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LOAD-BEARING COMPOSITE FRACTURE-FIXATION DEVICES OPTIMIZED WITH TAILORED FIBRE PLACEMENT

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ABSTRACT

Oliver Liesmäki: Load-bearing composite fracture-fixation devices optimized with tailored fiber placement
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Metallic fracture fixation devices have been the gold standard of internal fracture treatment since their introduction to clinical practice in the late 19th century. While metallic devices are a satisfactory treatment modality for fracture treatment, their utilization has a few intrinsic drawbacks. Biomechanically, their flexural stiffness is typically significantly higher than that of cortical bone. Applying such plates potentially causes mechanical underloading of the bone leading to adverse bone remodeling and complications of the bone healing. The problem is emphasized in veterinary orthopaedics where metals are the main material option for fracture fixation devices.

Fibre reinforced composites (FRCs) are an attractive material option for loadbearing orthopaedic devices. This is due to their good fabricability to match the biomechanical properties of cortical bone by combining lower elastic modulus than that of metals with high flexural strength of bone. Careful fabrication of FRCs also provides good biocompatibility, favorable osseointegration, and adequate wear resistance.

The aim of this thesis was to develop a novel FRC-based fracture fixation plate for antebrachial fractures in toy-breed dogs. Isoelastic properties with cortical bone were aimed to achieve by utilizing tailored fibre placement (TFP) pattern determined and optimized using finite element analysis (FEA) as a main tool. The plates investigated in this study consisted of bisphenol A glycidylmethacrylate-based matrix reinforced by E-glass fibres. A pilot version of TFP plate was mechanically tested with a control plate containing continuous unidirectional fibre reinforcement. The mechanical tests included four-point bending of the plates along with four-point bending and torsion tests conducted on osteotomized chicken tibiae reunited with a plate and metallic screws. Based on simulations of the mechanical tests, the structure of pilot TFP plate was iteratively optimized by FEA. Consequently, the mechanical tests were conducted on the optimized TFP plate designs with the biostable and a semiresorbable consistency comprising polylactide (PLA) matrix and PLA/E-glass hybrid fibres. These tests were followed by a final FEA assessment.

The maximum torque resistance of the optimized TFP plates (0.82 N·m) was statistically significantly higher than that of plates with unidirectional fibres (0.25 N·m). However, bending strength of 1.03 N·m in the plates with unidirectional fibres was superior to that of other plates. Bending strength of the optimized TFP plate (0.343 N·m) was still acceptable for its indication. Optimization was successful in reducing the critically loaded volume within the plate. Fibre volume distribution of the plates was improved, providing uniform thickness of the plate.

Keywords: fibre-reinforced composite, veterinary medicine, orthopaedics

TIIVISTELMÄ

Oliver Liesmäki: Räätälöidyllä kuituorientaatiolla optimoidut kuormaa kantavat murtumafiksaatiolaitteet
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Metalliset murtumafiksaatiolaitteet ovat olleet ensisijainen valinta sisäisessä murtumien hoidossa 1800-luvun lopulta lähtien. Metalliset laitteet ovat tyydyttävä hoitomuoto murtumien hoidossa, mutta niiden käyttöön liittyy olennaisia haittoja. Biomekaniikaltaan niiden taivutusjäykkyys on tyypillisesti merkittävästi suurempi kuin tiivisluun. Kyseisten levyjen käyttö johtaa mahdolliseen luun epäfysiologiseen alikuormittumiseen, mikä puolestaan altistaa luun pois sulautumiselle ja luutumisen komplikaatioille. Ongelma korostuu eläinlääketieteessä, jossa metallit ovat pääasiallinen materiaalivaihtoehto murtumalaitteissa.

Kuitulujitteiset komposiitit (FRC) ovat lupaava materiaalikandidaatti kuormaa kantavien ortopedisten implanttien valmistukseen. Tämä johtuu niiden hyvästä muokattavuudesta vastaamaan biomekaanisilta ominaisuuksiltaan kortikaalista luuta, jolloin niissä yhdistyy metallisia vaihtoehtoja matalampi kimmokerroin ja luun korkea taivutuslujuus. Komposiittien huolellinen valmistaminen mahdollistaa myös niiden hyvän bioyhteensopivuuden, suotuisan integraation luuhun ja adekvaatin kulutuskestävyyden.

Tämän työn tavoitteena oli kehittää uudenlainen FRC-pohjainen murtumalevy kääpiökoirien kyynärvarren murtumien hoitoon. Tiivisluun biomekaanisia ominaisuuksia tavoiteltiin levyn räätälöidyllä kuituorientaatiolla (TFP), jonka määrittämisessä ja optimoinnissa hyödynnettiin elementtimenetelmää pääasiallisena työkaluna. Työssä tutkitut levyt koostuivat bisfenoli-A-glysidyylimetakrylaatti-pohjaisesta matriisista, jota lujittivat E-lasikuidut. Pilottiversio TFP-levystä testattiin mekaanisesti yhdessä yksisuuntaisin kuiduin vahvistettujen kontrollilevyjen kanssa. Tutkimuksen mekaaniset testit kattoivat levyjen nelipistetaivutuksen lisäksi nelipistetaivutuksen ja torsiokokeen katkaisuleikatuilla kanan sääriluilla, jotka oli liitetty yhteen levyn ja metallisten ruuvien välityksellä. Mekaanisten testien simulaation pohjalta TFP-levyn rakenne optimoitiin iteratiivisesti elementtimenetelmää hyödyntäen. Optimoitua TFP-mallia testattiin mekaanisin kokein biostabiilin materiaalikoostumuksen lisäksi osittain bioresorboituvalla rakenteella, jossa polylaktidimatriisi oli lujitettu polylaktidin ja E-lasin yhdistelmäkuiduilla. Näiden testien jälkeen suoritettiin viimeinen elementtimenetelmäanalyysi.

Optimoitujen levyjen maksimaalinen torsion sietokapasiteetti (0,82 N·m) oli statistisesti merkitsevästi suurempi yksisuuntaisin kuiduin vahvistettujen levyjen vastaavaan nähden (0,25 N·m). Yhdensuuntaisesti vahvistettujen levyjen taivutuslujuus 1,03 N·m oli kuitenkin ylivertainen muiden levyjen vastaavaan nähden. Optimoitujen TFP-levyjen taivutuslujuus (0,343 N·m) oli silti asianmukainen niiden käyttöindikaatioon. Optimoinnilla onnistuttiin vähentämään levyn kriittisesti kuormittunutta tilavuusosuutta. Kuitujen tilavuusjakaumaa onnistuttiin parantamaan levyn yhtenäisen paksuuden aikaansaamiseksi.

Avainsanat: kuitulujitteinen komposiitti, eläinlääketiede, ortopedia

PREFACE

I would like to thank assistant professor Niko Moritz and senior researcher Julia Kulkova from Turku Clinical Biomaterials Centre for their supervision, support, and guidance throughout the years with this and our previous projects. I am grateful to doctoral candidate Artem Plyusnin for his cooperation in the study and his meritorious modelling endeavours for this project. I also want to thank assistant professor Oommen Oommen from Tampere University for his guidance and understanding through the course of my studies and this project.

Turku, 26th of May 2020

Oliver Liesmäki

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LIST OF ABBREVIATIONS

ASTM	The American Society for Testing and Materials
BisGMA	bisphenol A glycidylmethacrylate
BMSC	bone marrow-derived mesenchymal stem cell
CAD	computer aided design
CFR-PEEK	carbon fibre-reinforced polyether ether ketone
CT	computed tomography
DMAEMA	dimethylaminoethyl methacrylate
E-glass	electrical grade glass
FRC	fibre-reinforced composite
FEA	finite element analysis
ISO	International Standardization Organization
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell
NF-κB	nuclear factor kappa-B
OPG	osteoprotegerin
PAEK	polyaryletherketone
PCL-PLA	polycaprolactone-co-lactide
PDS	polydioxanone
PEEK	polyether ether ketone
PET	polyethylene terephthalate
PLLA	poly-L-lactic acid
PMMA	polymethylmethacrylate
PTFE	polytetrafluoroethylene
PTH	parathyroid hormone
PU	polyurethane
RANK	receptor activator of nuclear factor kappa-B
RANKL	receptor activator of nuclear factor kappa-B ligand
TEGDMA	triethylene glycol dimethacrylate
UHMWPE	ultra-high molecular weight polyethylene

1. INTRODUCTION

Long bone fracture treatment is one of the most remarkable fields of hard tissue surgery. This has been the case throughout the written history as the earliest recordings of bone setting procedures date back thousands of years (Brorson 2009). For millennia, fracture treatment was limited to external methods that do not penetrate skin. This was due to the restricted selection of methods enabling successful open surgery (Peltier 1990). Consequently, little progress in fracture treatment was made before the first modern standalone external fracture fixation devices were introduced in 1840s (Vidal 1983, Bick 1968). At the end of the 19th century, fracture treatment was revolutionized by the first generation of internal fracture fixation plates (Hansmann 1886).

Since the introduction of the first metallic internal fracture fixation devices, metals have been the golden standard among the materials applied in fracture treatment. Despite all the progress in treatment materials and techniques during the last century, a few fundamental problems especially with fracture sites in loadbearing conditions remain to be solved. The problems are even more emphasized in veterinary medicine among breeds with low bone quality (Farrell 2016).

Conventional rigid metallic fracture fixation devices are a satisfactory treatment modality for fracture treatment. However, their utilization has a few intrinsic drawbacks. Probably most notably, flexural stiffness of metallic devices is typically significantly higher than that of cortical bone. Applying such plate on the bone potentially leads to unphysiological underloading of the bone and to consequent adverse bone remodelling processes referred to as stress shielding (Huiskes et al. 1992, Uhthoff and Dubuc 1971).

The issues concerning utilization of conventional rigid metallic devices have been attempted to be solved by different surgical techniques and material selections (Woo et al. 1983). Fibre reinforced composites (FRCs) are an attractive material option for hard tissue reconstructions. Due to their good wear resistance and tailorability to match the mechanical properties of various body sites, FRC implants with different combinations of constituent materials have been studied in several tissue engineering applications. (Evans and Gregson 1998). Successful clinical application of FRCs in dental restorations

(Vallittu 1999, Karmaker et al. 1997) initiated interest towards their utilization in reconstruction of other tissues (Vallittu 2017, Piitulainen et al. 2015, Aitasalo et al. 2014, Ramakrishna et al. 2001, Evans and Gregson 1998).

As for a biomechanically ideal loadbearing orthopaedic device, two fundamental characteristics are desired. Firstly, the device should promote high loadbearing capacity. Secondly, the device's flexural and torsional stiffness values should be comparable to those of cortical bone. Such devices promote more physiological load sharing between the implant and the bone. This promotes more natural bone healing pattern as opposed to the one achieved by conventional rigid plating techniques (Evans and Gregson 1998, Huiskes et al. 1992). As FRCs promote high strength and lower flexural modulus than that of their metallic counterparts, they serve as potential candidates for loadbearing fracture fixation materials. The efficacy of FRCs in long bone fracture treatment have been shown in animal models (Kettunen et al. 1999, Gillett et al. 1985, Woo et al. 1983, Tayton et al. 1982, Akeson et al. 1975).

Favourable tensile properties and good chemical stability has made glass fibres a commonly utilized reinforcing agent in implantable medical FRCs (Vallittu 2017, Abdulmajeed et al. 2014, Aitasalo et al. 2014, Moritz et al. 2014, Abdulmajeed et al. 2011, Vallittu 1999, Evans and Gregson 1998). Carefully fabricated thermosetting polymers, such as the ones based on dimethacrylate, can be applied as composite matrix with good biocompatibility (Bae et al. 2012). Along with biocompatibility, osteoconduction and osteoinduction are other desired properties of an ideal orthopaedic device. These phenomena imply growth of bone tissue along material surfaces and induction of bone formation, respectively (Albrektsson and Johansson 2001). FRC implants can be fabricated to promote these properties by supplementing them with agents such as bioactive glass or changing their surface properties. Such bisphenol A glycidyl methacrylate (BisGMA) - based FRCs have been successively applied in non-loadbearing devices (Vallittu 2017, Piitulainen et al. 2015, Aitasalo et al. 2014) as well as in loadbearing conditions in animal models (Moritz et al. 2014, Zhao et al. 2009).

In this study, an FRC plate with unidirectionally oriented reinforcing fibres was used as control. Such highly anisotropic material possesses high flexural strength along the axis orthogonal to the plane of fibre layers. However, such plate design has a few intrinsic drawbacks in clinical applications. Drilling the screw holes through fibre layers exposes the fibre ends to abrasion with the screws. This potentially leads to fibre debris formation and to consequent inflammation of the surrounding tissues. The debris formation was a

leading reason for clinical failure of, for instance, the earliest total hip arthroplasty implants consisting of carbon FRCs (Gillet et al. 1985) along with implant failures resulting from poor implant design and surface properties (Adam et al. 2002, Allcock and Ali 1997).

Secondly, the holes interfere the integrity, and hence the stress distribution pattern of the device. The stress concentrations can be decreased, and the plate structure enhanced by means of tailored fibre placement (TFP) method (Mattheij et al. 1998). Together with computer aided design methods TFP can be utilized to produce customized fibre preforms that can be refined into FRCs for a variety of applications (Breier 2015, Hahner et al. 2015a, Hahner et al. 2015b, Hoyer et al. 2014, Rentsch et al. 2014, Rentsch et al. 2010).

The aim of this thesis was to develop a novel fracture fixation device applicable in load-bearing conditions. Isoelastic behaviour compared to cortical bone was aimed to achieve by means of TFP pattern determined and optimized with finite element analysis. All the studied FRC plate designs consisted of dimethacrylate-based polymer matrix reinforced with E-glass fibres. In addition, a plate group with optimized TFP pattern was prepared from polylactide matrix and hybrid yarn of polylactide and E-glass fibres. The research was conducted in Biomedical Engineering Research Group of Turku Clinical Biomaterials Centre.

2. REVIEW OF THE LITERATURE

In this section the main elements related to the scope of this thesis are discussed. The fundamentals of bone tissue, evolution of fracture fixation materials, and computer aided designing tools are reviewed.

2.1 Bone

As for bone tissue's fundamental structural consistency, it can be considered as a composite material involved in a whole variety of physical and physiological functions. As tissue and furthermore as organ, bones are essential for body movement while they also serve as an internal mineral reservoir, account for maintenance of body's structural orientation and mechanical sheltering of vital internal organs and bone marrow (Grabowski 2009).

At the macromolecular level bone tissue can be interpreted as a composite material consisting of two distinguishable phases possessing specific mechanical and chemical properties. The reinforcing phase responsible for bone's compressive strength consists of the its mineral content. The main constituent of this is carbonated hydroxyapatite which in bone appears as flat, plate-shaped crystallite structures aligned parallelly to each other (Fratzl and Weinkamer 2007). The mineralization occurs as a result of extracellular fluid calcium ions bonding with phosphates released from matrix vesicles excreted by osteoblasts (Katsimbri 2017).

The nucleation points for bone tissue's mineralization are provided by macromolecules of its organic matrix that mainly consists of proteins. As 50 to 70% of total bone mass in adult skeleton is comprised of mineral content, the remaining proportion contains organic matter and water. Accounting for 20 to 40% of its total mass, the most abundant group of organic molecules in bone tissue is proteins. The majority of these, from 85 up to 90% belong to group of collagenous proteins. As the most abundant protein in human body, type I collagen is the most plentiful protein in bone extracellular matrix also. As hydroxyapatite crystallites account for bone tissue's stiffness and load-bearing strength, its flexibility and elasticity are provided by organic matrix proteins. Hence, the correct ratio of inorganic mineral content to organic matrix is crucial for optimal mechanical properties at the organ level (Katsimbri 2017). Beside of aforementioned constituents, bone contains around 3% of lipids and 5 to 10% of water (Clarke 2008).

Despite bone can be described as a composite material of hydroxyapatite crystallites embedded in a protein matrix, a division into distinctive subcategories of bone can be made based on its hierarchical structure and porosity at a more macroscopic level. Functions of different bones strongly affect their appearance. Hence, bones accounting for locomotion, such as femurs, and mechanical protection of vital organs, like skull bones, usually tend to consist of denser cortical bone. More porous trabecular bone is observed surrounded by cortical bone structures in bones that are frequently exposed to compressive mechanical stress, such as vertebrae, or contain haematopoietically active bone marrow, for instance pelvic bones. (Fratzl and Weinkamer 2007) Usually, individual bones contain both types with differing ratios of cortical to trabecular bone. For instance, long bones are often comprised of diaphysis, a hollow stem in the middle consisting of primarily cortical bone, transforming into metaphysis and eventually epiphysis around the growth plates at the both ends. The outermost regions comprise cortical bone shell filled with trabecular bone (Clarke 2008).

At a microscopic level, both cortical and trabecular bone are composed in an organized lamellar structure, where collagen fibres are aligned parallelly within a thin sublayer. This gives rise to mechanical properties of lamellar bone superior to those of woven bone where the fibres are aligned arbitrarily. In cortical bone, the alignment of adjacent layers of parallel collagen fibres rotates resulting to construction referred to as plywood structure in literature (Clarke 2008, Fratzl and Weinkamer 2007). Even though the lamellar structure of trabecular bone is somewhat different from that of cortical bone, the orientations of mineral particles and protein fibres are well-organized and distinguishable from those of woven bone. In a normal adult skeleton, bones are composed in a lamellar pattern. However, woven bone formation takes place at early stages of fracture healing in an adult as well as in intramembranous ossification associated with *de novo* osteogenesis in a foetus. Moreover, woven bone formation is observed in pathological states and bone remodelling disorders such as Paget's disease (Fratzl and Weinkamer 2007).

The relatively rapid formation of woven bone and its gradual replacement with lamellar bone is a remarkable consequence of bone modelling and remodelling tendencies regulated by interplay of bone resorbing and forming cells. The event of bone remodelling involves bone resorption, where the old bone tissue is removed, as well as bone formation where osteoid, new organic bone matrix, is first deposited and eventually mineralized to form new bone tissue. Even though bone modelling is the prevalent process during individual's development and growth, bone remodelling starts already before birth lasting for the entire lifetime. While remodelling mechanisms account for healing of sites

prone to microdamage, they also constantly renew the whole skeletal system in an arbitrary manner (Katsimbri 2017).

At a tissue level, a remarkable structural motif in both cortical and trabecular bone is osteon structures. Cortical bone osteons, referred to as Haversian systems, are cylindrical, longitudinally oriented canals running within the bone. Such canals are composed of concentric lamellae with nerves and blood vessels passing within them, therefore enabling nourishment and innervation of the tissue (Clarke 2008). As cortical bone is relatively dense tissue with typically less than 5% porosity, occurrence of metabolically active Haversian canals accounts for a remarkable proportion of its pore structures. Along with Haversian systems, the canalicular network connecting mature bone cells, osteocytes, accounts for the rest of the porosity in cortical bone. Residing distributed throughout bone tissue surrounded by it in fixed positions within their cavities called lacunae, the osteocytes interact with each other and other bone cells through this network (Fratzl and Weinkamer 2007).

As porosity in trabecular bone is around 80%, the structure of its osteons differs from that of Haversian canals in cortical bone. Trabecular bone consists of trabeculae, thin and spiny bone processes, thoroughly embedded in bone marrow. Due to the immediate presence of bone marrow, trabecular bone cells are provided with nourishment locally. Furthermore, trabecular bone osteons, referred to as packets, do not contain blood vessels (Fratzl and Weinkamer 2007). However, the packets are comprised of concentric lamellae in a similar manner as Haversian canals. Moreover, the remodelling cycle in both bone types eventually leads to formation of a new osteon (Clarke 2008).

Bone tissue's ability to remodel and reinforce according to mechanical circumstances was first proposed in 1892 by Julius Wolff (Wolff 1892). According to his observation known today as Wolff's law, the incident mechanical stress will lead to increase in strength and density of the exposed bone tissue along the axis of the force. On the contrary, areas with reduced mechanical loading end up losing strength and density. At the cellular level, osteocytes serve as mechanosensors and endocrine cells mediating the functions of osteoclasts that resorb bone and osteoblasts that account for formation and mineralization of bone matrix (Fratzl and Weinkamer 2007). At the molecular level, numerous systemically and locally acting factors regulating bone remodelling have been discovered (Grabowski 2009).

It still remains uncertain whether the mechanosensing in osteocytes occur through direct cellular deformation or resulting from shear forces caused by interstitial fluid movement

during the bone deformation. However, the mechanisms enabling detection of mechanical stimuli has been identified in osteocytes as well as in osteoblasts and osteoclasts. However, as osteocytes constitute the majority of all bone cells and they are distributed all over the bone tissue, they can be considered as the most remarkable mechanosensing cells of bone tissue. The signalling is mediated through various G-protein, MAPK/ERK, integrin, connexin and nitric oxide coupled pathways. (Rubin et al. 2006)

In addition to mechanical signal transduction, effects of various other physiological factors on bone remodelling have been identified. Such factors include balances in calcium and phosphate metabolism and acid-base homeostasis, for instance. (Grabowski 2009) Despite trabecular bone only accounts for about 20% of total bone mass in a grown-up individual, its high porosity enables significantly higher metabolic activity compared to that of cortical bone. Hence, due to significantly higher surface area to volume ratio compared to that of cortical bone, the majority of bone's functions related to mineral metabolism are carried out by trabecular bone (Katsimbri 2017).

A remarkable cellular signalling pathway involved in regulation of bone turnover cycle is the one resolving in activation of nuclear factor kappa-B (NF-κB). In osteoclasts and their progenitor cells, activity of the pathway is mediated by the activation status of receptor activator of nuclear factor kappa-B (RANK) located at their cell membrane. The key activator of RANK is receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL is encountered with osteoprotegerin (OPG) that serves as a decoy receptor for RANKL, hence inhibiting RANKL-mediated osteoclastogenesis and activation of mature osteoclasts. (Grabowski 2015) As both RANKL and OPG are produced in osteoblasts and their progenitor cells, they can regulate osteoclast activity by varying the ratio of expressed RANKL to OPG. Consequently, these cells account for the balance between bone resorption and formation. Moreover, certain endocrine and paracrine messengers have direct stimulatory or inhibitory effects on either of the events. (Clarke 2008)

A case in point of a systemic endocrine signalling agent directly affecting calcium metabolism is parathyroid hormone (PTH) whose main function is to increase calcium content in blood. PTH's major mechanism of action is to enhance reabsorption of calcium and reduce that of inorganic phosphate at renal tubules. PTH also indirectly stimulates bone resorption by enhancing expression of RANKL and inhibiting that of OPG in osteoblasts. Moreover, PTH accelerates production of biologically active vitamin D which occurs in renal tubules. Calcitriol is a biologically active form of cholecalciferol, known as vitamin D_3, that promotes increasing effects on calcium levels in plasma. This occurs as calcitriol increases calcium and phosphorus absorption from gastrointestinal tract.

(Hadjidakis and Androulakis 2006). Moreover, cholecalciferol indirectly enhances bone anabolism by stimulating differentiation of osteoblasts and their expression of enzymes required for organic bone matrix mineralization (Clarke 2008).

Oestrogens act by maintaining bone tissue, as they enhance osteoblast proliferation and decrease their apoptosis. They prevent osteoclast maturation by decreasing their sensitivity to RANKL and upregulate Fas ligand expression in osteoblasts hence accelerating apoptosis of osteoclasts and their precursors (Krum et al. 2008, Hadjidakis and Androulakis 2006). Consequently, the decrease in oestrogen levels in perimenopausal women has been shown to promote increased bone resorption (Krum et al. 2008).

As for normal physiological bone remodelling, bone resorption and formation are tightly coupled. Hence, disturbances in these opposing processes will eventually lead to either pathologic decrease in mechanical strength and mineral density of bone, osteoporosis, or alternatively to excess bone formation, osteopetrosis. The bone remodelling cycle occurring over time frame of several weeks consists of five consecutive phases called activation, resorption, reversal, formation, and termination. (Katsimbri 2017)

As discussed above, the stimulus initializing bone remodelling cycle can be either mechanical, endocrinological or paracrinological. The detection of bone remodelling enhancing signal leads to recruitment of mononuclear monocyte-macrophage lineage precursors cells from circulation and their activation into preosteoclasts (Teitelbaum and Ross 2003). Consequently, several of these mononuclear cells fuse forming multinucleated preosteoclasts (Clarke 2008). Simultaneously, bone lining cells retreat and endosteal membrane is penetrated by collagenase enabling attachment and maturation of multinucleated preosteoclasts on the surface of trabeculae or Haversian canal walls (Katsimbri 2017).

During the actual resorption phase, osteoclasts attached on the bone surface start to expose it with hydrogen ions by vacuolar-type H^+-ATPase, carbonic anhydrase II and H^+/Cl^- exchange transporter (Teitelbaum and Ross 2003). This leads to formation of acidic cavities, referred to as Howship's lacunae, with pH decreased to 4.5. As the acidic pH environment accounts for mobilization of bone's mineral content, osteoclasts start to excrete proteolytic enzymes, predominantly cathepsin K and matrix metalloproteinase 9, that in turn degrade the organic matrix. (Clarke 2008) Eventually, the multinucleated osteoclasts undergo apoptosis, hence completing the resorption phase (Katsimbri 2017).

The transition from bone resorption to bone formation occurs through reversal phase where several mononuclear cell types become present at the resorption site previously

occupied by multinucleated osteoclasts. Such cells include monocytes and osteocytes released form the resorbed bone matrix (Clarke 2008). The newly revealed bone surface gets prepared for attachment of osteoblasts by means of coupling mechanisms that eventually lead bone resorption to formation. However, these pathways still remain unclear. (Katsimbri 2017). During reversal phase, the present mononuclear cells also enhance osteoblast recruitment and differentiation (Hadjidakis and Androulakis 2006).

Osteoblasts are the major bone forming cells whose main function is to deposit new osteoid and enhance its mineralization. Osteoblasts are derived from pluripotent mesenchymal stem cells (MSCs), predominantly from those of bone marrow (BMSCs). Beside of osteoblasts, MSCs have potency to differentiate into several other cell types, including chondrocytes, adipocytes, and myocytes, for instance. (Katsimbri 2017) The determining factor for MSC differentiation into osteoblasts and chondrocytes is activity of bone morphogenic proteins (BMPs), especially BMP2, BMP6, BMP7 and BMP9. Beside of hard tissue development, growth factors belonging to BMP superfamily mediate tissue development of several organs. (Beederman et al. 2013)

The capability to modulate differentiation status of dual potent common osteochondroprogenitor cells enables occurrence of two distinct ossification patterns, intramembranous and endochondral ossification. As both modalities originate from a mesenchymal condensate, they differ from each other in respect of stages in cellular differentiation. Essentially, the cells of mesenchymal condensate directly differentiate into osteoblasts in intramembranous ossification, whereas the process occurs by means of a cartilaginous precursor in endochondral ossification. During embryonic development, intramembranous ossification typically occurs in formation of axial skeleton, such as in bones of calvaria. The majority of human bones form by endochondral ossification that also takes place in natural fracture healing process. (Day et al. 2005)

In intramembranous ossification, activity of canonical Wnt signalling pathway and consequent recruitment of β-cathenin and Runx2 to the osteocalcin promoter directs MSC differentiation into osteoblasts (Tang et al. 2009). Upon this, gene expression of transcription factors leading to chondrocyte differentiation, Sox9 being one of the most remarkable, are downregulated (Beederman et al. 2013). In turn, upregulation of Sox9 and absence of canonical Wnt/β-cathenin signalling activity is characteristic of endochondral ossification (Tang et al. 2009, Akiyama et al. 2004).

During the formation phase, osteoblasts synthesize new bone matrix predominantly consisting of collagenous proteins. The most remarkable of these is type I collagen that is

highly abundant in tissues throughout the body. In addition to collagenous proteins, osteoblasts produce several growth factors and cytokines, such as insulin-like growth factors, platelet-derived growth factor, and BMPs (Hadjidakis and Androulakis 2006). Type I collagen is a triple helical protein consisting of two α_1 and one α_2 polypeptide chains. During biosynthesis of collagen, the individual chains are directed into endoplasmic reticulum for assembling of procollagen that contains three chains. Consequently, procollagen is translocated into Golgi apparatuses where it is further modified and prepared for secretion to extracellular space. Following secretion, both N- and C-termini of procollagen molecules are cleaved and resulting tropocollagen molecules organize into collagen fibrils by self-assembly. (Canty and Kadler 2002)

Consequently, the newly synthetized osteoid is mineralized as a result of matrix extracellular vesicle release from osteoblasts (Katsimbri 2017). Matrix vesicles have high phosphates and calcium ion concentrations and provide preformed hydroxyapatite crystals serving as nucleating points for further hydroxyapatite development. Moreover, matrix vesicles contain enzymes, such as alkaline phosphatase, adenosine triphosphatase and pyrophosphatase, that inactivate mineralization inhibitors therefore enhancing the osteoid mineralization (Anderson 2003).

Bone formation phase lasts usually from 4 to 6 months and it terminates as bone forming osteoblasts either undergo apoptosis, as in 50 to 70% of cases, or differentiate into mature osteocytes (Clarke 2008). Alternatively, osteoblasts may also differentiate into bone lining cells that constitute a blood-bone barrier regulating the mineral influx and efflux between bone cells and bone extracellular fluid. Furthermore, bone lining cells are capable of redifferentiating into osteoblasts under certain circumstances. Such environmental factors include various signalling modalities such as mechanical, chemical and endocrinological, predominantly PTH, stimuli (Hauge et al. 2001).

2.2 Fracture healing

Fracture treatment has belonged to core areas of medicine throughout the written history. The Edwin Smith Papyrus, among the other earliest documented surgical bone setting procedures date back to 1500 B.C. and beyond (Brorson 2009). As open fractures with extensive wounds and bleeding formed a severe risk of infections and consequent sepsis, fracture treatment was mainly based on external fixation methods (Peltier 1990). Numerous procedures for external fixation have been documented ranging from the Hippocratic corpuscle, complied around 300 B.C., to the modern traditional external fixation apparatuses first introduced by Jean-Francois Malgaigne in 1840 (Vidal 1983, Bick

1968). Despite more advanced treatment options, external fixation methods still have their indications in modern clinical practice (Paul 2003).

From the viewpoint of modern medicine, a revolutionary innovation for fracture treatment was the introduction of internal fracture fixation. The first surgical procedure based on internal fixation was documented in 1886 by Carl Hansmann (Hansmann 1886), where a metallic plate was directly implanted onto the fracture site hence unifying the bone ends. Several studies relying on resembling methods with slight changes in material choices and implant design were conducted during the following decades (Müller et al. 2012). Despite the improvements in corrosion resistance of the devices, the initial clinical experiments often tended to be unsuccessful due to fatiguing and subsequent failure of the plate or inflammatory response caused by the resulting metal debris (Uhthoff et al. 2006).

In addition to inadequate mechanical stiffness of these plates, they were also remarkably prone to electrolysis (Woo et al. 1983). As Venable et al. (1937) introduced stainless steel plates and screws for internal fracture fixation, their clinical utilization was generalized, and development of fracture fixation constructions subsequently advanced. This resulted in remarkable decrease in proportion of plate breakdowns among all the implant failures during the following decades (Woo et al. 1983).

As the available materials for fracture fixation devices improved, it led to general aim of increasing the rigidity of resulting fixation by different plating techniques. The first attempt at a compression plate system was introduced in 1948 by Eggers (Eggers 1948). The idea behind such compression plating was to induce axial pressure between the fractured bone ends and consequently increase stability of the fracture site. The Eggers plate contained two longitudinal slots along which the screws could slide. This enabled adjusting the compression according to the resorption of the fractured bone ends. However, due to the mechanical weakness of this construct, compression over the fracture site could not be maintained in long-term (Uhthoff et al. 2006).

At around the same time Eggers introduced his construct, Danis noticed that compression over fracture site was necessary in achieving healing process where periosteal callus was not formed (Müller et al. 2012). In 1949, Danis developed a fracture fixation construct that produced axial force on the fracture fragments applying an additional compression generating device attached to the plate itself. This method enabled rigid fixation with no radiological findings of periosteal callus formation during any stage of healing

process. Danis called this kind of ossification process *soudure autogène*, autogenous welding, which is at the present time referred to as primary healing (Uhthoff et al. 2006).

As the callus formation was at that time thought to be a sign of fixation's mechanical instability, works of Danis vastly affected the development of compression plating concepts (Müller et al. 2012). Consequently, compression plating and fracture fixation methods promoting primary healing were studied and developed further in the following decades. In 1958, Bagby and Janes introduced their compression plate with oval screw holes enhancing compression between fracture fragments once the screws were tightened (Bagby and Janes 1958). This concept utilizing eccentric holes in compression plates was popularized later in dynamic compression plate (DCP) design. Despite the plate was named dynamic, the amount of provided compression could not be adjusted afterwards. (Uhthoff et al. 2006)

In 1965 Müller et al. (1991) introduced a compression plate construct that essentially popularized their clinical utilization. As opposed to earlier designs enhancing interfragmentary compression, Müller's construct was based on a sturdier plate that was tightened by means of an external tensioner momentarily attached to the plate and the bone.

As fracture fixation device designs evolved, effects of long-term rigid fixation on bone were discovered. A dog study conducted by Uhthoff and Dubuc (1971) demonstrated how a bone prolongedly exposed to a rigid compression device is prone to plate-induced osteopenia, that is lowering of bone mineral density. The study showed that in such cases natural bone remodelling mechanisms, resorption and formation, are disturbed leading to prolonged prevalence of trabecular bone at the implantation site and absence of lamellar orientation in new bone. Based on bone remodelling mechanisms initially introduced by Julius Wolff back in 1892 (Wolff 1892), stress shielding phenomenon is observed as a result of unphysiological mechanical loading of the bone subject to a rigid fracture fixation device (Huiskes et al. 1992). Hence, proportion of local mechanical strain targeted to a bone is decreased significantly in the presence of a device with remarkably higher flexural modulus than that of cortical bone.

Moreover, it was noticed that in comparison with natural healing through periosteal callus formation, primary healing by direct Haversian reconstruction is significantly slower process (McKibbin 1978). As primary healing is more of a remodelling process occurring in the later stages of natural ossification, the support provided by a fixation device in protocols aiming for primary healing is required for a remarkably elongated time. This in turn

increases the risk for plate-induced osteopenia. In addition, it was observed that biomechanical properties of fractures healed by closed weight-bearing treatment resting on periosteal callus formation were superior to those fixed with compression plating (Sarmiento 1980).

Consequently, this led to increased attention in development of new methods for less rigid fracture fixation promoting natural healing. Alternative treatment procedures utilizing rigid plates for an abbreviated time, less rigid plates, and biodegradable plates were proposed as solutions for drawbacks encountered with conventional rigid fixation (Woo et al. 1983). In addition to new materials, studies introducing new assessment methods for implant behaviour were also conducted. Finite element analysis (FEA) became a common tool for optimization of relation between stress shielding effects and reasonable interfacial stresses between implant and the adjacent bone (Huiskes et al. 1992).

Despite problems associated with stress shielding can be avoided to some extent by timing the removal of a rigid plate correctly (Ganesh et al. 2005, Woo et al. 1983), clinical utilization of metallic implants still has some intrinsic drawbacks. Metallic implants can cause cytotoxic effects *in vivo* due to liberation of metal ions and corrosion products (Uhthoff et al. 2006). In load-bearing sites where the implant is permanently inserted, such as in the case of total hip arthroplasty, these long-term effects should especially be considered (Huiskes et al. 1992). Moreover, metallic implants in general interfere with medical ionizing imaging methods, such as roentgenography and computed tomography, as well as magnetic resonance imaging. This is observed as imaging artefacts in these diagnostic modalities (Nawaz et al. 2014, Shellock 2002). The predominant causes of artefacts resulting from interactions between metallic implants and ionizing radiation are beam hardening, scattering and increased noise (Giantsoudi et al. 2017). Consequently, the presence of metallic implants complicates both diagnostic and therapeutic procedures utilizing high-energy electromagnetic radiation and magnetic field (Nawaz et al. 2014, Shellock 2002).

Consequently, non-metallic materials were introduced to orthopaedic research. Since 1960s, ceramics have been proposed and studied as potential materials for hard tissue replacement and regeneration. Due to their good biocompatibility, high strength, toughness, and corrosion resistance, bioinert alumina and zirconia ceramics became extensively utilized in dental restorations and later in loadbearing applications, such as in femoral stems (Ramakrishna et al. 2001, Hulbert 1993). However, in a similar manner to metallic implants, such inert ceramics cause foreign body reactions at the implantation site. This is observed as acellular collagen encapsulation of the implant isolating the

device from its surroundings (Vallet-Regí 2010). The remarkably higher elastic modulus of these bioinert ceramics compared to that of cortical bone also promotes similar stress shielding effects as observed with stiff metallic implants (Hulbert 1993). On the other hand, bioactive calcium phosphate and hydroxyapatite ceramics promote osteoconductive properties, which has made them popular in regeneration of nonloadbearing hard tissue. Such applications include dental restorations, maxillofacial reconstructions and cranial implants (Brie et al. 2013, Vallet-Regí 2010). Moreover, they are widely used as coating materials and as composite blends (Hench and Polak 2002, Suchanek and Yoshimura 1998). However, the main concern of wider utilization of such ceramics as bigger implants and at loadbearing sites is their brittleness and low fatigue resistance (Vallet-Regí 2010).

In 1980s, the use of polymeric materials popularized in maxillofacial reconstructions. For instance, polyethylene was approved by the Food and Drug Administration for treatment of orbital fractures, and it has been utilized in various formulations since (Suh et al. 2018, Qian and Fan 2014). Production of new thermoplastic polymer materials, such as those belonging to polyaryletherketone (PAEK) family, was commercialized for industrial applications in late 1980s. At the same time, the interest towards orthopaedic devices with comparable flexural properties to those of cortical bone was blooming. Polyaromatic ketone polymers possessing excellent flexural properties, resistance to chemicals and radiation, and compatibility with several reinforcing constituents were extensively studied in orthopaedic applications (Kurtz and Devine 2007). In the 1990s, polyetheretherketone (PEEK) based composites became commonly utilized materials especially in spinal implants and started to replace metals in loadbearing applications (Merola and Affatato 2019, Kurtz and Devine 2007).

Hence, fibre-reinforced composite (FRC) materials were adopted to orthopaedic practice. The interest in applying FRCs in load-bearing devices instead of metals was due to their good wear resistance and formability to closely correspond to the mechanical properties of any specific implantation site. Hence, FRC implants could potentially enable such local distribution of mechanical load that reduces adverse peri-implant bone resorption caused by stress shielding (Evans and Gregson 1998, Huiskes et al. 1992). Due to potentially superior biocompatibility of carbon composites, initial FRC materials were predominantly based on composites with carbon fibre reinforcement. Still, in many instances such implants turned to be unsuitable for orthopaedic applications that require more com-

plex design approaches. For instance, carbon fibre reinforced total hip arthroplasty prostheses often tended to be unsuccessful due to implant failures (Adam et al. 2002, Allcock and Ali 1997) or inflammatory reactions caused by carbon fibre debris (Gillet et al. 1985).

However, due to successful clinical applications of FRC devices in non-load-bearing conditions, such materials are regarded as potential candidates for replacing metals in skeletal reconstructions (Evans and Gregson 1998). FRCs consisting of glass fibres and matrix material based on bisphenol A glycidyl methacrylate (BisGMA) have become routinely used in various dental restorations (Vallittu 1999, Karmaker et al. 1997). Moreover, non-loadbearing BisGMA-based FRC implants have successfully been applied in cranial reconstructions (Vallittu 2017, Piitulainen et al. 2015, Aitasalo et al. 2014).

In addition to FRC implants favorable mechanical properties, they possess other pleasant features as for clinical practice. For instance, unlike metallic implants that interfere with medical imaging methods (Shellock 2002, Nawaz et al. 2014) non-metallic FRC implants do not produce artefacts in these imaging modalities, but instead they can be observed in all of them (Zhao et al. 2009). Moreover, FRC implants allow postoperative radiation therapy as opposed to their metallic equivalents.

The stress shielding related problems are even more emphasized in some small domestic animals. Such species, like dogs, are often inbred and hence suffer from hereditary disorders that are not observed in their relative equivalents that have been evolved by natural selection. In addition, as treatment methods utilized in veterinary medicine are generally adopted from human medicine with delay, the same issues are being addressed. (Farrell 2016)

In small domestic cats and dogs diaphyseal fractures of antebrachium are common and often involve both antebrachial bones, radius and ulna (Denny and Butterworth 2000). Among toy-breed dogs, i.e. breeds with bodyweight less than 5 kg, such fractures may occur due to minimal trauma, for instance in jumping falling injuries (Harasen 2003, Muir 1997) and they comprise approximately 17% of all canine fractures (Phillips 1979). The reasons for prevalence of such fractures in small domestic animals are related to the low body weight, reduced intraosseous blood supply, and breed-specific differences in bone geometry and mineral density (Welch et al. 1997, Sumner-Smith 1974).

2.3 Composites

In this subchapter composite materials and their evolution are introduced generally. The main focus is kept in implantable composite devices and their development.

2.3.1 Introduction to composite materials

Composites are materials that consist of two or several constituents that are representing different phases. Commonly the constituents possess distinct physical features which leads to unique properties of the composite material in comparison with those of the constituent materials by themselves (Hull and Clyne 1996). As for mechanical behaviour of composites, they often contain mechanically stiff component, accounting for rigidity of the material, surrounded by more elastic medium, maintaining the position of other constituents and distributing the incident mechanical strains between the reinforcing structures (Mallick 1993). Reinforcing agents in naturally occurring composite materials, bone and wood for instance, are crystalline hydroxyapatite and fibrous cellulose, respectively (Fratzl and Weinkamer 2007). In turn, type I collagen serves as the matrix material in bone as hemicelluloses and lignin do in wood (Hull and Clyne 1996).

In both naturally occurring and synthetic composite materials, alternative orientation of the reinforcing phase may promote vastly different mechanical behaviour in comparison to another. In bone tissue, the remodelling results in reinforcement of the tissue along the axis of incident strain. This results in superior mechanical performance to an arbitrary orientation along the axis of incident strain (Clarke 2008, Fratzl and Weinkamer 2007). However, such anisotropic materials cannot withstand mechanical strains equally effectively in all directions (Hull and Clyne 1996). This addresses the demand for optimizing the alignment of reinforcing phase within materials intended for loadbearing, such as orthopaedic implants.

A variety of composite material combinations with different reinforcing phases and matrix materials have been developed for certain applications. In industrial use, the most commonly utilized matrices include polymers, metals and ceramics. In medical composites, polymers are the predominant material choice due to diverse selection of different material properties (Mallick 1993). In synthetic composites, the matrix polymer can either be thermosetting or thermoplastic (Evans and Gregson 1998).

An advantageous feature of thermoplastic polymers, such as PEEK, is their tight intermolecular forces. This results in a structure withstanding both mechanical and chemical strains which gives rise to good biocompatibility of such composite materials (Mallick 1993, Brown et al. 1990). However, the downside of thermoplastic polymers is their meticulous processing especially with long unidirectional fibre reinforcement required in loadbearing implants. For processing of FRCs, thermosetting polymers allow more accurate implant fabrication. In order to apply thermosetting polymers safely and effectively

in medical applications, appropriate polymerisation is required to avoid release of residual monomers to surrounding tissues. (Evans and Gregson 1998) However, careful polymerization of BisGMA resins containing dimethacrylate monomers has resulted in polymer composition with good biocompatibility (Bae et al. 2012).

As opposed to selection of matrix materials, the variety of reinforcement fibres in wider industrial and medical use is more restricted (Evans and Gregson 1998). As for FRC material development for orthopaedic applications, carbon fibres have been one of the most notable reinforcing agents. This is due to potentially excellent biocompatibility of carbon and high stiffness to mass ratio of the fibres (Evans and Gregson 1998, Hastings 1978). Such features suggest that carbon fibre composites are potential materials for developing devices with high strength and reduced stiffness. However, implant failure (Adam et al. 2002) and release of carbon fibre debris (Gillet et al. 1985) are among the reported concerns observed in wider utilization of loadbearing carbon fibre composite implants. Aramid fibres consisting of aromatic polyamides are also studied as reinforcement agents for FRCs due to their high tensile strength. However, due to low compressive strength and stiffness of aramid fibres, such as Kevlar, their applicability in loadbearing implants is restricted (Evans and Gregson 1998).

In industrial applications, glass fibres are the most commonly utilized reinforcing agents of polymer matrices (Mallick 1993). Consequently, their utilization has generalized in medical applications as well (Vallittu 2017, Abdulmajeed et al. 2014, Aitasalo et al. 2014, Moritz et al. 2014, Abdulmajeed et al. 2011, Vallittu 1999). Glass fibres promote good tensile properties and chemical stability for implantable medical devices (Evans and Gregson 1998) despite their inferior mechanical performance to carbon fibres (Hastings 1978). Performance of implants that contain mechanically inferior fibres can be optimized by applying various anisotropic fibre orientations simultaneously (Mallick 1993). A case in point of such construct in the field of orthopaedics is an FRC intramedullary nail with improved torsional strength (Moritz et al. 2014). These nails contained a biaxial glass fibre sleeving surrounding the core of longitudinally oriented unidirectional glass fibres.

2.3.2 Evolution of composite implants

Metal alloys remained as an unarguable gold standard for material choice in internal fracture fixation plates prior to exploring the effects of plate-induced osteopenia and stress shielding (Uhthoff and Dubuc 1971). Thereafter, alternative materials and surgical practices (Woo et al. 1983) have been examined as potential solutions for optimizing the relation of apparent stress shielding and stresses at the proximal interface between bone

and the implant. That is, the stiffer the loadbearing orthopaedic device, the lower the related interface stresses, and vice versa. This implies that the aim to minimize both stress shielding and interfacial stresses is intrinsically contradictory. As the increment of proximal interface stresses may lead to defective bonding between bone and the implant, the device should restrict interface stresses at a moderate level while producing acceptable amount of stress shielding. (Huiskes et al. 1992)

To overcome challenges with bulk metallic implants, multiphase materials have been adopted in orthopaedic practice. Among such materials, fibre reinforced composites (FRC) with polymer matrix are the ones that are utilized the most (Scholz et al. 2011). Concurrently with development of composite materials, the mindset of utilizing biomaterials in clinical applications and patient treatment has gone through a vast revolution. In the last few decades, the vision of ideal biomedical material has shifted from the one having the most minimal effect on its biological and chemical environment in a living tissue into ones with biodegradable or otherwise bioactive properties. Consequently, remarkable development of biodegradable and biostable composites has been made during that time (Holland et al. 1986).

At present, biomaterials are commonly defined as any natural or synthetic materials intended to interact with biological matter within or at a surface of a living individual (Arcos and Vallet-Regí 2013). Traditionally, four *in vivo* material response patterns have been represented in the literature (Hulbert 1993). In addition to materials that promote direct toxic tissue effects, nearly inert, surface-active, and resorbable biomaterials are distinguished, forming three biomaterial generations (Hench and Polak 2002).

Initially, medical device's biological compatibility was considered as absence of debris formation that would lead to inflammation and toxic effects caused by the material (Hulbert 1993). The main aim of so-called first-generation biomaterials was purely substituting the defects of already existing tissue. This material group consists of materials that behave in a nearly inert manner within living organisms, like stainless steel or zirconia and alumina ceramics, for instance. While such materials are non-toxic, they still cause foreign body reactions to a varying extent (Vallet-Regí 2010).

In addition to drawbacks of metallic implants in terms of their mechanical behaviour, the naturally occurring acellular collagen encapsulation of a foreign body became a more acknowledged phenomenon due to its decreasing effects on implant integration to the surrounding tissues. Consequently, this may give rise to instability of metallic fracture

fixation plates (He et al. 2017). New biomaterial research induced by the aim of minimizing implant encapsulation led to studies around more natural implant integration into body. In addition to modifying implant's topology to enhance its integration into the surrounding tissues (Hou et al. 2018), new methods relied on making the implant material bioactive, like bioactive glass, or biodegradable, like polylactide, to reduce problems at the interface between implant and surrounding tissue. Second-generation biomaterials are constituted of such surface-active and resorbable biomaterial groups (Hulbert 1993).

The idea of utilizing glass in hard tissue replacement was initiated in the late 1960s when it was first used for hard tissue replacements and bone regeneration (Hench and Polak 2002). In general, glass is a solid amorphous material whose disordered molecular structure enable its high reactivity (Vallet-Regí 2010). The concept of silica-based bioactive glass devices is based on a molecular composition that promotes remarkable ion exchange with body fluids that surround the implant. The series of sequential reactions leads to formation of nucleation centres for bioactive hydroxy apatite crystallization along the implant surface. Consequently, the resulting hydroxy apatite layer supports advancement and differentiation of hard tissue forming cells, predominantly osteoblasts, on the implant surface, and implies further implant integration to the surrounding tissues. (Arcos and Vallet-Regí 2013)

Besides its osteoconductive properties, further studies on bioactive glasses have revealed that topology, porosity and composition of the material can be optimized to produce an osteoinductive implant that activates bone regeneration stimulating genes (Hench and Polak 2002). The technique of immersing bioactive glass granules into the bulk or on the surface of an implant has been applied to FRC materials also to improve their osteoconductivity and osteogenicity (Piitulainen et al. 2015, Aitasalo et al. 2014, Moritz et al. 2014, Zhao et al. 2009, Tuusa et al. 2008, Tuusa et al. 2007).

Another case in point of second-generation biomaterial technology is the variety of bioresorbable materials. In the late 1960s, applying of synthetic biodegradable polymers in clinical practice took off as first synthetic, bioresorbable sutures were developed (Joseph et al. 2017). The success of sutures based on plain polyglycolide (PGA) and copolymer of polyglycolide and polylactide (PLGA) popularized the use of these materials in medical field and induced new research on their use in different applications (Talbot et al. 2002). In body conditions, these polymers degrade principally by nonspecific hydrolysis. The resulting monomers are excreted in urine or, predominantly, as carbon dioxide produced in tricarboxylic acid cycle. Resultant carbon dioxide is eventually eliminated during respiration. (Agrawal et al. 1995) Consequently, polylactic acid is currently widely utilized in

various formulations and clinical applications ranging from resorbable sutures to load-bearing fracture fixation devices (Saikku-Bäckström et al. 2005, Veiranto et al. 2002, Saikku-Bäckström et al. 2000, Böstman et al. 1993, Hench 1980).

In the 1980s, biomaterial research took steps towards the modern biomaterial technology due to aroused interest of making bioactive materials bioresorbable, and vice versa (Vallet-Regí 2010). The aim of such modified bioresorbable polymer materials is to activate genes that account for cellular proliferation and differentiation. Consequently, this leads to enhanced production of extracellular matrix and tissue regeneration. The two major modalities of applying third-generation biomaterials rely on *ex vivo* tissue engineered constructs or direct *in situ* tissue regeneration. A case in point of such tissue engineered construct is a modified bioresorbable scaffold system where progenitor cells from the host tissue are seeded onto prior to construct's implantation into its target site. The scaffold itself is eventually resorbed and placed with host tissue cells and extracellular matrix. In turn, direct tissue regeneration techniques include locally administered microparticle solutions, for instance (Hench and Polak 2002).

At present, such third-generation biomaterials are utilized in tissue engineering and tissue regeneration applications in several forms, including resorbable scaffolds, sutures and fracture fixation plates (Saikku-Bäckström et al. 2005, Hench 1980). In clinical practice, third-generation biomaterials allow more sophisticated and targeted implant functions. For instance, drug delivery systems are such constructs that simultaneously apply implant's bioactive and bioresorptive properties. This kind of composite devices enable targeting of drug delivery into a specific area in the body. Consequently, these properties allow controlled and steady therapeutic levels of drug release (Arcos and Vallet-Regí 2013).

In loadbearing applications, FRC materials provide superior tailorability to match the mechanical properties with that of bone. Isoelasticity of the device and bone can be optimized by means of finite element analysis applied in the development process of the implant. The controllable FRC material features include the volume ratio of fibres to matrix material and the arrangement pattern of the fibres (Kharazi et al. 2010).

As traditional FRC materials are considered nearly inert biomaterials, FRC derivatives enriched with granules of bioactive glass improve their surface-active properties. Moreover, steps towards third generation FRC implants have been taken as the bioinert polymer matrix itself or together with reinforcement phase has been substituted with bioresorbable materials (Piitulainen et al. 2015, Aitasalo et al. 2014, Saikku-Bäckstöm et al.

2005). In this study, one plate group consisted of partially resorbable FRC plates with bioresorbable polylactide matrix reinforced with bioinert E-glass fibres.

As less rigid bioinert fracture fixation plates have been developed in attempt to enhance more natural bone healing pattern and to postpone the need of secondary plate removal surgery, resorbable materials have become progressively more appealing options in development of such devices. Completely bioresorbable fracture fixation devices enable gradual increment of bone straining as degradation of the implant proceeds. Moreover, the need for secondary surgeries is avoided as the device is completely resorbed. However, the major concerns restricting wider utilization of such fully bioresorbable loadbearing implants are toxic by-products of degradation process and difficulties in achieving suitable degradation rates. (Scholz et al. 2011)

2.3.3 Clinical applications of composite materials

Besides fracture fixation devices that are in the focus of this thesis, composite technology is widely utilized in other applications on biomedical field as well. One of the composite material constructions with notable clinical applicability is an ensemble of cells seeded onto an artificial, biodegradable matrix known as scaffold (Mironov et al 2009). Together with living cells and growth controlling signals the scaffold forms an entity referred to as tissue engineering triad (O'Brien 2011). In tissue engineering applications, the scaffold material and structure should meet a few requirements as for structure, composition, and biocompatibility to enhance cell proliferation while simultaneously degrade in a controlled manner. Scaffold technique serves as a promising treatment for healing bone defects, for instance (Breier 2015, Rentsch et al. 2014, Rentsch et al. 2010).

Serving as a template for cell growth, scaffold should provide the cells with sufficient mechanical strength and shelter as well as with topological features that induce cell spreading, proliferation and production of extracellular matrix characteristic to cells involved (Breier 2015, O'Brien 2011). Eventually, the aim is to make the degradation rate of the scaffold material identical to the production rate of new matrix material. This applies likewise to other loadbearing skeletal reconstructions, such as fracture fixation plates. Therefore, the scaffold construct should initially share similar mechanical properties with the tissues at the implantation site. Moreover, the newly formed tissue should be able to progressively carry bigger proportion of the mechanical load as the resorption of scaffold material proceeds. (Hutmacher 2000)

In addition to enhancing the spreading of parenchymal cells into the scaffold, the material porosity is vitally important likewise for transportation of secondary degradation products and immunological cells in and around the scaffold. A substantial prerequisite is also that the by-products are not biotoxic and that they can be eliminated without detrimental effects on other tissues (O'Brien 2011).

Production of the scaffolds has been revolutionized by the methods relying on computer aided design (CAD) and additive manufacturing. Such methods include for instance 3D printing modalities like selective laser sintering and stereolithography. The bulk object is produced by depositing raw material particles and binder matrix on assembling template followed by curing of the particles and layers together by radiation or heat, for example (Breier 2015). The described method can also be applied to positioning biomolecules and living cells into the matrix material (Mironov et al 2009). Such biofabrication has enabled production of complex and multifunctional drug delivering implants (Arcos and Vallet-Regí 2013).

However, the mechanical properties of textile scaffolds are insufficient for load bearing applications which restricts their use. Therefore, a technique involving an intramedullary rod as a mechanical support is needed while utilizing textile scaffold in load bearing tissue engineering applications, such as in fixation of fractures in tibia or femur. In such constructs, the scaffold serves as a template for patient's autologous mesenchymal stem cells which can spread, proliferate, and differentiate into osteogenic and vasculogenic cells while an intramedullary rod provides the fracture site with sufficient stability. (Breier 2015). Such constructs have successfully been applied in treatment of critical size bone defects in animals by utilizing embroidered bioresorbable polycaprolactone-co-lactide (PCL-PLA) scaffolds (Rentsch et al. 2014, Rentsch et al. 2010).

In addition to treatment of hard tissue defects, composite materials have also been studied as potential candidates for several soft tissue replacement applications. Due to their unique mechanical and biological features, ligaments and tendons are one of the most challenging and broadest targets for tissue replacement. Ligaments are essentially strands of connective tissue joining bones at a synovial joint hence contributing to the stability of the union. In turn, tendons link muscles to the bone in a similar manner therefore enabling force transmission and locomotion.

Ligaments and tendons can be considered as composites of collagen fibres surrounded by matrix material consisting of elastin and glycosaminoglycans. Due to their restricted vascularization, their regenerative potential is limited, and the regeneration process

takes time (Kellomäki et al. 2015). As for their mechanical properties, ligaments and tendons are unique in the sense that their stress-strain curves do not obey Hookean law. Instead, further material deformations are achieved with greater tensile forces leading to a convex curve shape. This contrasts with concave stress-strain curves observed in traditional engineering materials. (Ramakrishna et al. 2001) To respond to this demand, multidomain composite constructs have been developed for artificial replacement of ligaments and tendons (Breier 2015, Hahner et al. 2015a, Hahner et al. 2015b). Such constructs aim to mimic all the regions distinguished in a natural ligament, namely the sections anchoring to bone and the midsection exerting ligament's stabilizing function. Materials currently suggested as potential candidates for artificial manufacturing of load-bearing ligaments include embroidered single-, bi-, and multicomponent scaffolds containing PLA, PCL-PLA, and polydioxanone (PDS) enriched with collagen (Hahner et al. 2015a, Hahner et al. 2015b, Hoyer et al. 2014).

Another case in point of a meticulous tissue to artificially engineer is blood vessels. As ligaments and tendons, the behaviour of blood vessels does not obey Hookean law under physiological strains as they contain overlapping layers of fibrous macromolecules oriented in an anisotropic manner (Ramakrishna et al. 2001). The most remarkable feature for successfully engineered vascular graft is appropriate porosity enhancing recruitment and proliferation of host tissue progenitor cells while still inhibiting leaking of the vessel. (Scholz et al. 2011). The current synthetic vascular grafts include tubular woven or knitted polytetrafluoroethylene (PTFE) and electrospun randomly oriented networks of either PLGA, PCL, PLA or PLA-PCL with mixture of collagen and elastin (Lee et al. 2007, Ramakrishna et al. 2001).

A potential site to apply composites for another kind of physiological demand is a stress adapted hernia mesh. Hernia is a protrusion of tissue or organ through the wall of body cavity where it originates. Such mesh is intended to provide adequate mechanical stability while enhancing recruitment of surrounding tissues to the site of hernial opening. The composite materials have shown to be superior to uncoated polymer equivalents as for their biocompatibility. (Ramakrishna et al. 2001) Embroidered polypropylene meshes and collagen-covered polyethylene terephthalate (PET) fabrics have been suggested as candidates for treatment of abdominal hernias (Hahn et al. 2017, Werkmeister et al. 1998).

Composites have also been studied in auditory applications. Efforts have been made to develop composite material replacements for tympanic membrane transmitting sound waves from external to middle ear (Teoh et al. 1999) and for ossicles accounting for

auditory conductivity between tympanic membrane and cochlea (Ramakrishna et al. 2001). Such constructs have utilized for instance carbon fibre reinforced PTFE and composite of polyurethane and ultra-high molecular weight polyethylene (UHMWPE) and polyurethane (PU), respectively.

While FRC materials are still clearing their way for wider utilization in loadbearing orthopaedic applications, carbon fibre reinforced composites (CFRC) have served as golden standard for prosthetic limb materials for decades (Ramakrishna et al. 2001). This is due to their lightness and capacity to efficiently store and release energy (Scholz et al. 2011).

2.3.4 Fibre-reinforced composites

The interest towards utilization of FRCs in hard tissue reconstructions was initiated by their wear resistance properties and good formability to make their mechanical properties match with those of the given implantation site (Evans and Gregson 1998). The composite of polymer matrix reinforced with E-glass fibres have shown to promote lower flexural modulus to that of metallic implants while they still possess high flexural strength and good wear resistance tendencies (Ballo et al. 2007, Vallittu and Lassila 1992). BisGMA-based FRC materials have been studied extensively in dental applications (Abdulmajeed et al. 2014, Ballo et al. 2014, Abdulmajeed et al. 2011, Shinya et al. 2011, Ballo et al. 2009, Ballo et al. 2007, Vallittu 1999). Utilization of these FRCs have spread from dental applications to other non-loadbearing restorations (Vallittu 2017, Piitulainen et al. 2015, Aitasalo et al. 2014, Tuusa et al. 2008, Tuusa et al. 2007). Moreover, glass fibre reinforced BisGMA composites have been studied progressively as potential loadbearing implants (Liesmäki et al. 2019, Moritz 2014, Zhao et al. 2009), spanning also to a large-scale EU-project (NEWBONE, Final report 2010).

Due to abundant hydrogen bonding between hydroxyl groups of the monomer units within the polymer, BisGMA has remarkably high viscosity. The viscosity can be lowered by mixing BisGMA with lower viscosity fluids such as triethylene glycol dimethacrylate (TEGDMA) to ease implant processing with it. (Ferracane 1995) BisGMA resin can be polymerized utilizing a thermochemical of photochemical initiator whose function is to induce production of free radicals within the fluid. Actions of these eventually result in crosslinking between the molecules. (Ramakrishna et al. 2001)

2.4 Evaluation of mechanical properties required for loadbearing

The aim of this study was to further develop FRC plates with BisGMA/TEGDMA polymer matrix that were previously hypothesized as potential candidates for treatment of antebrachial fractures in toy-breed dogs (Liesmäki et al. 2019). The desired properties of a loadbearing fracture fixation device include adequate mechanical strength and elasticity to maintain both micromotion of the fracture site and stress shielding of the bone at an acceptable level. As traditional rigid metallic implants often promote mechanical strength superior to those of their non-metallic equivalents, the clinically significant quest concerning material choices is to optimize the relation of implant related micromotion and stress shielding. Alternate techniques to achieve the same clinical outcome comprise utilization of less rigid plate materials or reduction of plate dimensions when more rigid materials are applied (Huiskes et al. 1992, Akeson et al. 1975). This implies that the mechanical properties of the plate material can be either superior or inferior to those of cortical bone as the plate design determines ultimately the performance of the construct.

A case in point of a successful application of less rigid fracture fixation plates in conditions comparable to the interest of this study is the use of self-reinforced bioresorbable poly-L/D-lactide (70/30 wt%-ratio) plates in treatment of distal radius fractures in small-breed dogs (Saikku-Bäckström et al. 2005). In previous studies, flexural strength, the physical quantity describing the ultimate load that the construct can bear, has shown to range from 166 MPa to 176 MPa in these composites (Veiranto et al. 2002). In contrast, flexural strength of canine cortical bone has determined to be 185 MPa (Acevedo et al. 2015).

Another physical property describing the suitability of a plate to perform in loadbearing conditions in concert with cortical bone is its structural stiffness. While geometry of the device also contributes to ultimate structural stiffness of the plate, the physical quantity serving as comparable measure between plate materials is flexural modulus. Flexural modulus of aforementioned poly-L/D-lactide composite was observed to range from 4.0 GPa to 5.0 GPa (Veiranto et al. 2002). Flexural modulus of canine cortical bone has been observed to be 18 GPa (Acevedo et al. 2015).

Beside bioresorbable poly-L/D-lactide composites, a variety of less rigid FRC materials have been studied extensively for fracture fixation in animals (Kettunen et al. 1999, Gillett et al. 1985, Woo et al. 1983, Tayton et al. 1982, Akeson et al. 1975). Among these stud-

ies concerning commonly examined carbon fibre composites, flexural strength of the devices ranged from 251 MPa (Gillett et al. 1985) to 450 MPa (Kettunen et al. 1999) and flexural modulus 8.52 GPa (Gillett et al. 1985) to 40 GPa (Kettunen et al. 1999). Despite these materials promoted superior flexural strength and flexural modulus to those of self-reinforcing poly-L/D-lactide plates (Veiranto et al. 2002), these values were still observed to be lower in comparison with the most commonly utilized metal alloys. For instance, flexural strength and flexural modulus of stainless steel have been observed to be 977 MPa and 210 GPa (Losertová et al. 2016), those of cobalt-chromium (Co-Cr) 1403 MPa and 283 GPa (Baron and Ahearne 2017), and those of titanium alloy Ti6Al4V 1034 MPa and 106 GPa (Losertová et al. 2016).

Consequently, review of the studies around the subject suggests that clinically acceptable lower boundary for flexural strength of a fracture fixation plate is 166 MPa and its flexural modulus should settle within the range from 4 GPa to 40 GPa. Moreover, as dogs are observed not to fully lean on their injured leg during the early stages of healing process, mechanical straining and hence required strength of the limb is not as high as expected in physiologically normal locomotion (Anderson et al. 1993).

2.5 Finite element analysis

Finite element analysis is a numerical tool commonly applied in the field of engineering sciences. The application areas of finite element analysis range from continuum mechanics to thermal physics and electromagnetism, for instance. In such applications, the involved materials, strain distributions and geometries are often complex. This causes analytical solutions describing the entire problem to be virtually impossible to determine. Whereas an analytical mathematical solution is a mathematical sentence yielding the value of the examined variable at any point of the investigated body, finite element analysis relies on discretizing the body into smaller units, finite elements. By formulating algebraic equations describing the behaviour of each element, the necessity to solve differential equations involved in analytical solutions can be avoided. Consequently, equations of adjacent units, interconnected by means of nodal points, boundary lines and surfaces, can be combined to describe the entire problem. (Logan 2011)

The idea of representing one-dimensional structural analysis problems in regionally discretized elements was introduced in 1940s (Prager and Synge 1947). However, the equations were cumbersome to calculate by equipment available at that time (Clough 1990). Concurrently with development of digital computers in 1950s, two-dimensional matrix formulation of structural theory was derived (Turner 1956). In 1960, the term finite

element was first used by Clough in plane stress analysis (Clough 1990). Consequently, utilization of tetrahedral stiffness matrices enabled extension of finite element analysis to three-dimensions (Melosh 1963).

In a mathematical point of view, structural finite element analysis is based on derivation of individual element stiffness matrices in the coordinate system of their own. These local stiffness matrices are assembled into global stiffness matrix in global coordinates. In stiffness method approach, the global stiffness matrix is essential in the relation

$$\{F\} = [K]\{d\},$$

where $[K]$ is the global stiffness matrix, $\{F\}$ the vector of incident forces and $\{d\}$ the vector of displacements (Hughes 2000).

With the modern computational tools, the process flow behind solving a structural problem by finite element analysis begins with defining geometrical features of the studied object. The material in question and the physical quantities describing its flexural properties, for instance Young's modulus and Poisson's ratio, are determined and inserted into the model. With the material properties determined, the incident loads acting on the modelled object are defined. Consequently, the finite element mesh is defined over the examined body. This can be executed manually or by means of a mesh-generator programme. As the finite element mesh is generated, the boundary conditions are set. In practice, such conditions are known values of stress or strain on certain points at a given time, for instance. This reduces the complexity of the problem. (Logan 2011)

Based on the local stress-strain relations and the aforementioned quantities, the global stiffness matrix can be assembled. Subsequently, the set of linear systems is ready to be solved. (Hughes 2000) Smaller elements as well as higher order elements are required in the areas of the macroscopic object with high gradients. As utilization of smaller elements provide better accuracy and closer resemblance to the physical problem, they require longer computation times as opposed to larger elements. Hence, the end user is expected to do the justification of the smallest element number possible. As for the possible element types to be combined in the meshing, there are seven fundamental element geometries with alternative number of intermediate nodes along their edges available. These include beams, triangular and quadrilateral planar elements, triangular and quadrilateral torus elements as well as tetrahedral and hexahedral elements. (Logan 2011)

As the finite element analysis run has finished, the solution is ready for post processing and visualization. Character of the results depend on the application as they can be interpreted as stresses, strains, displacements, or forces, for instance (Hughes 2000). In structural problems, the finite element analysis describes the displacement at each node and stress within each element (Logan 2011).

In biomechanical loadbearing applications, finite element analysis has been applied extensively in bone remodelling analyses and prediction of stress shielding effects, for instance (Zhao et al. 2009, Huiskes et al. 1992). Moreover, finite element analysis can be utilized as a tool for optimizing mechanical performance of devices intended for complex loadbearing conditions. Fibre reinforced composite implants serve as a case in point of an engineering problem where finite element analysis enables computed assessment of the problem. The fibre placement and fibre to matrix volume fraction can be optimized in iterative finite element analysis replicating the initial mechanical studies (Kharazi et al. 2010).

In this study, finite element analysis was utilized iteratively to improve the mechanical properties of the fracture fixation plates with tailored fibre placement pattern. Assessment of critical volume fraction was conducted after each simulation and corresponding changes made to the currently existing TFP pattern for next simulation.

2.6 Tailored fibre placement technology

Popularized by the textile industry, tailored fibre placement (TFP) technology has offered new methods for manufacturing medical devices that require unique features as for their topology and mechanical structure. For instance, embroidering enables accurate production of optimized porous networks that are ideal for scaffold materials in tissue engineering applications (Breier 2015, Rentsch et al. 2014, Rentsch et al. 2010). As embroidery technology can be utilized jointly with CAD techniques, TFP method enables production of patterns containing curves with small radii (Spickenheuer et al. 2008, Mattheij et al. 1998). The possibility to produce stress adapted fibre orientations by TFP was first applied in several nonmedical applications requiring light-weight parts with resistance to complex stresses (Mattheij et al. 1998). Moreover, iterative finite element analysis can be utilized in optimization of the fibre orientation and hence, the mechanical performance of the embroidered construct (Kharazi et al. 2010). Consequently, several features of textile preform, such as porosity, fibre volume content and mechanical properties, can be modified with textile parameters. These include stitch length and orientation, for instance (Breier 2015). A potential application site of such tailored fibre placement network,

a reinforcing textile preform for a loadbearing orthopaedic composite implant, is examined in this study.

The embroidery machine relies on similar fundamental operating mechanism as a sewing machine with two threads running on the opposite sides of a base material. The machine produces lock stitches by winding the upper thread around the lower one. As the needle driving the upper thread pierces the base material, the two threads are laced by a rotary hook. Consequently, the needle is pulled back and the framing system directs the base material to the desired new position. Placement of materials that cannot be deposited with the needle, such as fibre rovings, occurs by means of a rotating roving pipe. Deposition of the roving is directed by rotation of the roving pipe as well as translation of the framing system. Eventually, the roving is fixed onto the base material with zigzag stitches by upper and lower thread. Framing system and roving pipe are navigated by a computer programmed unit. (Breier 2015, Spickenheuer et al. 2008, Mattheij et al. 1998)

The applicable thread materials for embroidery technology cannot simply be determined based on the mechanical properties of the raw thread material. While high tensile strength is crucial for smooth processing of the desired pattern and its eventual performance, various other thread qualities should also be considered. For instance, strength of the material in bending and torsion as well as surface properties of the yarn determine its processing and performance potential. It has been proposed that mono- and multifilament yarns with diameters within the range of 50 µm to 250 µm can be applied in embroidering technology. (Breier 2015)

In this study, intelligent fibre placement method was applied in development of fibre preforms of the plates. The chosen additive manufacturing technique for producing TFP pattern was embroidery technology. Together with additive manufacturing techniques computer aided design methods enable production of mechanically optimized structures without manual processing of the fibres and postprocessing of the cured plates. This approach provides several advantages over low technology methods relying on manual labour (Kang and Fang 2018).

Embroidered fibre pattern with continuous rovings can be optimized to reduce the stress concentrations at critically loaded areas of the plates, such as around the holes (Kharazi et al. 2010). The fibre pattern possessing desired mechanical features can be accurately produced by additive manufacturing based on the CAD model. This enhances predictability of the plate performance and replicability of the manufacturing process as potential

sources of human error are reduced. Moreover, this also enables production of the devices on an industrial scale. (Kang and Fang 2018).

Drilling holes to already cured plates result in suboptimal stress distribution within the plates and consequent development of stress concentrations. This is especially observed with intermittent unidirectional fibres between the holes. The stress concentrations can be reduced, and the plate structure enhanced by means of TFP method (Mattheij et al. 1998). While TFP pattern is optimized considering presence of the holes, it removes the need for drilling and other postprocessing. This also eliminates the exposure of fibre ends to abrasion with the screws. The abrasion may lead to fibre debris formation and eventual inflammation of the surrounding tissues (Uhthoff et al. 2006, Hulbert 1993, Gillet et al. 1985).

3. AIMS OF THE STUDY

Metallic fracture fixation devices have served as golden standard for internal fracture fixation in loadbearing sites since their introduction. As their utilization has a few intrinsic drawbacks, novel methods for fracture treatment are sought after. As for a biomechanically ideal orthopaedic device, flexural stiffness comparable to that of cortical bone is desired along with sufficiently high flexural strength. Such device can provide the fracture site with adequate stability to promote natural ossification while minimizing adverse stress shielding effects. The acceptable flexural strength and flexural stiffness values can be determined by comparing those of clinically applied non-metallic, polymer-based fracture fixation devices. For instance, these quantities are known have significantly lower values in self-reinforced polylactide implants used in canine radial fracture treatment (Saikku-Bäckström et al. 2005, Veiranto et al. 2002). Comparable values have also been achieved with dimethacrylate-based glass fibre reinforced composites (Moritz et al. 2014, Zhao et al. 2009).

Four-point bending is a mechanical testing modality recommended by ISO standard for assessment of metallic fracture fixation plates. In comparison with more isotropic material compositions, highly anisotropic material such as FRCs with unidirectional fibre orientation can be expected to possess superior flexural strength along the axis orthogonal to the plain of the fibres. However, such bending test is a simplification of a clinical situation. In physiological locomotion, the stresses are commonly combinations of tensile and shear elements (Yang et al. 2014). Hence, plates with unidirectional fibre layers have relatively higher flexural strength at the expense of lower torsion resistance. Intermittent unidirectional fibre orientation between the holes is prone to development of stress concentrations at these areas. This may further lead to delamination of the layers under shear forces. Moreover, drilling holes after curing the plates exposes the fibre ends to abrasion with the screws.

The aim of this study was to develop a novel FRC-based load-bearing implant especially for small mammalian antebrachial fracture fixation. TFP technology was the chosen approach to produce the plates. Finite element analysis (FEA) was used as a main tool for optimization of the plate design. Suitability of TFP method and achieved FEA outcomes were assessed by mechanical tests conducted on physical replicas of the studied plate designs.

4. MATERIALS AND METHODS

In this section the process flow of this study is described. Moreover, preparation process of the specimens, methodology of their mechanical testing and the utilized methods are discussed.

4.1 Methodology of the study

This study consisted of three phases including mechanical experiments in phases 1 and 3 as well as a finite element analyses (FEA) in each phase. In phase 1, the FRC plate with unidirectional fibres and pilot TFP plate were mechanically tested. This was followed by simulation of the mechanical tests by FEA, verifying that the model of pilot plate obeys the mechanical data. In phase 2, the structure of pilot TFP plate was optimized by FEA in an iterative process. Consequently, an optimized TFP pattern was achieved. In phase 3, the optimized TFP plate was mechanically tested in a similar manner as the plates in phase 1. This was followed by a final FEA. The workflow of the study is illustrated in Figure 1.

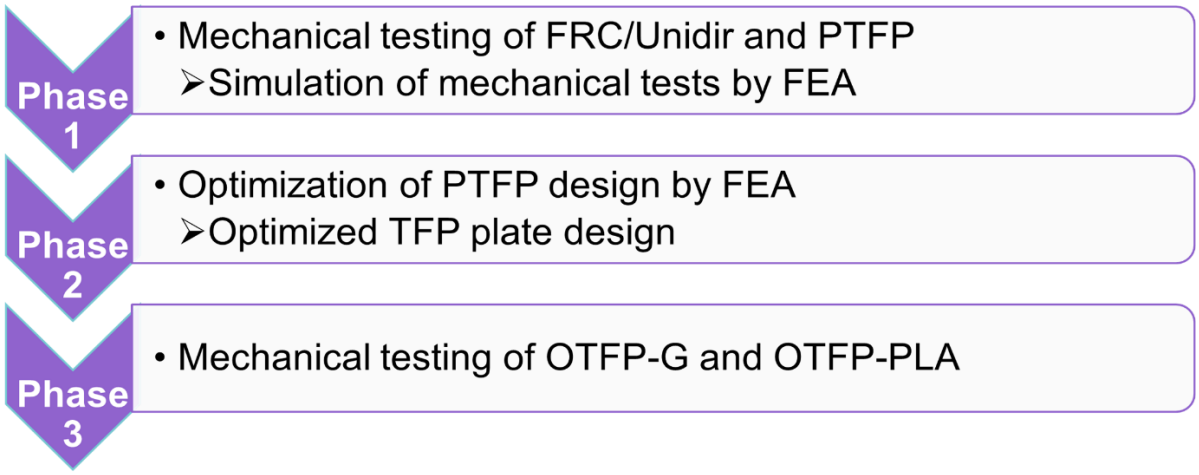


Figure 1. *A flow chart of the study design*

In this study, three different mechanical tests were carried out for each plate group. The plates were tested as such in four-point bending according to the ISO 9585-1998 standard. In addition, constructs of osteotomized chicken tibiae reunited with a plate and six metallic screws were tested in four-point bending and torsion. Testing protocol of the constructs was modified from the one applied by Sod and co-workers (Sod et al. 2005). A summary of the specimen groups tested in this study is represented in Table 1.

Table 1. Summary of the tested specimens

Specimen group	Matrix	Reinforcement phase	Number of plates tested in four-point bending	Number of constructs tested in torsion	Number of constructs tested in four-point bending
FRC/Unidir	Bis-GMA/TEGDMA (60/40)	Continuous unidirectional E-glass fibres	5	5	5
PTFP	Bis-GMA/TEGDMA (60/40)		5	5	5
OTFP-G	Bis-GMA/TEGDMA (60/40)		5	5	5
OTFP-PLA	PLA		5	5	5
Intact chicken tibia				9	7

In phase 2, optimization of the TFP plate design was done by FEA. The optimization was performed as an iterative manner starting from the pilot TFP (PTFP) plate design. During each repetition, the currently existing plate design was assessed in simulations of mechanical tests executed in phase 1. Based on the simulation outcome, the plate design was modified accordingly and tested in the subsequent iteration round. With this approach optimized TFP (OTFP) plate design was achieved. The plate performance was assessed by means of a novel method relying on critical volume fraction comparisons.

Critically loaded volume fraction within each plate was determined and the numerical volume fraction values were utilized in plate design comparisons.

Altogether six groups of FRC plates were prepared and mechanically tested. In phase 1, the plates with unidirectional glass fibre reinforcement (FRC/Unidir group) and plates with the initial tailored fibre placement pattern (PTFP group) were tested. In phase 3, optimized tailored fibre placement plate (OTFP-G) was tested. These plates also contained glass fibre reinforcement and same matrix as FRC/Unidir plates. In addition, plates with optimized tailored fibre placement geometry consisting of polylactide matrix and PLA/E-glass hybrid yarn (OTFP-PLA) were tested. Beside the plates and constructs, intact chicken tibiae were tested in four-point bending and torsion. In each of the mechanical tests, the failure was determined to occur at the first instant with decrease in corresponding mechanical load.

4.2 Preparation of FRC plates

All the composites contained the same polymer matrix consisting of bisphenol A dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) copolymers with respective mass percentages of 60 wt% and 40 wt%. Photoinitiator-activator system applied in polymerization of the resin consisted of camphorquinone (0.7 wt%) and dimethylaminoethyl methacrylate (DMAEMA) (0.7 wt%). Fibre volume content was 0.5 for all plate designs. The plate measurements were 39.9 mm in length, 5.4 mm in width, 1.5 mm in thickness and curvature with radius of 14 mm. The plates had six screw holes the distance between midpoints of adjacent screw holes being 6.9 mm.

The plates with unidirectional fibre reinforcement contained E-glass fibres with diameter of 15 µm and tex number of 2400 (R338-2400, Ahlstrom Glassfibre Oy, Finland). To achieve the aimed volume fraction of 0.5, five fibre rovings were mixed with corresponding amount of resin. The preforms for pilot TFP plates with resin matrix were made of E-glass fibres with tex number of 600. For TFP plates with optimized fibre pattern, E-glass fibres with and tex number of 300 (EC14 300 TD44C, Saint-Gobain Vetrotex, Aachen, Germany) were used. These preforms were also impregnated with the amount of resin resulting in volume fraction of 0.5 as in the case of FRC/Unidir group. A hybrid yarn containing one roving of 300 tex E-glass fibres and 16 rovings of 167 dTex (decitex) PLA was utilized in production of preforms for plates with PLA matrix. The preforms for plates with PLA matrix were embroidered according to the optimized fibre pattern on a PLA film with thickness of 0.2 mm. The embroidered textile preforms were fabricated utilizing an

embroidery machine (ZSK JCZ 0209-550, ZSK Stickmaschinen GmbH, Germany). A summary of the materials used for preparation of the FRC plates is represented in Table 2.

Table 2. Constituents of the FRC plates

Material	Type of material	Manufacturer
Bisphenol-A-glycidyl-methacrylate (BisGMA)	Comonomer	Röhm Chemische Fabrik GmbH, Darmstadt, Germany
Triethylene glycol dimethacrylate (TEGDMA)	Comonomer	Aldrich Chemie GmbH, Steinheim, Germany
Camphorquinone	Photoinitiator	Sigma-Aldrich GmbH, Buchs, Switzerland
Dimethylaminoethyl methacrylate (DMAEMA)	Activator	Fluka Chemie GmbH, Buchs, Switzerland
R338-2400	E-glass fibre	Ahlstrom Glassfibre Oy, Kotka, Finland
EC14 300 TD44C	E-glass fibre	Saint-Gobain Vetrotex, Aa-chen, Germany

The manufacturing process of all BisGMA/TEGDMA composites followed a similar protocol. The glass fibres for FRC/Unidir group were impregnated with resin in a heat cabin (BE600, Memmert GmbH + Co.KG, Germany) at 39 °C for one hour. The prepregs were consequently transferred into individual moulds (92 x 5.9 x 1.7 mm) that were wrapped in aluminium foil and set into a vacuum oven (VO400, Memmert GmbH + Co.KG, Germany) at atmosphere of 10 mbar and temperature of 25 °C for 38 min. The aim of this was to get rid of air pockets between fibres. The prepregs were thereafter covered with additional layer of resin and precured with a dental hand curing device (Elipar S10, 3M ESPE, Seefeld, Germany) for 2 min. The plates were post-cured in a vacuum light oven (Visio Beta vario, 3M ESPE, Seefeld, Germany) for 15 min and in a light oven (Liculite,

Dentsply De Trey GmbH, Dreieich, Germany) for 25 min at ambient temperature. The plates were consequently cut to the right length and screw holes were drilled with a computer programmed milling machine (MDX-40A, Roland, USA).

The preforms for TFP plate groups were impregnated with resin in a heat cabin at 39 °C for one hour residing in their individual moulds (39.9 x 5.4 x 1.5 mm) that were wrapped in aluminium foil. The latter steps of manufacturing process were identical to the ones followed in the preparation of FRC/Unidir plates.

The plates with PLA matrix were produced by hot pressing in a two-piece mould made of aluminium alloy. Before hot pressing, the preforms were dried for three hours at 80 °C and surfaces of the mould were treated with a releasing agent. Consequently, the preforms were transferred into their mould and to a hot press machine. Air from the working zone of the hot press was deflated to 50 mbar and the mould was exposed with pressure of 8 bar. Temperature of the working surface was incrementally risen to 200 °C at the rate of 15 °C/min after which the pressure was increased to 10 bar. After maintaining the temperature of 200 °C for five minutes, the mould was let to cool down to 25 °C within two hours. Consequently, pressure of the mould and vacuum of the working surface were released, and the plates collected.

4.3 Preparation of cadaver tibiae for mechanical testing

The bone samples utilized in this study were chicken tibiae extracted from commercially available chicken drumsticks from four Finnish producers of Atria company (330, A. ja V. Rantalan tila, Atria). The soft tissues along with epiphyseal cartilage were removed by hand and the bones were stored in refrigerator at 8 °C wrapped in wet tissues. The bones were tested within 24 hours after the extraction from soft tissues and were kept moisturized throughout the preparation processes.

From the bones tested in four-point bending, proximal and distal ends were cut off at metaphyseal region with resulting bone length of 70 mm. Consequently, six screw holes with diameter of 1.6 mm were drilled through the diaphyseal section of the bone and an osteotomy dividing the bone into two equally sized halves along its transverse axis was performed. The bone ends were joined with an FRC plate placed on the anterior surface by means of six surgical screws with diameter of 2.0 mm and length of 12 mm (MF Cortex Screw, Synthes, Switzerland).

The bones that were tested in torsion were first embedded in polymethylmethacrylate (PMMA; Vertex Dental B.V., The Netherlands) in a special mould from their both ends.

Prior to embedding, length of the bones was adjusted to 90 mm, resulting in 70 mm span between the PMMA blocks. Thereafter, the screw holes were drilled, osteotomy performed and FRC plate attached in a similar manner as with the bones for bending test.

In addition to the constructs of osteotomized bones joined with FRC plates, control group of bones was prepared for both mechanical testing methods. That is, the diaphyseal proportions remained intact with neither holes being screwed nor osteotomy being performed.

4.4 Mechanical testing of FRC plates in four-point bending

The FRC plates were tested in mechanical four-point bending according to ISO 9585-1990 standard. Consequently, the load span *k* was set to 13.8 mm and the support span (*2k + h)* to 27.6 mm. The radius of cylindrical shaped loading noses and supports (*D1* and *D2*, respectively) was 5.0 mm. A schematic illustration of a four-point bending setup is represented in Figure 2.

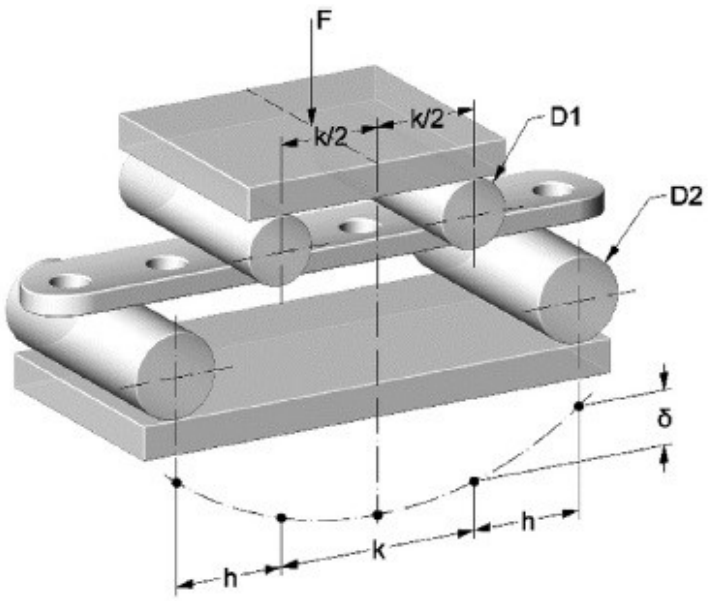


Figure 2. *A schematic illustration of a four-point bending test setup with relevant measures of length labelled (Liesmäki et al. 2019)*

Lloyd material testing machine (model LRX, Lloyd Instruments Ltd., Fareham, England) was utilized to produce loading velocity of 1.0 mm/min applied in posterior-anterior direction. The load-deflection curves were documented by means of a commercial computer software (Nexygen, Lloyd Instruments Ltd., Fareham, England).

The measurement was continued until failure of the plate. Maximum load *F* (N) and the slope of the linear region *S* (N/m) were determined from the load-deflection curves. Based on these quantities, the equivalent bending stiffness *E* (N·m^2) and bending strength *B* (N·m) of the plates were consequently derived for each group according to the formulas

$$E = \frac{(2h + 3k)Sh^2}{12}$$

and

$$B = 0.4Fh,$$

where *E*, *h*, *k*, and *S* are as determined above.

4.5 Mechanical testing of cadaver tibiae with plates in torsion

The constructs of osteotomized chicken bones fixed with FRC plates were tested in torsion according to the setup utilized by Sod and co-workers (Sod et al. 2005) with modifications due to different plate dimensions. A material testing machine (Avalon Technologies, Rochester, MI, USA) was utilized to produce torsion rate of 64°/min applied in earlier studies with similar setup (Zhao et al. 2009). A torsion test in progress is represented in Figure 3.

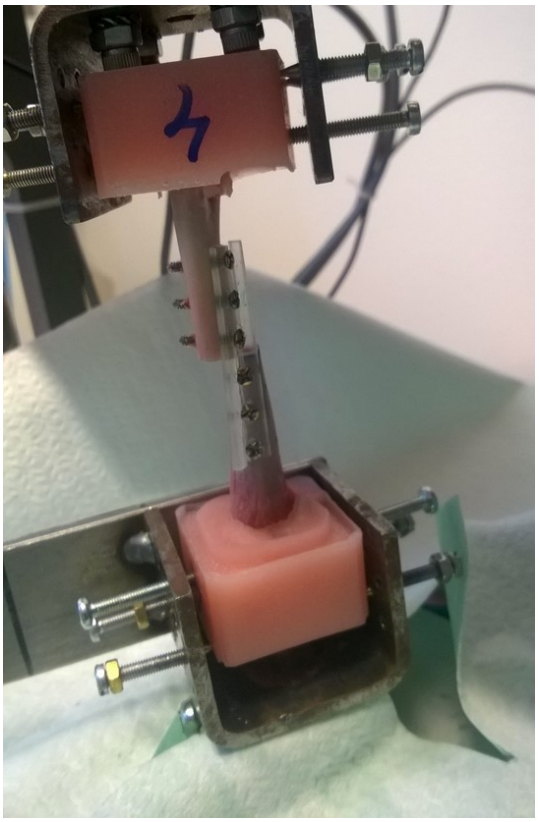


Figure 3. *A torsion test in progress*

To mimic the physiological loading of tibia, the direction of axial torsion was determined so that the proximal end rotates externally in relation to the distal (Yang et al. 2014). The measurement was continued until failure of the bone or the plate, depending on which occurred first. Maximum torque τ (N·m) and angle of rotation α (deg, °) at failure were determined from the moment-angle curve. Based on these quantities, torsional stiffness S_τ (N·m/rad) of the constructs was consequently derived for each group according to the relation

$$S_\tau = \frac{\tau c}{\alpha},$$

where c is the conversion coefficient yielding newton-metres per radian (180°/π) while S_τ, τ, and α are as above.

4.6 Mechanical testing of cadaver tibiae with plates in four-point bending

The constructs of osteotomized chicken bones fixed with FRC plates were tested in mechanical four-point bending according to the setup utilized by Sod and co-workers (Sod

et al. 2005) with modifications due to different plate dimensions. Hence, the load span was set to 27.6 mm and the support span to 55.2 mm. The radius of cylindrical shaped loading noses and supports was 5.0 mm. Lloyd material testing machine (model LRX, Lloyd Instruments Ltd., Fareham, England) was utilized to produce loading velocity of 1.0 mm/min applied in posterior-anterior direction. The load-deflection curves were documented utilizing a commercial computer software (Nexygen, Lloyd Instruments Ltd., Fareham, England). A construct tested in four-point bending is represented in Figure 4.

Figure 4. *A construct tested in four-point bending*

The measurement was continued until failure of the bone or the plate, depending on which occurred first. Maximum load *F* (N) and the slope of the linear region *S* (N/m) were determined from the load-deflection curves. Based on these quantities, the equivalent bending stiffness *E* ($N \cdot m^2$) and bending strength *B* (N·m) of the constructs were consequently derived for each group.

4.7 Optimization of the TFP pattern

The models applied in finite element simulations were engineered according to the test setups applied in the corresponding mechanical test modality. A commercial computer aided engineering programme Abaqus/Standard 2017 (Dassault Systemes Simulia Corp., USA) was utilized.

Identical two-dimensional finite element mesh was used for all plate designs. The two-dimensional pattern was then applied to each layer in third dimension. Due to varying fibre alignments, the total number of elements was different in each plate design. In FRC/Unidir plates, thickness of the layers was set to correspond to the horizontal dimension of the elements. In TFP plates, the meshes were generated utilizing a meshing

software package (AOPS, IPF Dresden, Germany). In this approach, each fibre roving loop was represented by a separate layer of elements whose thickness was determined by Tex number of the fibres and fibre volume content.

All the simulations were performed as general static problems. The element type for plates was C3D8. Values of mechanical quantities describing resin and fibre properties were taken from literature. The bones were modelled as elastic bodies. The element type utilized was C3D8. In all simulations, the screws were modelled as rigid bodies. The osteotomy site was modelled as hard frictionless contact without a gap.

In four-point bending simulations, loading and support noses were modelled as rigid bodies. The element types applied were rigid tri- and quadrilateral 3D elements (R3D3 and R3D4). No movement of the supports was allowed, and the loading noses could move in vertical plane only. In the simulation of the plates, total concentrated force of 100 N was directed through the loading noses. In the simulation of the constructs, the corresponding force was 200 N. Interactions between the testing specimens and loading and support noses were modelled as hard contacts with friction coefficient of 0.05.

In torsion test, one end of the construct was fixed in all directions. In respect of the point laying on construct's longitudinal central axis at the other end, torque of 1 N·m was applied.

Failure mode concept (Cuntze 2006) was applied in assessment of plate performance in simulations. UVARM subroutine was programmed to calculate the five composite failure modes introduced in failure mode concept as well as their mode interaction. The five failure modes are represented in Figure 5.

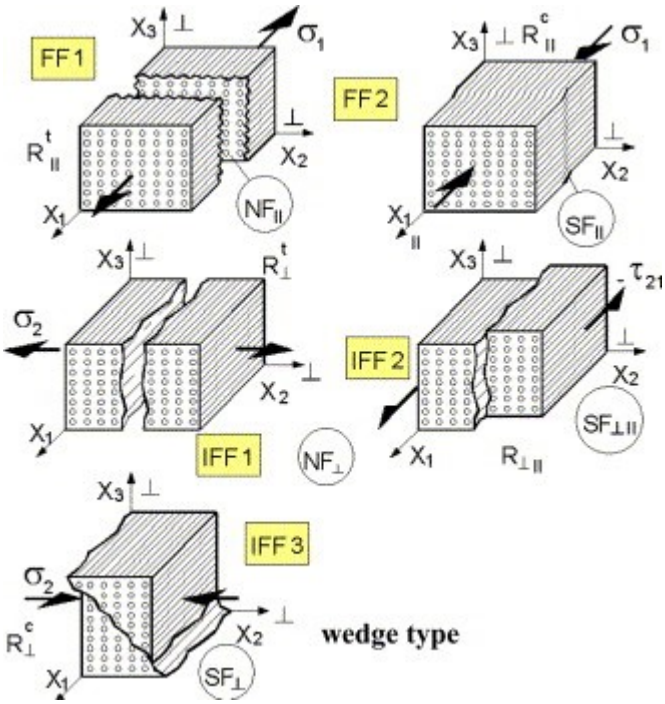


Figure 5. *The five composite failure modes represented in failure mode concept: Tension fibre failure (FF1), compression fibre failure (FF2), and three inter-fibre failure modes (IFF1 – IFF3) (Cuntze 2006).*

A novel assessment method for plate performance was developed. The fundamental concept with this approach was to calculate the volume fraction of critically loaded elements in each plate design. The assessment was based on occurrence of failure mode concept's mode interactions. The elements in each layer were categorized by volume in an ascending order and were then divided into ten groups. Critical volume fractions were first determined for each group of each element layer. Consequently, values of corresponding groups in each layer were averaged. To control the changes in element volumes, average element volumes of each group in each layer were normalized in respect of the average element volume of the group with biggest element volumes in the given layer. Normalized average element volumes of corresponding groups in each layer were then averaged.

Optimization of the plate design was done as an iterative process. Based on the simulation of mechanical tests, changes to the existing finite element mesh representing the loops of the fibre rovings within the plate were made. Consequently, new simulation was conducted. Nine rounds of iteration were run prior to achieving the mesh applied for producing the optimized TFP plates. During the optimization process, the main aims

were achieving acceptable values of structural stiffness, optimizing the fibre volume distribution to provide uniform thickness of the plate, and reducing the critically loaded volume fraction within the plate. Moreover, the proximity of the holes was aimed to reinforce from the mechanical strain resulting from the screws.

As for their contribution to the overall mechanical performance of the plate, the rovings were divided into three group. Fibres oriented along the longitudinal axis of the plate were expected to give rise to flexural strength of the plate. These plates were located at the edges of the plate. The overlapping fibres at the areas between the holes were intended to contribute to torsion resistance of the plate. Lastly, the fibre loops around the holes were expected to give resistance to the strain caused by the screws.

4.8 Statistical analysis

Statistical analysis of the mechanical data was executed with a commercial computer software (SPSS Inc., Chicago, Illinois, USA). Normality of the data was tested with Kolmogorov-Smirnov test and homogeneity of variances with Levene's test. For normally distributed data groups with equal variances, one-way ANOVA and consequent Tuckey's post hoc t-tests were applied while comparing the groups. In the case of non-normal data distribution or unequal variances, Kruskal-Wallis test and consequent post hoc Mann-Whitney *U* tests were performed. The considered level of statistical significance was 0.05.

5. RESULTS

In this section results of the mechanical tests are represented. Results of each testing modality are organized to their respective subchapter.

5.1 Mechanical testing of FRC plates

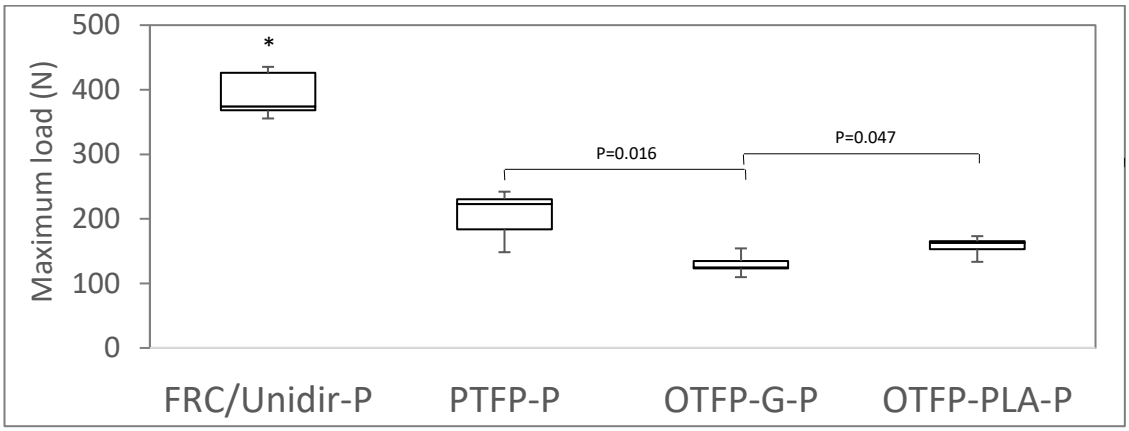
In respect of each studied parameter, Kolmogorov-Smirnov test confirmed the data to be normally distributed. Requirement of equal variances was met in slope and equivalent bending stiffness. One-way ANOVA and consequent Tuckey's post hoc t-test was applied in comparison of the groups in respect of these parameters. Due to unequal variances, distributions of maximum load and bending strength were assessed by Kruskal-Wallis test and consequent post hoc Mann-Whitney pairwise comparisons. The mechanical parameters determined in the four-point bending tests conducted on different FRC plate designs are represented in Table 3. Results of the statistical analysis conducted on mechanical data are represented in Figure 6.

Table 3. Mechanical properties of the different plate groups in four-point bending

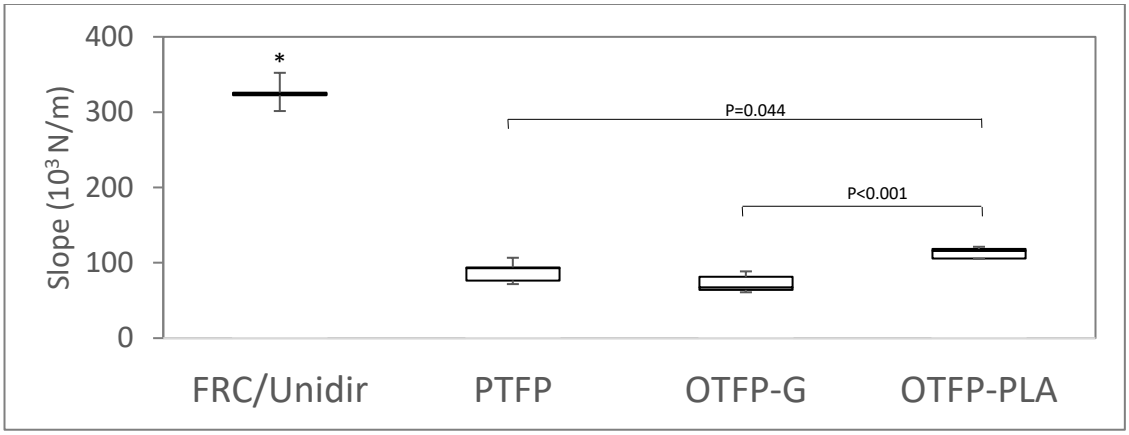
Plate group	**Maximum load *F*, N** [b]	**Slope *S*, N/m** [a]	**Equivalent bending stiffness *E*, N·m²** [a]	**Bending strength *B*, N·m** [b]
FRC/Unidir	373.9 (368.0, 426.1)	325.2 ± 18.1	0.071 ± 0.004	1.03 (1.02, 1.18)
PTFP	222.8 (183.8, 230.5)	88.4 ± 14.2	0.019 ± 0.003	0.63 (0.61, 0.80)
OTFP-G	124.6 (123.2, 134.7)	72.4 ± 12.0	0.016 ± 0.003	0.343 (0.340, 0.372)
OTFP-PLA	162.7 (152.7, 165.1)	113.4 ± 7.4	0.025 ± 0.002	0.45 (0.42, 0.46)

[a] Data represented as mean ± standard deviation

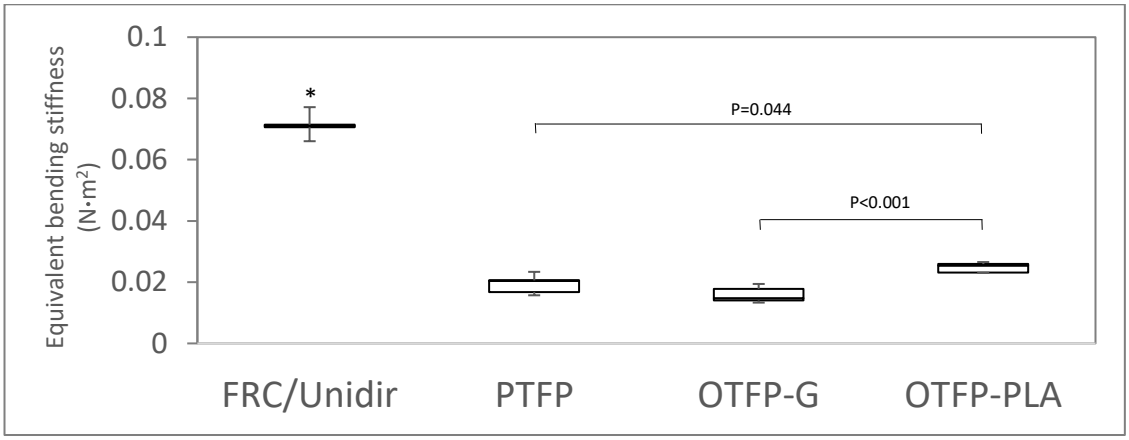
[b] Data represented as median (1st, 3rd quartile)



*P=0.008



*P<0.001



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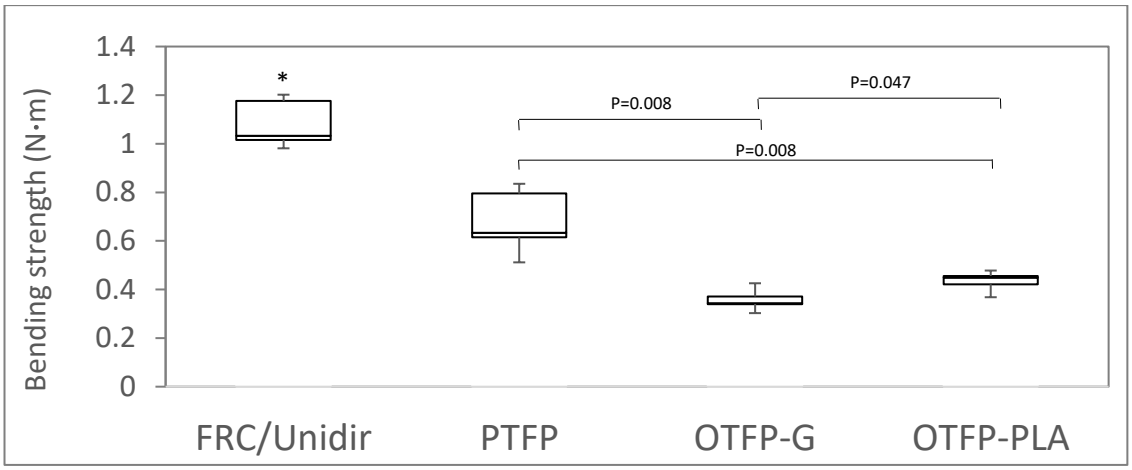


*P=0.008

Figure 6. *Boxplots for maximum load, slope, equivalent bending stiffness, and bending strength of the plates assessed in the four-point bending*

Maximum load in group FRC/Unidir was statistically significantly greater than that in any other group (P=0.008). Maximum load in group OTFP-G was statistically significantly lower than that in groups PTFP and OTFP-G (P=0.016 and P=0.047, respectively). There were no other statistically significant differences in maximum load.

In group FRC/Unidir, the slope of load-deflection curve was statistically significantly greater than that in any other group (P<0.001). Slope of the group OTFP-PLA was statistically significantly greater than that in groups OTFP-G and PTFP (P<0.001 and P=0.044, respectively). There were no other statistically significant differences in slope.

Equivalent bending stiffness of the group FRC/Unidir was statistically significantly higher than that in any other group (P<0.001). Equivalent bending stiffness of the group OTFP-PLA was statistically significantly higher than that in groups OTFP-G and PTFP (P<0.001 and P=0.044, respectively). There were no other statistically significant differences in flexural stiffness.

Bending strength in group FRC/Unidir was statistically significantly higher than that in any other group (P=0.008) Bending strength in group PTFP was statistically significantly higher than that in groups OTFP-G and OTFP-PLA (P=0.008 and P=0.008). In group OTFP-PLA, bending strength was statistically significantly higher than that in group OTFP-G (P=0.047). There were no other statistically significant differences in bending strength.

5.2 Mechanical testing of cadaver tibiae with plates in torsion

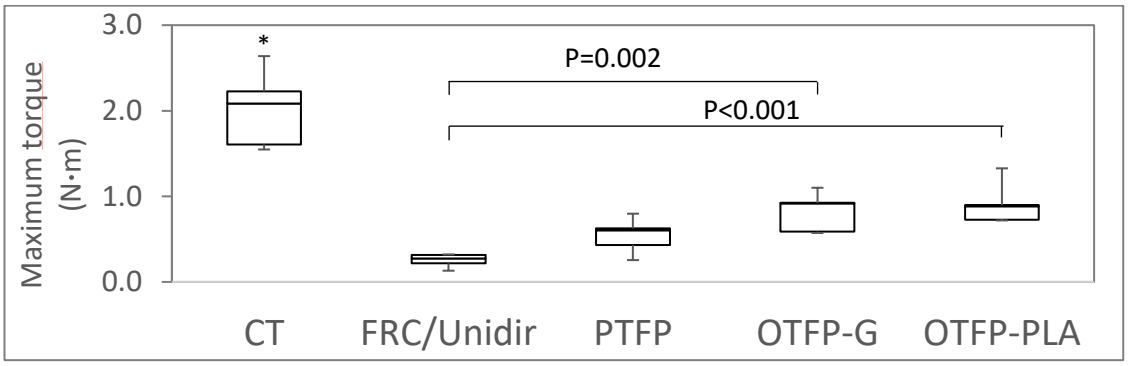
In respect of each studied parameter, Kolmogorov-Smirnov test confirmed the data to be normally distributed. Requirement of equal variances was met in maximum torque. One-way ANOVA and consequent Tuckey's post hoc t-test was applied in comparison of the groups in respect of this parameter. Due to unequal variances, distributions of angle at maximum torque and torsional stiffness were assessed by Kruskal-Wallis test and consequent post hoc Mann-Whitney pairwise comparisons. The mechanical parameters determined in the torsion tests conducted on constructs of osteotomized chicken tibia reunited with different FRC plates are represented in Table 4. Results of the statistical analysis conducted on mechanical data are represented in Figure 7.

Table 4. Mechanical properties of the different constructs in torsion

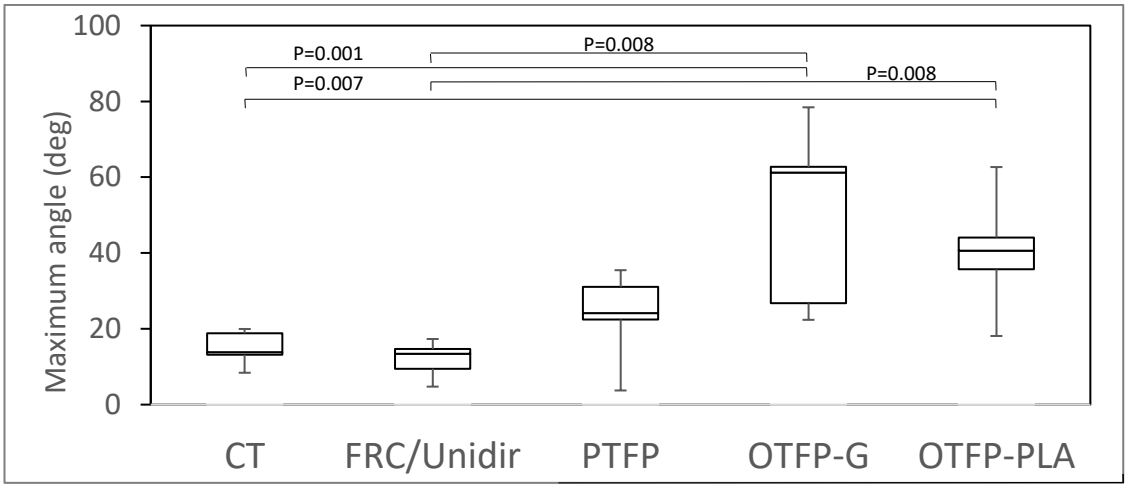
Construct group	**Maximum torque τ, N·m** [a]	**Angle at τ α, °** [b]	**Torsional stiffness S_τ, N·m/rad** [b]
FRC/Unidir	0.25 ± 0.08	13.42 (9.48, 14.64)	1.07 (1.04, 1.58)
PTFP	0.54 ± 0.21	24.12 (22.47, 31.10)	1.41 (1.06, 2.17)
OTFP-G	0.82 ± 0.23	61.18 (26.73, 62.73)	1.51 (1.33, 2.46)
OTFP-PLA	0.91 ± 0.25	40.58 (35.68, 44.09)	2.28 (2.25, 2.41)
Chicken tibia	2.01 ± 0.37	1.07 (1.04, 1.58)	13.80 (13.15, 18.80)

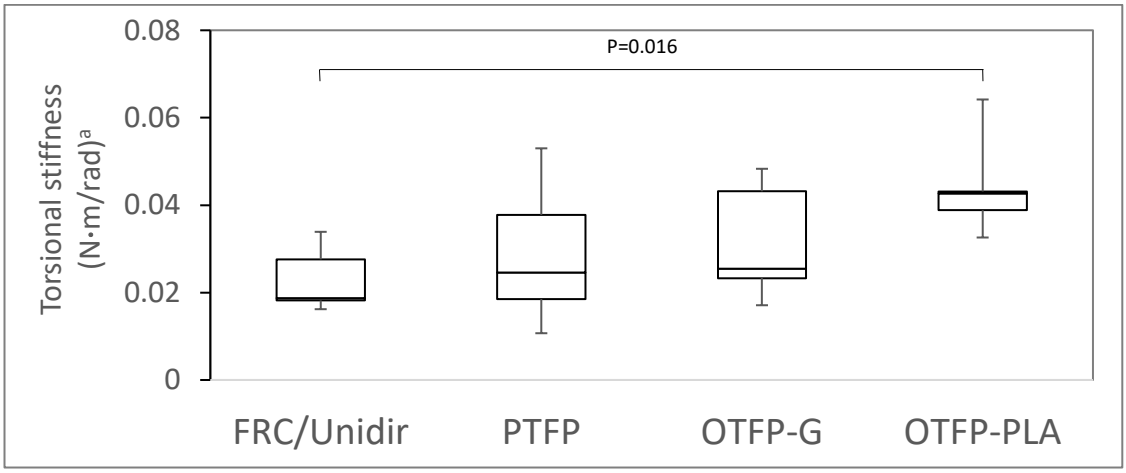
[a] Data represented as mean ± standard deviation

[b] Data represented as median (1st, 3rd quartile)



*P<0.001





[a] Group intact chicken tibia excluded from the boxplot (13.80 (13.15, 18.80) N·m/rad, P=0.001)

Figure 7. *Boxplots for maximum torque, angle of rotation at maximum torque, and torsional stiffness of the constructs assessed in the four-point bending*

In groups OTFP-G and OTFP-PLA, the maximum torque resistance was statistically significantly higher than that of group FRC/Unidir (P=0.002 and P<0.001, respectively). In addition, maximum torque resistance was statistically significantly higher in intact chicken tibia group than in any other group (P<0.001). There were no other statistically significant differences in maximum torque.

In groups OTFP-G and OTFP-PLA, the angle at maximum torque was statistically significantly higher than that in group FRC/Unidir (P=0.008 and P=0.008, respectively). The angles at maximum torque in groups OTFP-G and OTFP-PLA were also statistically significantly higher than that in intact chicken tibia group (P=0.001 and P=0.007, respectively). There were no other statistically significant differences in angle at maximum torque.

In group OTFP-PLA, torsional stiffness was statistically significantly higher than that in group FRC/Unidir (P=0.016). Torsional stiffness of the group intact chicken tibia was also statistically significantly higher than that in any other group (P=0.001). There were no other statistically significant differences in torsional stiffness.

5.3 Mechanical testing of cadaver tibiae with plates in four-point bending

In respect of each studied parameter, Kolmogorov-Smirnov test confirmed the data to be normally distributed. Requirement of equal variances was met in every parameter. One-way ANOVA and consequent Tuckey's post hoc t-test was applied in comparison of the groups. The mechanical parameters determined in the four-point bending tests conducted on constructs of osteotomized chicken tibia reunited with different FRC plates are represented in Table 5. Results of the statistical analysis conducted on mechanical data are represented in Figure 8.

Table 5. Mechanical properties of the different constructs in four-point bending

Construct group	**Maximum load *F*, N**	**Slope *S*, N/m**	**Equivalent bending stiffness *E*, N·m^2**	**Bending strength *B*, N·m**
FRC/Unidir	225.8 ± 83.6	177.0 ± 31.0	0.31 ± 0.05	1.25 ± 0.46
PTFP	266.3 ± 30.4	135.1 ± 42.2	0.24 ± 0.07	1.47 ± 0.17
OTFP-G	262.4 ± 55.1	218.8 ± 62.5	0.38 ± 0.11	1.45 ± 0.30
OTFP-PLA	234.8 ± 79.8	180.8 ± 37.3	0.32 ± 0.07	1.30 ± 0.44
Chicken tibia	308.3 ± 54.2	167.4 ± 43.6	0.29 ± 0.08	1.70 ± 0.30

All data represented as mean ± standard deviation

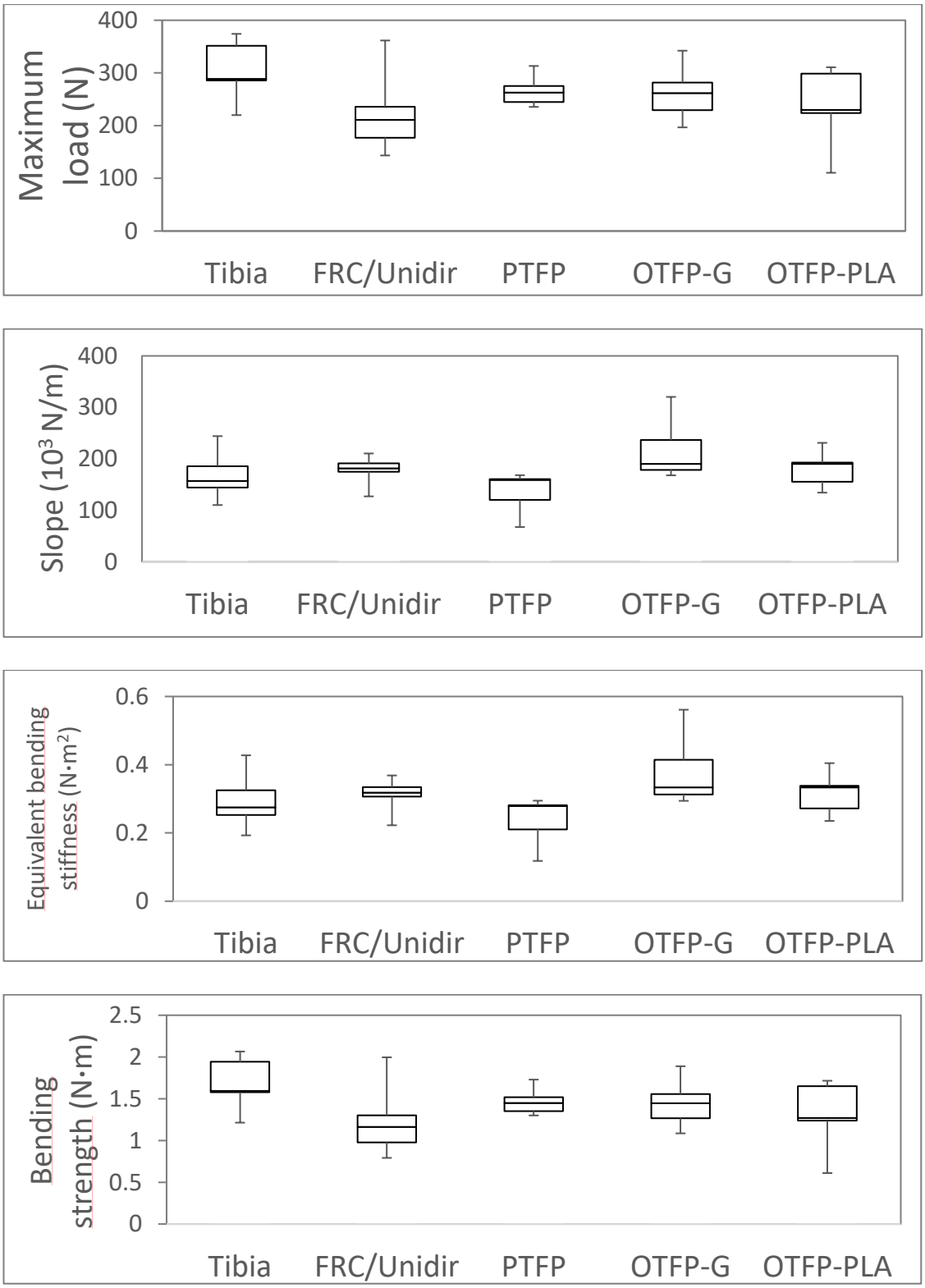


Figure 8. *Boxplots for maximum load, slope, equivalent bending stiffness, and bending strength of the constructs assessed in the four-point bending*

No statistically significant differences between the groups were observed in four-point bending of the constructs in respect of any measured parameter.

6. DISCUSSION

The aim of this study was to develop a composite-based load-bearing fracture fixation device that could resolve biomechanical issues related to their metallic equivalents. Unphysiological underloading of the bone and development of plate-induced osteopenia is an acknowledged major drawback of the conventional metallic fracture fixation devices (Huiskes et al. 1992, Uhthoff and Dubuc 1971). Successful efforts made in decreasing the stress-shielding effects include applying different surgical techniques, engineering conventionally used materials into shapes with reduced structural stiffness, as well as discovering novel material choices with lower rigidity (Woo et al. 1983).

The clinical demand for fracture fixation devices with biomechanical properties comparable to those of cortical bone is emphasized in the patient groups that are especially prone to stress shielding and effects of long-term exposure to rigid plating. Such groups include individuals with pre-existing decrease in bone mineral density and impaired bone quality due to high age or underlying medical conditions, along with skeletally immature paediatric and adolescent patients (Tinubu and Scalea 2015, Kelly et al. 2013, May et al. 2013, Hollevoet et al. 2011, Hollevoet and Verdonk 2003). In the field of veterinary medicine, stress shielding is a notable problem among small domestic mammals (Farrell 2016, Harasen 2003, Muir 1997).

Commercially available universal biomaterial for treating different kinds of hard tissue lesions does not exist (Puska et al. 2013). This sets the stage for study of novel material choices. Fibre reinforced composites have been successfully utilized in various clinical applications (Vallittu 2017, Ramakrishna et al. 2001, Vallittu 1999, Evans and Gregson 1998, Karmaker et al. 1997). This is due to their tailorability to promote isoelastic properties with cortical bone along with good biocompatibility and osseointegration (Piitulainen et al. 2015, Aitasalo et al. 2014, Moritz et al. 2014, Zhao et al. 2009, Tuusa et al. 2008, Tuusa et al. 2007). Both biostable and bioresorbable material candidates have been proven to be suitable for load bearing FRC-based devices (Piitulainen et al. 2015, Aitasalo et al. 2014, Saikku-Bäckstöm et al. 2005, Saikku-Bäckström et al. 2000). FRCs can be fabricated into highly anisotropic material compositions high load bearing capacity (Moritz 2014, Zhao et al. 2009).

The growing market of implantable medical devices has revealed the shortcomings of conventional manufacturing methods in delivering products with high quality and consistency in an efficient manner (Kang and Fang 2018). Despite new fabrication technologies are adopted to biomaterial research in an accelerating pace, few have become generally applied in industrial production. Hence, production of composite implants with continuous fibres and thermosetting matrix is still heavily centred around hand lay-up fabrication method where manual labour is in key position (Migliaresi 2013).

In this study, isoelastic properties with cortical bone were aimed to achieve by means of tailored fibre placement pattern determined and optimized with finite element analysis. Finite element analysis is commonly utilized in biomechanical applications for analysing and optimizing stress-strain relations of complex geometries (Kharazi et al. 2010, Zhao et al. 2009, Huiskes et al. 1992). Tailored fibre placement is an additive manufacturing modality enabling fabrication of physical structures determined by FEA or other CAD methods (Breier 2015, Spickenheuer et al. 2008, Mattheij et al. 1998). The approach was utilized in this study to produce mechanically optimized plates with reduced need for their manual processing.

In mechanical four-point bending tests, the plates with unidirectional fibres promoted superior mechanical properties to other plate designs as anticipated. However, strains in physiological conditions contain both tensile and shear elements. Under loads directed to the fibres in angles differing from orthogonal, performance of unidirectional plates highly relies on contact forces of the matrix material and the fibres. This makes them prone to delamination and development of inter fibre fissures. In bending tests of the plates, the plates with unidirectional reinforcement were observed to develop a crack propagating through the plate before the eventual material failure. This collapse of the longitudinal curvature of the plate would implicate failure in a clinical context. To complement the tensile test, a torsion test was chosen as the other mechanical testing modality.

In torsion tests, breaking of unidirectionally reinforced plates into two or more individual pieces was emphasized. According to the torsion test, optimized TFP plate design provided statistically significantly higher ultimate torsion resistance as opposed to the unidirectional plate. This was observed with both studied material compositions of the optimized plate. Along with improved torsional resistance, reinforcement of the screw holes in the optimized plate was achieved. However, this was achieved at the expense of equivalent bending stiffness and flexural strength.

Bending tests conducted on constructs of chicken tibiae reunited with an FRC plate revealed no statistically significant differences. Considering the differences observed in other mechanical testing setups, this was rather unexpected. Most probably the reason for nondifference between the groups was due to the insufficient structural rigidity of the chicken bones. As the device under development is intended to be utilized in small mammals, especially toy-breed dogs, mammalian bone would have been an ideal material option for testing. However, due to poor accessibility of sufficiently many similar bones to provide results with acceptable statistical reliability we decided to opt for chicken bones. The reason for this was good accessibility of commercially available chicken drumsticks and high resemblance in size and shape of tibiae between individuals.

Due to our future aim to develop a completely bioresorbable FRC-based plate with controlled degradation-related strength reduction, a semi-resorbable plate design was tested in this study along its biostable replica. PLA was the biopolymer of choice due to its proven applicability for load bearing applications (Saikku-Bäckström et al. 2005, Saikku-Bäckström et al. 2000). Plates with both material compositions promoted comparable properties to each other in the mechanical tests. Prospects of this development process involve studies of plate designs with increased level of bioresorbability, cyclic mechanical loading tests, and studies of plate properties during the degradation process along with further optimization of the plate design.

Tailored fibre placement by embroidery technology was proven to be a suitable approach for loadbearing fracture fixation plates. In the upcoming studies, production methods with even higher level of automation, for instance stereolithographic 3D printing of impregnated fibres, could be experimented on.

7. CONCLUSIONS

Tailored fibre placement method can be applied to producing loadbearing orthopaedic fracture fixation devices with predetermined fibre pattern possessing optimized mechanical properties. TFP decreases need of manual processing while providing higher consistency and efficacy for industrial production. Torsional properties of the plate were succeeded to enhance with TFP method. Despite flexural properties of the optimized plate were inferior to those of the pilot plate they were still at acceptable level in comparison with those of cortical bone.

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