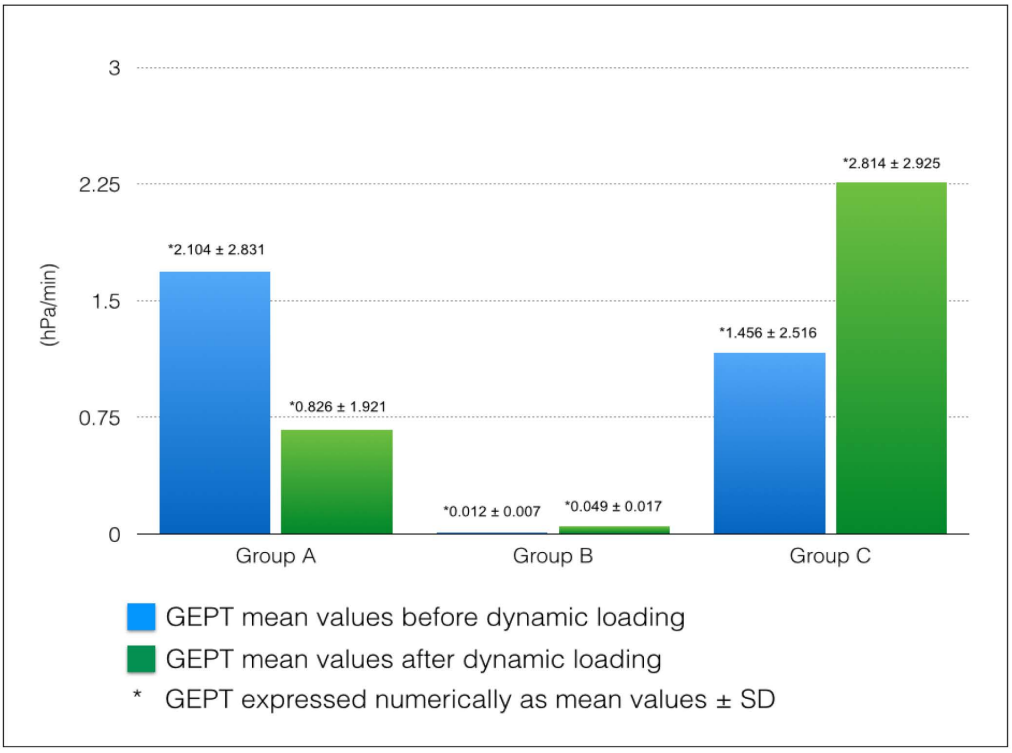


**Fig. (4).** A graph showing a comparison of implants performance before and after dynamic loading presented as mean values ± SD.

design as compared to the micro-motion in a internal hexagonal design. The authors suggested that this fact might therefore lead to more bacterial penetration, which was actually corroborated by the findings of the current investigation.

In the present study, gap analysis was not quantitatively performed, but was used to visualize the situation of implants which showed GEPT values close to the a value of 0.09 hPa/min, which was arbitrarily defined in our previous study to be the cut-off value below which no bacterial leakage occurs [5]. The SEM confirmed the presence of small gaps only and thus the high sensitivity of GEPT measurements in detecting implant leakage over bacterial leakage. If spatial analysis is to be conducted, we suggest 3D analysis as a valuable tool to assess this attribute. The current model setup was the first study in which the non-loaded performance of an implant system was correlated to its performance under dynamic loading conditions. It provided a continuous analysis of implants testing before, during and after dynamic loading. The bacterial leakage model (turbidity detection), per se, has a long history of use in leakage evaluation for both conventional dentistry [13] and implant dentistry [14, 15]. To our knowledge, it is the first time that it was applied in a dynamic model. The ideal model in which bacterial leakage can be directly detected during dynamic loading was challenging. It required replicating the conditions of an isolating split chamber system design, but which also allows direct detection of turbidity through a clear or semi-clear wall on the detection medium side and finally, has an elastic wall which does not interfere with the dynamic loading process. Cell cultures were taken to ensure leakage related to the used bacteria only and to confirm exclusion of any external contamination. In addition, it also proved that the bacteria could survive the whole testing period. Furthermore, the mounting quality after all experimental steps was re-tested: Theoretically, the difference between the GEPT at baseline and at the end of all experiment after re-sealing the implant apices should be zero. Indeed, we found very small differences only ranging from -0.010 to +0.009 hPa/min, which reflects an intact mounting and sealing quality at all times.

Implants failure due to the prosthetic assembly fixed on top depends on the following considerations: Mechanical factors, related to the load applied [16], the abutment retention type (cemented vs. screw retained) [17], and prosthesis retention (cemented vs. screw retained) [18]. The second important factor is the bacterial inhabitation in uncleansable niches [19].

Clearly, studies found that implant failure was correlated to the presence of gaps and their size at the implant-abutment interface [20-22]. Leakage provides an indirect indication of gaps present at the implant-abutment interface and can therefore be considered as a quantitative parameter to assess the quality of the connection at the implant-abutment interface [23]. The long-term survival of implants has been linked to the precision of the overall assembly of dental implant parts and thus the preservation of the surrounding supporting bone level [24, 25]. Nakazato and co-workers 1989 [26] have elaborated bacterial colonization at the prosthetic connector 4 hours after exposure to the oral environment, whereby gaps allowed fluid and bacteria shifts through the implant-abutment interface in both directions. Thus, the presence of gaps at the implant-abutment interface presents a risk factor which jeopardizes the prognosis of an implant [27]. Histological studies highlighting the importance of gap levels in relation to the bone crest demonstrated that the closer the gap was to the bone crest, the higher the risk of peri-implantitis [27, 28]. Quirynen and co-workers 2002 showed that persistent bacterial inoculation of the implant-abutment interface is related to a chronic inflammatory response at the bone crest.

## CONCLUSION

This study elaborated a methodology to investigate the leakage of implant systems under static conditions which highly correlated to implants performance under dynamic loading. Implants with a flat-to-flat interface and internal hexagonal mating surfaces showed the best performance with regard to leakage under both static and dynamic conditions. This study has also provided a proof that tight implants under static conditions will provide better sealing characteristics under dynamic conditions, which in turn highlights the importance and relevance of in-vitro implant system leakage testing under static conditions, as a preclinical assessment parameter. It can also be concluded that the design of the implant-abutment interface and its stability plays a determinant role against bacterial leakage under dynamic loading.

## CONFLICT OF INTEREST

The study design was conceived and executed independently, however the corresponding author PhD fellowship is supported – in part - by Biomet 3i.

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# Evaluation of a novel repetitive gas-enhanced permeation test for restoration leakage determination after thermo-mechanical loading

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ORIGINAL ARTICLE

# Evaluation of a novel repetitive gas-enhanced permeation test for restoration leakage determination after thermo-mechanical loading

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## Abstract

*Objectives*: To assess leakage of class-I restorations using a gas-enhanced permeation test (GEPT) as compared with conventional SEM or dye analysis. *Materials and methods*: Pressure differences over time and penetrating water volumes were measured simultaneously in a two-chamber system (GEPT) before and after class I cavity preparation in 30 molars. Ten teeth were restored with a composite restoration without bonding (A1), a composite restoration with bonding (A2) or a ceramic indirect restoration (B). Five intact teeth served as controls (C). Another GEPT measurement was performed and impressions were taken. Teeth were subjected to thermodynamic loading (1 200 000 cycles) and final GEPT measurements and impressions were made. SEM evaluation of the marginal continuity was performed and teeth were subjected to a Fuchsin dye penetration test. Spearman's rank test was used to compare results from different tests. *Results*: The GEPT and SEM values did not correlate before loading (0.359, $p = 0.051$), but significantly correlated afterwards (0.662, $p < 0.0001$). The correlations between the Fuchsin dye penetration test and GEPT and SEM surface marginal analysis were significant (0.777 and 0.534, $p$-values $< 0.0001$ and 0.002, respectively). *Conclusions*: SEM marginal analysis was mainly limited in reflecting the surface restoration integrity. GEPT evaluation may, therefore, serve as a tool to non-destructively assess restoration sub-surface integrity over time. *Clinical relevance*: The current study provided proof that restoration margin quality does not necessarily reflect its leakage behaviour.

**Keywords:** *Thermodynamic loading*, *leakage*, *marginal analysis*, *restoration*

## Introduction

Restoration marginal integrity is crucial for predictable long-term clinical success, especially of adhesively placed restorations [1]. In this context, polymerization shrinkage reflects a major problem and may initiate early failures of the restoration–tooth interface, resulting in interfacial gaps [2,3]. This might lead to a decreased marginal quality and consequently to microleakage, post-operative sensitivity, marginal discolouration and secondary caries [4]. Therefore, restoration quality improvement by material-related and technical means remains an important aspect in pre-clinical dental research.

Microleakage evaluation can be defined and classified according to the type of substrate used to study the penetration processes, e.g. air, bacterial, fluid, or molecular or ion penetration within the tooth–restoration interface [5]. *In vitro* air testing to assess restoration integrity was introduced in 1912 [6] when compressed air was forced through roots and bubble development at the tooth–restoration interface on the coronal side was observed with a microscope, which indicated restoration leakage. The same principle using compressing dyes was later implemented to assess sealability [7]. These methods were able to determine the quality of restorations by detecting the time point by which the leakage started.

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To evaluate the performance of the surface margin morphology, replica techniques were also established to screen the complete marginal circumference of restorations under SEM in order to describe and quantify the quality of dental restoration margins [8]. This method, however, was limited to the evaluation of the surface conditions only. Overall, the marginal adaptation must be regarded as an important area to study surface and sub-surface quality, which remain the main areas for bacterial retention. Increased bacterial retention at defective interfaces can lead to secondary caries development [9].

Due to the limitation of the visual assessment of restoration marginal adaptation, dye penetration models were developed to assess the penetration depths of fluids within defective intra-coronal interfaces in order to score the extension of dye perfusion in the dentinal tubules system within sectioned samples [10]. The later technique also bears some limitations, mainly the aggressive cutting of specimens and the ability to evaluate the margin only at a single time point, thus preventing understanding of the leakage process.

Unfortunately, analyses of the interactions among adhesive and resin composite materials, such as marginal analyses or dye penetration, do not necessarily correlate with clinical findings [11]. However, such *in vitro* tests are important to screen materials and techniques and to compare results before use in the clinic, taking into account the possible limitations of such set-ups.

It has been conceivably demonstrated that, if leakage occurs, bacteria, along with their by-products and irritants, will follow the path of the dentinal tubules into the pulp, which could be one possible cause of hypersensitivity, pulpal inflammation, or even pulpal death [12]. Recently, a gas-enhanced permeation test (GEPT) method allowing for fluid infiltration and pressure difference determination under standardized conditions was introduced for dentin fluid infiltration [13]. This system allows multiple non-destructive leakage measurements after different treatments, e.g. restoration placement, with high precision and reproducibility. To our knowledge, no study yet conducted has correlated surface marginal adaptation with leakage after thermodynamic loading using such an approach. Therefore, this study aimed to compare the outcomes of the three testing models, namely the novel GEPT, SEM surface marginal analysis and Fuchsin dye penetration tests, when applied to the same set of samples. For this purpose, standardized class-I restorations of different interface qualities were assessed and correlations between the restoration surface quality of the margins and the leakage were established. It is hypothesized that a positive correlation exists between the amount of leakage (assessed by GEPT and Fuchsin dye penetration) and the surface marginal quality of the restoration assessed by SEM.

## Materials and methods

Three test methods were used and compared with the same set of teeth without affecting the integrity of the sample embedding (Figure 1). For this purpose, 35 third molars were selected from the department collection of teeth of known age. The teeth were extracted from patients aged 18–21 years for reasons not related to the study and were stored in 0.2% thymol at a temperature of 5 °C. To be included in this study, the teeth had to be free of caries and cracks and have incomplete root formation with a wide pulp chamber to ensure dentinal tubule patency. The teeth were randomly allocated into three test groups ($n = 10$) and one control group using a randomization program (www.randomizer.org) ($n = 5$).

### *Embedding procedures*

Samples were embedded in brass rings with an outer diameter of 15 mm, an inner diameter of 10 mm and a thickness of 3 mm. The inner surface was sandblasted with aluminium oxide with a particle size of 50 μm (Benzer-Dental AG, Zurich, Switzerland). A conventional light-cured nail build-up material was used for fixation; primer, build up gel and glaze (Sina, Shenzhen Cyber Technology Ltd, Mainland, China) [13]. After primer application, samples were incubated for 2 min in a light-curing unit (Spectramat, Ivoclar Vivadent, Schaan, Liechtenstein). All parts then were assembled in a custom-made silicone putty carrier (Optosil, Heraeus Kulzer GmbH, Hanau, Germany). The nail build up gel material was applied to seal the space between the ring and the tooth sample. The gel material was extended to cover the root surface down to the last millimetre of the root tip. Samples were then light cured again for 4 min. A final layer of glaze material was applied to the top of the sample to improve the embedding and was light cured for another 4 min.

### *Cavity preparation*

Thirty teeth were randomly assigned to one of three test groups (A1, A2 and B; detailed description below) and class-I preparations were prepared using a parallelometer on a XY table (Cendres & Metaux SA, Biel, Switzerland) with a diamond bur with a grit size of 80 μm (Bur 837 KR, 8614, Intensive SA, Grancia, Switzerland). The preparations were 6 mm long (in mesio-distal direction), 3 mm wide (in bucco-oral direction) and 2 mm deep, as measured from the middle fissure level (Figure 1C). Five intact teeth without preparation served as negative controls (C).

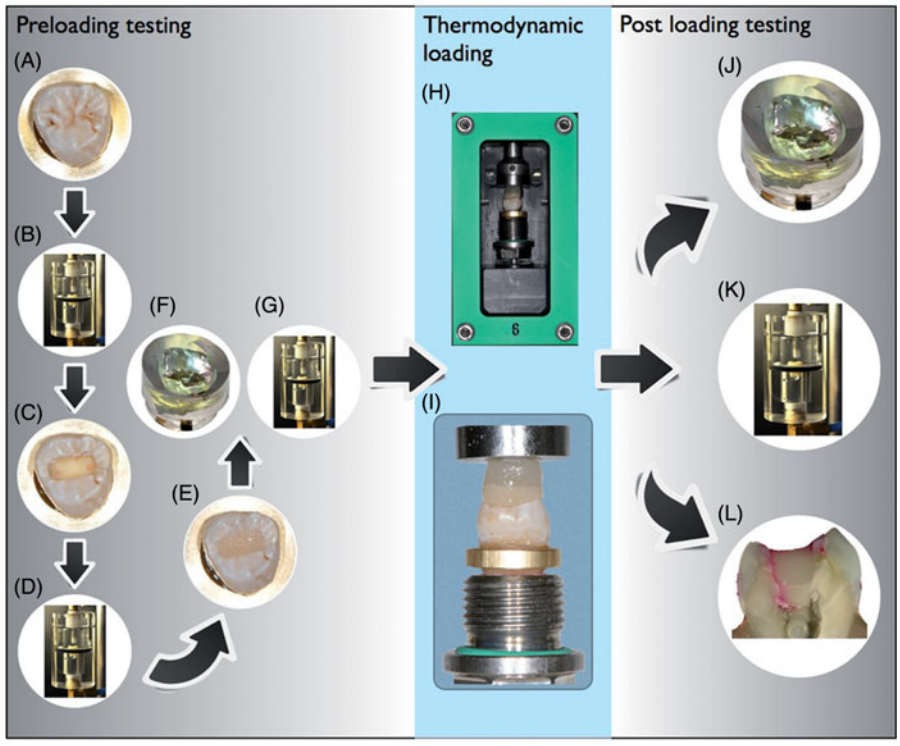


Figure 1. Overview of the different testing phases: after mounting of the samples (A), GEPT measurements were taken (B) and preparations were drilled (C). GEPT was re-assessed (D) and restorations were placed (E). Leakage was determined by SEM (F) and GEPT (G). Thermodynamic loading was performed in a loading chamber (H, I) and the final evaluation was made with SEM surface marginal analysis (J), GEPT (K) or Fuchsin dye penetration testing (L).



*Restorative treatments*

*Group A1: Composite restoration without bonding*

Teeth in this group were restored with resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) without any acid etching or bonding procedures, i.e. without any priming and bonding. The resin composite material was applied in three horizontal increments, which were polymerized for 20 s at 800 mW/cm$^2$ (Bluephase LED G2, Ivoclar Vivadent). Finishing of the restoration took place using specially designed finishing burs (Intensiv SA, Grancia, Montagnola, Switzerland) and polishing discs (Sofflex discs, 3M ESPE D) under a stereomicroscope (Stemi 1000, Zeiss, Oberkochen, Germany).

*Group A2: Composite restoration with bonding*

Restorations were placed as they were in group A1, but with an etch and rinse approach using a 3-step adhesive system (Syntac Classic, Ivoclar Vivadent) prior to composite placement. The enamel was selectively etched for 60 s with 35% phosphoric acid (Ultra Etch, Ultradent, South Jordan, UT) followed by a 40-s wash with water spray. After drying with air, a self-conditioning maleic acid-containing primer (Syntac Primer, Ivoclar Vivadent) was applied for 15 s and gently air-dried before the application of the adhesive (Syntac Adhesive, Ivoclar Vivadent) for 20 s. After gentle air drying, an unfilled bonding resin (Heliobond, Ivoclar Vivadent) was applied for 20 s and light cured for 40 s (Bluephase LED G2). Resin composite (Filtek Supreme, 3M ESPE) was applied in three horizontal increments, which were polymerized for 20 s each. The samples were then finished and polished.

*Group B: Ceramic indirect restoration (inlay)*

Ceramic inlays were designed using a Cerec 4D program, milled with a CEREC MCXL milling unit (Sirona Dental GmbH, Salzburg, Austria) utilizing a glass-ceramic material (IPS Empress CAD Multi, Ivoclar, Vivadent).

Teeth were conditioned as described above using the same adhesive system (Syntac Classic, Ivoclar, Vivadent). The ceramic indirect restorations were acid-etched with hydrofluoric acid (Vita Ceramics Etch, Vita Zahn Fabrik, Bad Säckingen, Germany) for 60 s. After extensive water spray application, a silane was applied (Monobond Plus, Ivoclar Vivadent) for 60 s and the ceramic inlay was dried. Then, an unfilled bonding resin was applied (Heliobond, Ivoclar Vivadent) to the inlay base without light curing. Resin composite material

(Filtek Supreme XT, 3M ESPE) was pre-warmed in an oven (AdDent Inc., Danbury, CT) to 37 °C before application to the inlay and the cavity. The inlay was first positioned by finger pressure. Subsequently, ultrasound was applied (mini Piezon, EMS, Nyon, Switzerland) for 10 s to finalize the placement of the inlay and the excess material was carefully removed. Light polymerization was performed from five aspects for 60 s each from the occlusal, mesial, distal, buccal and oral directions.

*The gas enhanced permeation test (GEPT)*

Details of the device and its set-up were described in a previous validation study [13].

In brief, the apparatus consisted of a two-chamber system where a sample was placed in the middle separating the two chambers. The embedded sample was fixed between the two parts using a rubber O-ring with an outer diameter of 22 mm, an inner diameter of 15 mm and a thickness of 3.5 mm, which resulted in two fully separated and hermetically sealed chambers with the embedded sample in between.

The temperature was controlled and constantly held at 35 °C in the inner chamber. This core system was installed in a second larger experimental box in which the temperature was stabilized at 31 °C.

A pressure-difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) was connected to the upper and lower chambers. Readings were recorded with a computer-unit running a proprietary program (V 4.2 SP2, Testo AG, Germany). The O-ring was lubricated with a silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) and the sample was positioned in place in the lower part of the permeability/leakage device and 2.5 ml of a pre-pressurized ($N_2$ gas 860 hPa) 0.9% saline solution was added on top. The upper part was repositioned and the three screws were tightened with a torque-controlled screwdriver. To achieve an effective pressure difference of 1030 hPa, the upper chamber was pressurized with $N_2$ gas to 860 hPa, whereas the lower chamber was negatively pressurized to −170 hPa. The pressure difference readings were initiated and continued over 40 min at a rate of 1 measurement/second. The resulting data were plotted as the rate of pressure change expressed as a drop in pressure difference over time. The slope between pressure value differences at two fixed time points (1200 s and 2400 s) was defined to present the sample leakage status:

$$\text{Slope} = \frac{P2 - P1}{T2 - T1}\text{hPa/min.}$$

where $P2$ is the pressure difference at time point 40 min; $P1$ is the pressure difference at time point 20 min; $T2$ is the time point 40 min; and $T1$ is the time point 20 min.

In addition, the infiltrating physiological saline solution was collected and weighed to calculate the volume that permeated the specimen.

For all samples, measurements were carried out at the following time points:

(a) At baseline, i.e. after embedding but before tooth preparation to assess tight sealing;
(b) After preparation, to determine the maximal leakage through the dentin wound;
(c) After restoration, to measure the restoration leakage value, which is expected to range between (a) and (b); and
(d) After thermodynamic loading.

In general, higher GEPT values implied more leakage, whereas lower values indicated improved tightness.

*Thermodynamic loading*

Samples were transferred to special carriers and embedded without interrupting the embedding disc mounting integrity (Figure 1). For this purpose, the stainless steel carriers had a separate cylindrical compartment (diameter of 11 mm and a depth of 12.5 mm), which was filled with heavy body impression material (3M ESPE Pentamix 2, 3M Deutschland GmbH, Seefeld, Germany). To maintain a space between disc and carrier, a rubber separator 1 mm in height was placed between the embedding disc and the carrier and was later removed. Thereby, any luxation of the disc was avoided and stress was transported only to the root ensuring no effect on the mounting integrity.

Antagonists were fabricated with resin composite material for each sample individually to allow full occlusal contact (Filtek Supreme XT, 3M ESPE).

The samples and their antagonists were mounted in a computer-controlled masticator and subjected to thermodynamic loading; 1 200 000 loadings at 20 N/cm$^2$ and 3000 temperature cycles, 5 °C/50 °C [14].

*SEM surface marginal analysis*

The quality of restoration margins was studied before and after the thermodynamic loading. After the restoration placement, occlusal surfaces were cleaned with alcohol, rinsed with water spray and dried with air. Impressions were made using low viscosity silicon impression material (President plus jet light body, Coltene, Altstätten, Switzerland).

After 24 h, epoxy resin was poured into the impressions (Stycast 1266, Emerson & Cuming, Henkel Eleotronlo Materials, Westerlo, Belguim), and 24 h later, the casts were trimmed and mounted on SEM holders (SCD 030, Balzer Union AG, Balzer-FL). The mounted samples were dried for another 24 h. With the aid of a sputtering device

(Oerlikon Balzers Coating AG, Balzer, Liechtenstein), casts were coated with a 90-nm gold layer under 0.08 mbar and current of 45 mA for 3 min.

The replicas were then analysed at a 200-fold magnification for gap presentation. A gap was defined as a pronounced defect in the continuity between the tooth and restoration surfaces, where the floor of the defect was non-detectable (Figure 2B). The total margin of the restoration was analysed in steps using a scanning electron microscope (SEM; Carl Zeiss Supra 50 VP FESEM, Carl Zeiss, Oberkochen, Germany). The total visual quality of the restoration margin was presented for each sample as a percentage of discontinuity, i.e. the percentage of defective restoration margin [8]. The surface marginal analysis was performed by one blinded and calibrated operator. The repeatability of identical samples at different time intervals (2 weeks) was 91%. The criteria for the marginal assessment were as follows:

(1) Perfect margin: No visible interruption of the interface continuity, i.e. no different levels visible.
(2) Marginal gap: the interface showed discontinuity, e.g. cracks or gaps.
(3) Non-assessable areas were defined as any deviation from the above-mentioned criteria. Non-assessable areas were mainly the result of impression inaccuracies and were mainly derived from bubbles, excess material, debris or contaminations at the restoration-tooth interface, which hampered the clear visualization and judgement of the respective margins.

All restorations were assessed before and after thermodynamic loading.

### *Fuchsin dye penetration test*

The Fuchsin dye penetration test was completed at the very final stage of the evaluation series described above because it required sectioning of the samples. Teeth were carefully demounted from the embedding medium for this purpose and were circumferentially sealed up to 1 mm from the restoration margin with nail varnish (Cover Girl, Nail slicks, Procter and Gamble, OXP, UK) and the samples were immersed in 0.5% basic Fuchsin dye solution for 20 h.

Under kerosene cooling, teeth were then sliced in the bucco-lingual direction utilizing a slow speed diamond saw (0.4 mm, Strures GmbH, Zweigniederlassung, Switzerland). In total, four sections were prepared for evaluation, which were photographed at a 25-fold magnification and digitized. Samples were dichotomously categorized as 'non-leaking' (= 0) when the dye did not reach the pulp chamber or 'leaking' (= 1) when the dye reached the pulp chamber (Figure 2C). All sections were independently evaluated by two blinded investigators. In cases of disagreement, sections were reassessed and discussed until an agreement was reached.

### *Statistical analysis*

Descriptive statistical analyses were completed separately for the three restorative treatments for the GEPT test (before and after the thermodynamic loading), the SEM surface marginal analysis (before and after the thermodynamic loading) and the Fuchsin dye penetration test (only after the thermodynamic loading). For the negative control, a descriptive analysis of the GEPT test and the Fuchsin dye penetration test (after the thermodynamic loading) was applied (Table 1). The following tests were applied to assess different statistical outcomes. The Kolmogorov-Smirnov test was applied to assess normality in the data distribution. To compare all test outcomes within the same treatment group before and after thermodynamic loading, the Wilcoxon signed rank test was applied. The Kruskal-Wallis test was used to compare different tests for before and after thermodynamic loading outcomes between different treatment groups. In order to further assess where differences appear, the Mann-Whitney U-test was applied. Finally, for all respective correlations, a Spearman's rank correlation test was applied. A significance level (probability for type I error) of 0.05 was used with the two-sided $p$-values.

## Results

For the GEPT results, the assumption of a normal distribution was rejected for all groups before ($p$-values 0.003, 0.027 and 0.013 for groups A1, A2 and B) and nearly all groups after the thermodynamic loading ($p$-values = 0.009, 0.200, 0.000, 0.026 for groups A1, A2 and B and negative control) by a Kolmogorov-Smirnov test. For the SEM surface marginal analysis, normality was never rejected. Nevertheless, due to the small group sizes, only non-parametric tests were used.

A comparison of the values before and after thermodynamic loading (separately within each group) revealed that only the GEPT results in group A1 were significantly different (Wilcoxon signed rank test, $p$-value = 0.016) and were improved after thermodynamic loading. For all other groups (A2, $p$-value = 0.084 and B, $p$-value = 0.129) as well as for the SEM surface marginal analysis (A1 $p$-value = 0.114, A2 $p$-value = 0.139, B $p$-value = 0.169), no significant changes were observed. For the negative control, this comparison was not meaningful.

A further examination showed that for GEPT and SEM surface marginal analysis (before and after the thermodynamic loading), the results among groups A1, A2 and B (and negative control for GEPT after thermodynamic loading) differed

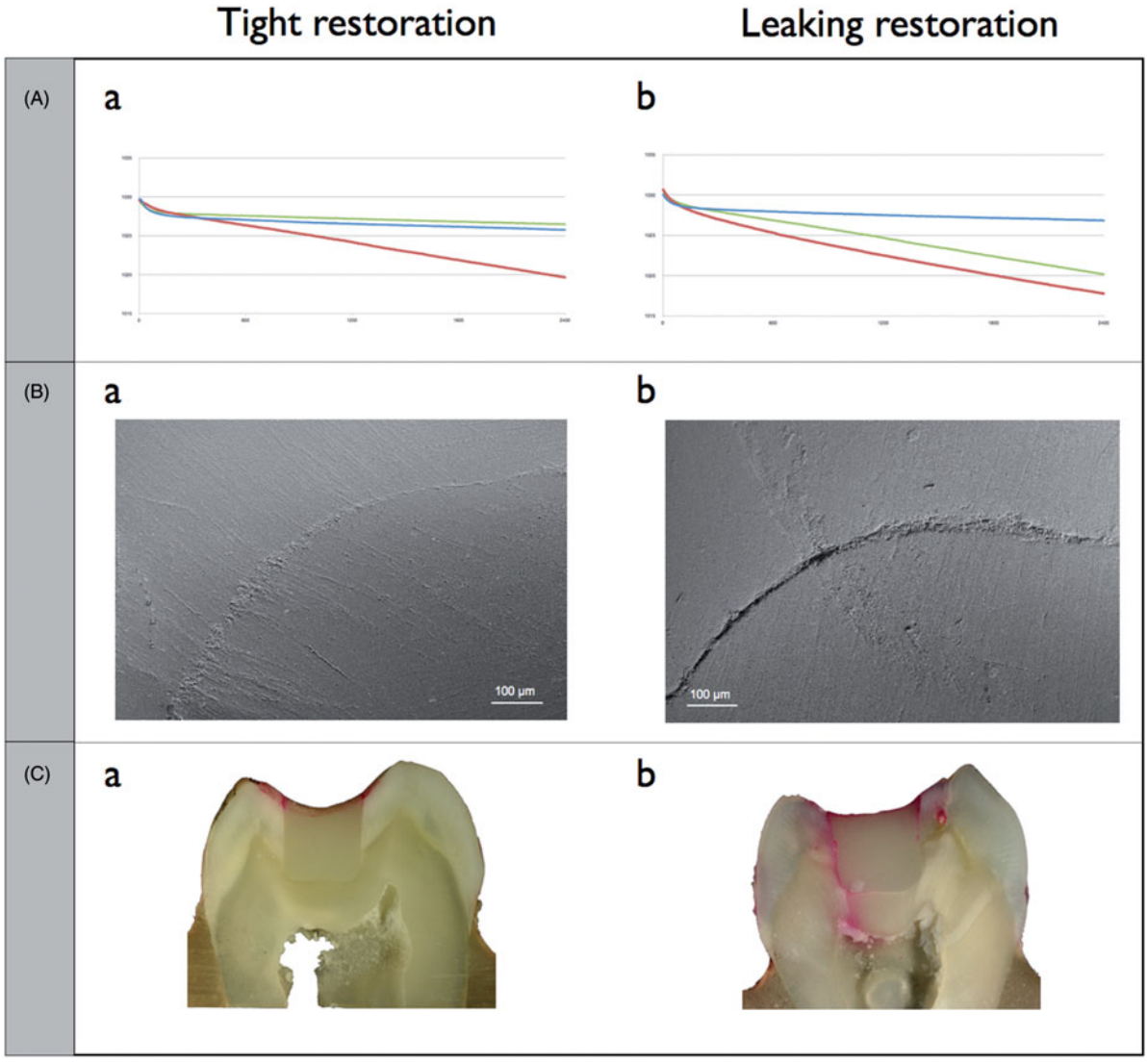


Figure 2. Illustration of the results of the three test methods (left: 'non-leaking', right: 'leaking'): GEPT evaluation with representative baseline pressure curves (A; blue = baseline, red = after preparation and green = after restoration); (B) SEM surface marginal analysis; (C) Fuchsin dye penetration test.

Table I. Results of the different test methods with regard to the respective treatment groups.

| Group | Before thermodynamic loading | | After thermodynamic loading | | |
|---|---|---|---|---|---|
| | GEPT hPa/min | SEM marginal defect analysis (%) | GEPT hPa/min | SEM marginal defect analysis (%) | Fuchsin (% of samples with dye reaching pulp chamber) |
| *Group A1* (Composite restoration without bonding) | $0.431 \pm 0.449^{A}$ | $18.9 \pm 9.2^{a}$ | $0.131 \pm 0.076^{A*}$ | $26.7 \pm 11.0^{a}$ | $80.0^{A}$ |
| *Group A2* (Composite restoration with bonding) | $0.074 \pm 0.020^{B}$ | $15.2 \pm 9.8^{ab}$ | $0.065 \pm 0.014^{B}$ | $11.2 \pm 6.5^{b}$ | $10.0^{B}$ |
| *Group B* (Ceramic indirect restoration) | $0.065 \pm 0.010^{B}$ | $3.6 \pm 4.3^{b}$ | $0.060 \pm 0.008^{B}$ | $5.7 \pm 4.4^{c}$ | $0.0^{C}$ |
| *Group C* (Negative control) | $0.062 \pm 0.005^{B}$ | – | $0.064 \pm 0.005^{B}$ | – | $0.0^{C}$ |

Test results are presented as mean values and standard deviations when applicable.
Different superscript capitals represent statistically significant differences in GEPT measurement/Fuchsin dye penetration, between the different treatment groups ($p < 0.05$; read vertically). Different superscript lower case letters represent statistically significant differences in SEM assessment between the different treatment groups ($p < 0.05$; read vertically). Asterisks indicate statistically significant change in the measured after thermodynamic loading value compared to the before thermodynamic loading measured value of a respective treatment group ($p < 0.05$; read horizontally).

significantly (Kruskal-Wallis tests). To see, where the differences appeared between groups, pairwise group comparisons were made using Mann-Whitney U-tests. The GEPT results between groups A1 and A2 were significant before or after loading or by SEM surface marginal analysis after thermodynamic loading. For groups A1 and B, all tests showed significantly different outcomes. For A2 and B, only SEM

surface marginal analysis after thermodynamic loading showed significant differences.

The Fuchsin dye penetration test results were significantly different among the four groups (Fisher's exact test, $p$-value $< 0.0001$). This finding was mainly due to the data from group A1 where a high number of leakage scores were observed (eight out of 10).

Finally, we looked at the correlation among the tests (globally over all groups). Spearman's rank correlation was used to correlate the different test results. For GEPT and SEM surface marginal analysis before thermodynamic loading, the correlation was only moderate (0.359) and not significant ($p$-value $= 0.051$), while it was significant after thermodynamic loading (0.662, $p$-value $< 0.0001$). Also, the correlations between the Fuchsin dye penetration test and GEPT and SEM surface marginal analysis (after loading) were significant (0.777 and 0.534, $p$-values $< 0.0001$ and 0.002), with a higher level of significance observed for the GEPT evaluation technique.



## Discussion

This study aimed to evaluate a new gas-enhanced leakage measurement system in comparison to a traditional SEM surface marginal analysis and a sub-surface Fuchsin dye penetration test. It was hypothesized that leaking restorations—as measured by the GEPT method—should, therefore, also display poorer marginal adaptation and an increase in their dye penetration profiles. This could be corroborated by the findings of the present study, where a significant correlation was found between the Fuchsin dye penetration test—considered as a gold standard in displaying the liquid penetration leakage pathways [15]—and the GEPT and the SEM. Although this correlation was significant, the SEM, which stands for the surface marginal and maybe sub-surface analysis, was shown not necessarily to represent the true performance of the restoration, especially before loading, which coincides with other studies where this evaluation method was judged to have controversial clinical relevance [11,16,17] and may have resulted in false negative conclusions with regard to tracer penetration and possibly caries formation [15]. Heintze et al. [18] compared SEM quantitative marginal analysis data with the penetration depth of the three most commonly used tracers for microleakage in Class II fillings *in vitro*, i.e. fuchsin, silver nitrate and methylene blue. In their study, teeth were subjected to occlusal loading and simultaneous thermodynamic loading in a comparable protocol as in the present study and the percentage of continuous margin of the cervical dentin and enamel was evaluated on replicas using SEM. They concluded that tracer penetration showed a moderate correlation with SEM quantitative marginal analysis at dentinal margins, but not at enamel margins. It must be highlighted at this point that a class I defect was prepared in the present study with all margins located in the enamel. Therefore, it may not be surprising that the correlation in our study was not significant before loading and became only significant after loading, but with a still rather low correlation. Nevertheless, despite the fact that the dye penetration method displays a high detection limit, it cannot assess and compare the exact effect of thermodynamic loading on restorations as this technique allows for a single time point measurement only because it requires sectioning of the samples.

In this context, the GEPT method may be a valuable measuring tool, as it displays a high sensitivity for detecting leakage without destroying the sample and also shows a low detection limit of 0.002 hPa/min for the pressure slope and 0.0225 µl/min for the fluid infiltration [13]. This was confirmed by the highly significant correlation with the Fuchsin dye penetration test. Unlike previous set-ups where the dye and fluid infiltration was applied in a reverse direction [7,19,20], the current study applied the normal possible direction of leakage (out-in) and, therefore, simulated the clinical situation more accurately where leakage is expected to occur from the oral cavity towards the pulp. This was also highlighted in a recent publication, which assessed the combined effect of cyclic loading and bacterial exposure on bacterial penetration at the interface between dentin and resin composite restorative material using a novel bioreactor system [21]. The study showed that gaps, which were as small as 15–30 µm, were enough to allow bacterial leakage to the full depth under thermodynamic loading. This fact highlighted the necessity of assessing leakage and also provided a new model, which was able to detect bacterial penetration and demineralization at the same time. However, this method also presented a limitation in its capability to allow multiple evaluation methods to compare and correlate results because this would require sectioning of the samples again. GEPT tries to overcome this problem, but other options for evaluation remain open for scrutiny.

When focusing on the different restorative treatments, the unbonded composite restorations showed higher GEPT values than did the bonded composite restorations and the adhesively placed ceramic indirect restorations. Surprisingly, the GEPT values improved after thermodynamic loading, which could be explained—in part—by some occlusion of the dentinal tubules by a dynamic frictional smear layer production. Another possibility could lie in the hygroscopic effects after fluid uptake [22].

As expected, both bonded restoration groups performed significantly better in contrast to the

non-bonded group at each evaluation stage, which underlines the importance of adequate bonding to ensure a tight and durable restoration interface. Overall, the results of the marginal quality evaluation corresponded to those in previously published studies that assessed marginal quality after loading with comparable evaluation techniques, i.e. SEM surface marginal analysis and dye penetration test [23,24]

The excellent performance of the negative control group before and after loading proved the adequate sample embedding procedures under thermodynamic loading conditions. A previous study using the same set-up, but under static conditions, also showed that repetitive measurements of identical samples resulted in reproducible readings [13].

## Conclusion

- Restorations with visually detectable deteriorated margins do not necessarily present higher subsurface leakage than do restorations with visually well-adapted margins.
- While SEM is a suitable method to judge surface marginal adaptation, it does not necessarily display the real leakage status of a restoration.
- The described GEPT method seems to be a suitable non-destructive approach to study the leakage behaviour of restorations and may, therefore, display interesting insights into leakage status.
- Bonding quality remains a determinant factor in the ability of a restoration to prevent leakage.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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