Tampereen yliopisto

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IN VITRO DEGRADATION TESTING OF BIODEGRADABLE POLYMERS

Physical and mechanical properties with applicable standards

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ABSTRACT

Riina Paalijärvi: *In vitro* degradation testing of biodegradable polymers: Physical and mechanical properties with applicable standards
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The use of polymers in biomedical applications has expanded significantly in the last decades due to their diversity and customizable properties. Moreover, the use of biodegradable polymers as an alternative to biostable polymers has grown rapidly due to numerous benefits associated with temporary applications, as well as the rapid development of modern technologies such as tissue engineering, gene therapy and controlled drug delivery. Controlling the degradation in a desired way, is one of the main objectives in the use of biodegradable polymers but due to complexity of the process, it remains to be one of the biggest challenges as well.

Hence, the purpose of this thesis is to explore test methods for physical and mechanical properties that could be used to test the *in vitro* degradation of biodegradable polymers. In addition, the aim is to analyze how the increased selection of materials has affected to chosen characterization methods and to investigate the current state of standardization of *in vitro* degradation testing. The scope of this thesis is limited to testing the physical and mechanical properties, and therefore do not include, for example, biological evaluation.

Based on the literature review, *in vitro* degradation testing is often performed by monitoring the percentual decrease in biomaterial mass and molecular weight, water absorption and changes in material- and application-dependent properties. The tests should be conducted mimicking the conditions of the final use. Especially, for characterizing the mechanical properties of the polymer, numerous static and dynamic test methods are available, from which the most appropriate ones for the application should be selected. Only a few standards are available for the implementation of *in vitro* degradation testing. Therefore, in many cases it is necessary to apply other standards to fit the purpose.

From this review, it is clear that the aim for the future of *in vitro* degradation testing should be creating more uniform and consistent guidelines that would make the tests more united and comparable with each other. In addition, further standardization for different materials is required. To make this possible, deeper understanding of the biological processes as well as technological advances are needed.

Keywords: Biodegradable polymers, degradation testing, *in vitro*, characterization methods, standardization

TIIVISTELMÄ

Riina Paalijärvi: Biohajoavien polymeerien *in vitro* -hajoamistestaus: Fysikaaliset ja mekaaniset ominaisuudet sekä soveltuvat standardit
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Polymeerien käyttö biolääketieteen sovelluksissa on laajentunut merkittävästi viime vuosikymmeninä niiden monipuolisten ja muokattavien ominaisuuksiensa ansiosta. Lisäksi biohajoavien polymeerien käyttö vaihtoehtoisena ratkaisuna biostabiileille polymeereille on kasvanut nopeasti johtuen väliaikaisten sovelluksien lukuisista eduista ja nykyaikaisten teknologioiden, kuten kudostekniikan, geeniterapian ja kontrolloidun lääkeluovutuksen nopeasta kehittymisestä. Hajoamisen kontrollointi halutulla tavalla on yksi tärkeimmistä tavoitteista biohajoavien polymeerien käytössä, mutta prosessin monimutkaisuuden vuoksi se on edelleen myös yksi suurimmista haasteista.

Tämän opinnäytetyön tarkoituksena on tutkia fysikaalisten ja mekaanisten ominaisuuksien testausmenetelmiä, joilla voitaisiin tutkia biohajoavien polymeerien *in vitro* -hajoamista. Lisäksi tavoitteena on analysoida, miten materiaalivalikoiman kasvaminen on vaikuttanut valittuihin karakterisointimenetelmiin ja tutkia *in vitro* -hajoamistestauksen standardisoinnin nykytilaa. Tutkielman laajuus rajoittuu fysikaalisten ja mekaanisten ominaisuuksien testaamiseen, eikä näin ollen sisällä esimerkiksi biologisia testejä.

Kirjallisuuskatsauksen perusteella *in vitro* -hajoamistestaus suoritetaan usein seuraamalla biomateriaalin massan ja moolimassan prosentuaalista häviötä, veden imeytymistä sekä sovelluskohteesta ja materiaalista riippuvien ominaisuuksien muutoksia. Testaus tulee suorittaa mahdollisimman tarkasti jäljitellen aiottuja käyttöolosuhteita. Erityisesti polymeerin mekaanisten ominaisuuksien karakterisoinnille on lukuisia staattisia ja dynaamisia testimenetelmiä, joista sopivat tulee valita yksilöllisesti tietylle sovelluskohteelle. Vain muutamia standardeja on löydettävissä *in vitro* -hajoamistestien toteuttamiselle. Näin ollen useimmissa tapauksissa on tarpeen soveltaa muita standardeja.

Kirjallisuuskatsauksen perusteella on selvää, että *in vitro* -hajoamistestauksen tulevaisuuden tavoitteena on yhtenäisempien ja johdonmukaisempien ohjeistuksien luominen, joka tekisi testeistä yhtenäisempiä ja vertailukelpoisempia keskenään. Lisäksi tarve on laajemmalle standardisoinnille eri materiaaleja varten. Jotta tämä olisi mahdollista, tarvitaan kuitenkin syvempää ymmärrystä biologisista prosesseista sekä teknologian edistymistä.

Avainsanat: Biohajoavat polymeerit, hajoamistestaus, *in vitro*, karakterisointimenetelmät, standardisointi

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Riina Paalijärvi

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LIST OF SYMBOLS AND ABBREVIATIONS

3D	Three-dimensional
DSC	Differential scanning calorimetry
DMA	Dynamic mechanical analysis
ECM	Extracellular matrix
FTIR	Fourier-transform infrared spectroscopy
GPC	Gel permeation chromatography
HA	Hyaluronic acid
PBS	Phosphate buffered saline
PCL	Polycaprolactone
PDLLA	Poly(DL-lactic acid)
PGA	Polyglycolide
PLA	Polylactic acid
PLGA	Poly(lactide-*co*-glycolide)
PLLA	Poly(L-lactic acid)
SAOS	Small amplitude oscillatory shear
SEC	Size exclusion chromatography
SEM	Scanning electron microscope
SMP	Shape memory polymer
TE	Tissue Engineering
TGA	Thermogravimetric analysis
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction analysis
α	Alfa
E	Modulus of elasticity, Young's modulus
E'	Storage modulus
E''	Loss modulus
E_c	Creep modulus
E_r	Relaxation modulus
G	Shear modulus
$G*$	Complex of shear modulus
G'	Shear storage modulus
G''	Shear loss modulus
M_n	Number-averaged molecular weight
M_v	Viscosimetric molecular weight
M_w	Weight-averaged molecular weight
η	Intrinsic viscosity
T_g	Glass transition temperature
tan δ	Loss angle
tan Δ	Damping coefficient

1. INTRODUCTION

One major change in the field of medical biomaterials in recent decades has been the increasing use of biodegradable polymers as an alternative to biostable polymers. One of the main factors in this change has been the numerous advantages that can be achieved with biodegradable polymers that are completely eliminated from the body compared to biostable ones. These advantages, such as avoidance of a second surgery and elimination of potential biocompatibility problems associated with long-term applications, make biodegradable polymers suitable for a wide variety of short-term applications. [1] Another major contributor in the increasing use of biodegradable polymers in medical applications is the continuous development of modern technologies that rely on biodegradable materials. For example, biodegradable polymers are important in tissue engineering, controlled drug delivery, gene therapy and other specialities that require biological substitutes as a basis. [2] The development of these specialties has greatly influenced the way polymers are seen in biomedical applications and what properties are required of them. As a result, material options have increased rapidly, and the selection is wider than ever before.

The wide range of biodegradable polymers can be roughly classified by their origin into natural and synthetic polymers or by their mode of degradation. All of these materials have their unique properties, but they all still require biocompatibility from both the material itself and the resulting degradation products, as well as controlled properties over the degradation time that should correspond to the healing process. However, a successful outcome depends on multiple factors on both the material and tissue side and even varies over time. Therefore, ideal biomaterial does not exist, but instead variety of materials are needed, and each application must be tailored individually. [2] Given to their complexity, predicting degradation process can be especially difficult and different factors and circumstances must be carefully considered. For example, mechanical properties should be optimized so that the mechanical strength is maintained long enough for the healing process [3]. This requires a number of tests designed for a specific application and choosing the right test methods is one of the many challenges when using biodegradable polymers as biomaterials.

Thus, this thesis provides a literature review on the topic of *in vitro* degradation testing for biodegradable polymers in biomedical applications with a focus on the characterization of physical and mechanical properties during the degradation. Other properties that affect the optimization of the degradation process as well as the testing of the biocompatibility are excluded from this review. The aim of this thesis is to explore physical and mechanical evaluation methods that could be used for an *in vitro* degradation characterization for biodegradable polymers. In addition, the aim is to analyse how the increased material selection has affected and will affect to the appropriate method choices and to investigate the extent to which these methods have been standardized for these applications or can be modified for their use.

The first part of this thesis reviews biodegradable polymers as biomaterials and introduces their degradation methods. In addition, the development of the material selection over the years and the most commonly used polymers in medical applications and their properties are presented. Next, the physical and mechanical test methods for *in vitro* degradation testing are examined. At the same time, possible existing standards as well as other standards that could be applied for this application are discussed. Finally, the future prospects of these methods are considered from the perspective of the growing range of biodegradable polymers and the results of this review are summarized.

2. BIODEGRABABLE POLYMERS

Since the beginning of times, people have been searching for materials and tools that would provide a healthier and longer life. Thus, although the discipline of biomaterials as we know it is considered to be a relatively new field, its applications can be traced back to thousands of years BC. [4, p. 4] In the present day, the term biomaterial is commonly defined according to Williams [5] as “material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body”. In other words, biomaterials are any materials intended to be biocompatible with the human body and are designed to mimic the body tissues as much as possible so that they can temporarily or permanently replace the necessary functions of the tissue or organ [4, p. 8]. Because the human body is extremely complex system, the design of suitable biomaterials requires continuous development, wide material selection and multidisciplinary expertise.

Polymers offer a lot of possibilities in the biomedical field due to their diversity and easily convertible properties. Generally, polymers have high biocompatibility and can be easily processed to complex forms, such as plates, fibres, membranes, tubes, and sheets [4, pp. 75–81]. The term biocompatibility generally means that the material is able to perform accordingly in specific application without causing an inappropriate host response [5]. They have a wide variety of mechanical, thermal, electrical, and chemical properties that can be tailored to suit different applications and the behaviour can be enhanced by combining them with different materials as composites [6]. Because of their cost-effective synthesis and tailorable structures that provide appropriate biomimetic, interfacial, and physical properties, polymers are extensively used in many biomedical applications including single-use devices. In addition, polymers have the advantages over other biomaterials in terms of lower density, advanced manufacturing techniques, such as three-dimensional (3D) printing, and the great potential for degradation. [7]

As the temporary existence of materials inside the body has increased preferability in medical applications, the paradigm has shifted from biostable polymers to biodegradable ones. The change has offered multiple benefits from overcoming long-term biocompatibility issues to rapid development of modern technologies such as tissue engineering (TE) and controlled drug delivery. The term biodegradable polymer refers to those polymers which degrade *in vivo* and *in vitro* either into the body’s normal metabolites or into end products that can otherwise be eliminated from the body though metabolism. [1]

However, the term “biodegradable” is not only used in the context of biomedical materials, and therefore the term “bioabsorbable” is often used instead. These terms are often used varyingly but bioabsorbable is more specific about what happens to the end-products and means polymers that can be absorbed into the human body by degradation or dissolution. In other words, “biodegradable” considers more about what happens to the material itself disregarding how end-products are eliminated and “bioabsorbable” focuses more on the host metabolism to end-products. [8]

Many properties are required of a biodegradable polymer and most importantly it should be biocompatible and not evoke any harmful interactions inside the body. In addition, the material should have acceptable shelf life, appropriate mechanical properties for specific application and degradation time, both of which correspond to the healing time of the tissue and the degradation products must be non-toxic and able to be completely eliminated from the body. [9] However, many factors, such as the chemical and physical properties of the material and its structure and shape affect degradation and the tissue response to the biomaterial, and therefore each application must be designed individually. Particular attention needs to be paid to degradation products, as the properties and biocompatibility of the polymer vary during degradation and over time, and the material must meet the needs of the tissue for the required time. [2] When designing a biodegradable polymer construct, biocompatibility, processability to the application and ability to degrade in a controlled manner under biological conditions are generally important considerations [7].

The use of polymeric biomaterials is rapidly increasing due to their versatility, ability to be synthesized with a wide range of properties and are suitable for many new medical applications. Each application requires complex and unique properties, so it is necessary to develop a wide choice of options that can properly mimic the tissue or organ under consideration. Current applications for biodegradable polymers vary from therapeutic devices to pharmacological and tissue engineering applications. Examples of these include all sized implants like screws and sutures, membranes, meshes, temporary prostheses, scaffolds, and drug-delivery vehicles. [2] Consequently, as technology and understanding of the human body advances, biodegradable polymers have potential use in almost any medical application and innovative solutions from new perspectives are constantly needed and developed.

2.1 Material selection

Biodegradable polymers can be categorized either by their origin to natural and synthetic polymers or by their degradation method to enzymatically degradable polymers and hydrologically degradable polymers. However, in general, most of the polymers of natural origin undergo enzymatic degradation whereas synthetic commonly degrade hydrolytically. [2] Both polymer groups have been extensively used in biomedical applications and have their own advantages and challenges. Natural polymers inevitably have an excellent biocompatibility, and their structure provides unique properties because it naturally mimics normal tissues and organs of the body. However, they have their disadvantages that has slowed down their use in the medical field. With natural polymers comes a bigger risk of infection and they can be difficult to implement due to batch-to-bath variations in properties and unstable availability of materials. Synthetic polymers provide an excellent option to address these problems. Their easy processability, ability to create a wide range properties to suit a particular application and possibility to control their degradation rate very precisely makes them very popular as biomaterials and has led to the rapid development of polymeric materials in biomedical applications. [1][2]

The first discoveries using natural polymers as biomaterials can be dated back to thousands of years, but modern uses of polymeric biomaterials only started during the second world war in the 1940s. Early uses of polymeric implants were rubber elastic and glassy polymers and creation of fibre formed polymers made, for example, vascular grafts possible. [4, pp. 1−14] For a long time, non-degradable, inert polymers with rigid structure dominated the field of polymeric biomaterials creating conventional medical implants for applications such as orthopaedics and dentistry. When the possible adverse effects of inert and stable polymers, such as removal of the implants and irritations were recognized, the research began to move towards temporary and advanced implants and medical devices by emphasizing biodegradable materials. [10] One of the first uses of biodegradable polymer in medical applications were as suture material by using polylactic acid (PLA). Furthermore, biodegradable implants were found to possibly increase the ultimate bone strength by transferring the load to bone in a controlled rate as the material degrades and the bone heals. Thus, it allows to remove a problem of stress-shielding, which is common issue with metal implants. [6]

In the 1990s the rise of tissue engineering increased the use of biodegradable polymers even more and in the last decades biodegradable polymers are being developed in many new areas such as surface treatments, nanotechnological approaches and drug delivery [4, pp. 1−14]. At the same time, the common requirements for the polymeric biomaterials

have become more complex and more active function is expected from the material side. In addition to providing mechanical support that gradually decreases during healing, more demanding requirements such as ability to induce tissue growth or ability to incorporate molecules like cells or growth factors are often needed [3]. Currently biodegradable polymers are often expected to have excellent biocompatibility while also being bioactive and biomimetic [11].

Current and future trends of biodegradable polymers are discussed in more detail later in section 2.2. First, however, some commonly used biodegradable polymers and their properties will be introduced.

2.1.1 Synthetic biodegradable polymers

Synthetic polymers are widely used in biomedical applications due to their tailorable and predictable properties as well as being generally biologically inert which helps to avoid many adverse effects associated with natural polymers. They have been extensively used in transient implants, drug or gene delivery vehicles and scaffolds especially because of their more predictable erosion kinetics. [2] Of all the synthetic biodegradable polymers, poly(α-esters) are the most extensively studied group, due to their wide availability and relatively easy synthesis process [2][9][12]. All polymers in this class have aliphatic ester bond located in their backbone and therefore are theoretically degradable. However, only those polymers with relatively short aliphatic chains can be tailored to suit biomedical applications. [12] The biodegradable polyesters that meet these criteria can degrade *in vivo* because of their suitable main chain structure, crystallinity, and hydrophilic/hydrophobic balance [13]. In biomedical applications, lactide, glycolide, and caprolactone are the most commonly used monomers, from which both homopolymers and different copolymers suitable for specific applications can be prepared [1].

Poly(glycolic acid) or Polyglycolide (PGA) is the simplest aliphatic polyester with linear structure [13]. It is a highly crystalline polymer, which gives it high tensile stress and modulus and was one of the first used biodegradable polymers for medical applications. Initially PGA was used for developing sutures, but it has been commercialized for internal bone fixation devices as well. However, PGA degrades relatively quickly due to its ability to produce acidic degradation products that catalyse the degradation process. Moreover, PGA has low solubility in organic solvents, and both of these issues have affected limitedly in its applications. [1] The solution to this problem is copolymerization, which allows PGA units to continue to have valuable uses in the medical field [2]. Another highly used polyester is polylactic acid (PLA) which is the smallest optically active organic molecule

with either L(+) or D(-) stereoisomers [13]. In medical applications, only poly(L-lactic acid) (PLLA) and poly(DL-lactic acid) (PDLLA) have been largely studied. Similar to PGA, naturally occurring PLLA has good tensile strength and modulus due to its crystallinity. The degree of crystallinity, however, depends on the processing of the polymer and offers interesting possibilities for example in orthopaedics and sutures. However, high crystallinity can be problematic for degradation as well. PLLA is also more hydrophobic than PGA which results to very low degradation rate. Complete degradation can take even more than 5 years which has affected its popularity. PDLLA, on the other hand, is amorphous and therefore loses its strength in few months and is completely degraded within 12−16 months. Thus, PDLLA has particular use as a drug delivery vehicle and as a scaffold for tissue regeneration. [2] In addition, both PLLA, PDLLA are often combined with other degradable polymers to create composites with unique properties [12].

The most extensively used synthetic polymer in biomedical applications is, however, combination of PLA and PGA called poly(lactide-*co*-glycolide) (PLGA). PLGA has applications in many areas such as sutures, drug delivery devices and tissue engineering because altering the copolymerization composition chances its properties significantly. Furthermore, PLGA has great cell adhesion and proliferation properties and cand shaped to complex designs. [12] The chemical structures of PGA, PLA, and PLGA are presented in Figure 1. Additionally, from the polyester group, polycaprolactone (PCL) has been especially used for long-term controlled drug delivery applications due to its low degradation rate. Therefore, PCL can already be seen on the market as a contraceptive device. [1]

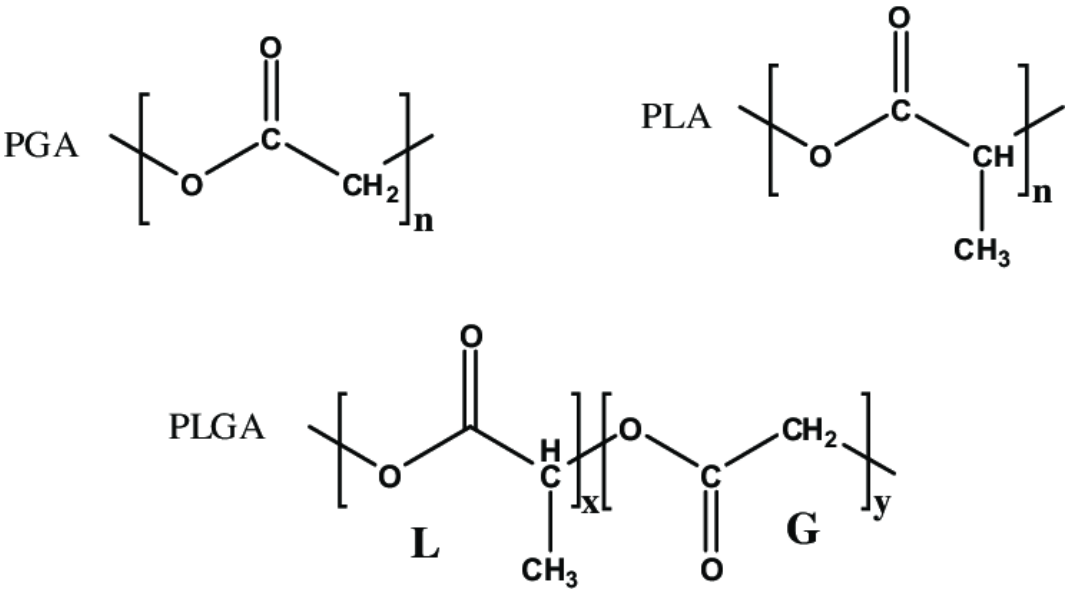

Figure 1. *Chemical structures of polyglycolide, polylactic acid, and poly(lactide-co-glycolide) [14].*

Polyesters are not the only synthetic polymer group used in biomedical applications but many other groups, such as polyurethanes, polyanhydrides, polyacetals, poly(ortho esters), polycarbonates and even some inorganic polymeric groups like polyphosphazenes have promising applications in the medical field as well. Whereas poly(α-esters) degrades by bulk erosion, polyanhydrides are class of surface eroding polymers. Surface degradation allows more precise release rate of the payload and therefore polyanhydrides have gained popularity in drug delivery applications. [12] Another group of polymers, that degrade by surface erosion are poly(ortho esters) and they have been found to be easily adjustable in terms of healing time and can increase bone growth. Polyurethanes then again are a large class of elastomers. They have good mechanical properties and biocompatibility and have been used especially for implants like cardiac pacemakers and vascular implants. More application areas have been studied as well but limited progress has been made due to many toxicity issues of the degradation products. [3] In addition, to organic polymers, polymers that contain non-organic backbone can also be used in the biomedical field and for example highly flexible polyphosphazenes have been studied for drug delivery and TE applications. Finally, it is important to keep in mind that although most of the synthetic polymers degrade hydrolytically, some synthetic polymers that degrade enzymatically, such as synthetic polyethers, have also been created to better mimic biological environments. [12] All in all, the range of synthetic homopolymers and their copolymers is extremely wide and choosing the correct choice for specific applications with suitable degradation behaviour is one of the main challenges in the field.

2.1.2 Natural biodegradable polymers

Additionally, to synthetic polymers, biotically derived natural polymers have been extensively studied for biomaterials due to their already natural occurrence in biological systems and thus being naturally biodegradable [1]. Natural polymers have significant bioactivity advantages, such as natural remodelling, ability to present ligands that can bind receptors and possibility to proteolytic degradation that has been induced by cells. However, high level bioactivity comes also with its issues like triggering immunogenic response, potential spread of diseases and difficulties in purification. In addition, compared to synthetic polymers, natural polymers have more unpredictable degradation process which varies notably with the implantation site and the concentration of enzymes. [2]

Most common used groups of natural polymers are proteins and polysaccharides. Proteins are a large group of molecules that are created by joining amino acid monomers

together by amide bonds. This creates complex 3D structures, which are hydrolytically stable but can be degraded by proteases. Proteins are one of the most common materials inside the body and have been used as a biomaterial in many applications from sutures to drug delivery and scaffolds. [12] Collagen is the most abundant protein, which can be found in ligaments, cartilage, tendons, skin, and bone. In addition, collagen forms structural frames for tissues, such as blood vessels. The name collagen, however, refers to a group of proteins, which have similar characteristics. At least 22 different types of collagens can be found in human body. [1] Excellent structural characteristics of collagen makes it one of the most extensively researched protein for various applications. Some advantages of collagen-based biomaterials are their great biocompatibility, mechanical strength and processability. Due to their fibrous nature, the focus of using collagen as a biomaterial has mainly been from TE scaffolds specially in load-bearing applications. Collagen, however, is investigated as well for more delicate applications, such as burn treatment and reconstructive surgery because of its ability to improve cell adhesion and proliferation. Additionally, combining collagen with other degradable polymers is common and provides even more potential uses. However, collagen has common issues related to natural polymers that limits its use as a biomaterial like uncertain availability and immunological issues. [12]

Other commonly used as a biomaterial and abundant proteins in humans are albumin, fibrin, and elastin. Albumin can be extensively found in blood plasma where it carries fatty acid molecules and maintains the blood pH. Due to its naturally exceptional blood compatibility, presence of functional groups, easy processability and solubility, albumin has been investigated for many applications, such as a drug delivery vehicle and as a coating material for cardiovascular devices. [2] Fibrin, on the other hand, is very much like collagen and is additionally involved in the blood clotting process. Some of the advantages of fibrin, such as its injectability and biocompatibility, which can also be seen as positive effect on cell adhesion and proliferation, have made fibrin one of the earliest used polymers in biomedical applications. Fibrin is used in particular as a sealant product but has also been investigated for controlled drug delivery. [9] Finally, elastin is an insoluble, cross-linked polymer that is a vital component for providing elastic properties of tissues such as vascular or lung tissue. These elastic properties make it intriguing in the biomedical field but due to its insolubility and possible triggering of immune response the use of native elastin is still limited as a biomaterial. [12]

Polysaccharides are also excellent candidates for many biomedical applications as a result of their wide range of properties, availability, and cost effectiveness. Composed

from one or more repeating monosaccharide units with multiple reactive functional groups in their structure, polysaccharides can be easily modified and possess great biocompatibility and water solubility. One advantage of them is also their ability to form hydrogels. [1] Polysaccharides can be derived from human origin or non-human and one of the most extensively studied human origin-based polysaccharide is hyaluronic acid (HA). HA is water-soluble, largest found glycosaminoglycan, which unlike other members of the glycosaminoglycan family does not bond to proteins, but instead forms highly viscous solutions. HA provides structural support in connective tissues but is also involved in their biological processes from moderating cell migration and differentiation to wound healing and regulating ECM organization. Due to its distinctive viscoelasticity and its other properties, such as immunoneutralinity and ability to promote angiogenesis, HA is considered one of the ideal biomaterials for TE and drug delivery applications. [2]

Polysaccharides from non-human origin have been also investigated for biomaterial purposes and one of the most important one is chitosan, which can be found in crustacean skeletons. Chitosan is a derivate of chitin which structure has similarities with HA. Chitin itself have been investigated as a wound dressing material, but its insolubility to many solvents limits its further use in other applications. To overcome these issues, a chitosan was created. [12] The differences and similarities in their chemical structures can be seen in Figure 2.

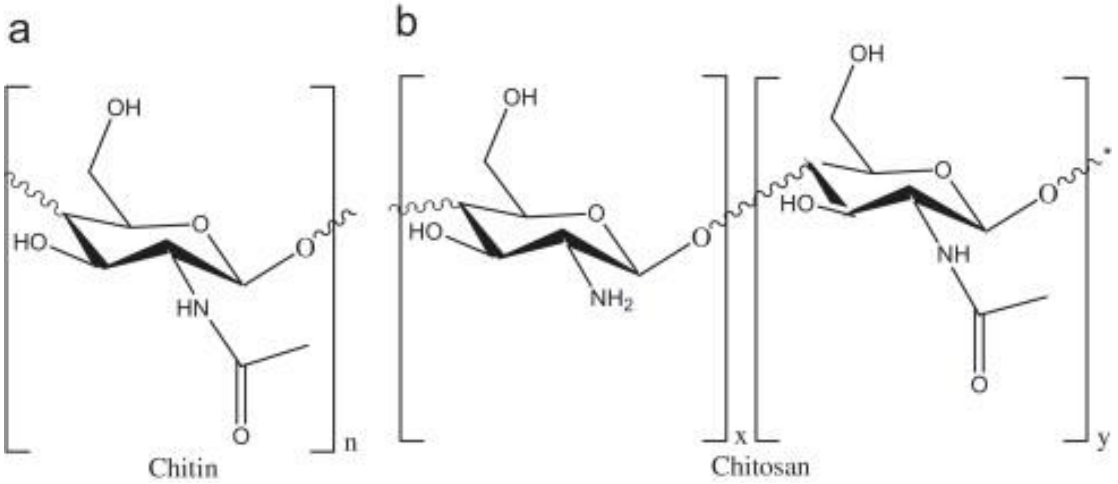

Figure 2. *Chemical structures of chitin (a) and chitosan (b) [2].*

Unlike chitin, chitosan is water absorptive, and provides versatility and processability that can be used in various applications, such as drug delivery, wound dressing, and scaffolds. Some advantages of chitosan include oxygen permeability, high bioactivity, mini-

mal foreign body reaction and improved wound healing. In addition to mentioned polymers other polysaccharides like, for example, dextran and agarose and proteins, such as poly(amino acids) and gelatin have been studied as biomaterials. [12]

2.2 Future trends and direction

As previously stated, due to fast development of advanced therapies such as tissue engineering, controlled drug delivery and gene therapy, the paradigm has shifted from rigid stable implants to preferring soft structures that mimic biological environment as closely as possible. Consequently, current trends of biodegradable polymers in biomedical applications rely heavily on these technologies, and the design aims to find more efficient material solutions in the form of composites, smart materials or new fabrications and moderation methods. One of the most intensively researched application areas for biodegradable polymers is the creation of porous scaffolds for tissue engineering. The aim of tissue engineering is to restore functions in damaged tissues, and this can be facilitated by the use of scaffolds, basic principle of which is to provide 3D environment that can mimic the real tissue and hence aid cells attachment and proliferation. Different tissues require different environments, which is why both synthetic and natural polymers are investigated for TE purposes. Both can be produced to different forms, such as films, 3D scaffolds, hydrogels, and 3D printed implants. Additionally, their potency in tissue regeneration can be improved by adding active molecules. [15]

One of the most attractive scaffold options for the future is hydrogels, whose structure and function mimic the extracellular matrix (ECM) very well [4, p. 74]. Hydrogels are dynamic crosslinked 3D networks which are highly hydrophilic and therefore can maintain significant amounts of water or other fluids in them. Thus, they effectively aid nutrient diffusion and provide biochemical and structural support for the cells due to their flexibility and elasticity which is similar to ECM. Many types of hydrogels from natural, synthetic and hybrid sources have been developed to meet complex criteria of different tissues. With natural polymers it is possible to create scaffolds with superior biocompatibility and low immunogenicity, but mechanical strength continues to be a challenge which synthetic polymers, on the other hand, can provide. To overcome these issues, mixtures of these groups have been developed, for example, in order to prolong the degradation time to better match the healing. However, improving the healing process remains to be the main goal in TE for the future as well and study with hydrogels and other scaffold materials will continue on improving their therapeutic effects. Great discoveries are already made with introducing nanoparticles into the structures and adding active agents like drugs or growth factors. [16] For soft tissues, injectable hydrogels, are also attracting

researchers for the future as they provide good carrier properties while being low invasive and adapting shape in real time. In practice injectable hydrogels are sol or pregel solution that are injected into a site and then allowed to gel. [15]

One of the challenges in the biomedical field with biomaterials, has been their inability to adapt to changes like normal biological environment would. To overcome this problem, smart polymeric materials have been developed and studied. The term smart materials describes a group of materials that respond to changes in the system, such as temperature, ionic factors, pH, biological molecules, light, electric or magnetic fields and so on. The stimulus can be external or internal and it reversibly changes the properties of the smart polymer. In TE, smart biomaterials have been found to maintain and control cellular behaviour and thus tissue regeneration. To date, the use of smart polymers is mostly focusing on pH- and temperature-responsive carriers of active agents to the cells but other functions are being studied as well. [17] For example, the so-called shape memory polymers (SMP) have interesting application possibilities not only in on-demand drug delivery but as implants, biosensors, biomedical devices, and smart textiles [4, p. 77][15]. They get their name from their ability to remember their original shape meaning that, when they are exposed to external stimulus, SMP temporarily changes its shape but return to the original shape after the stimulus has been removed. SMPs show promising results in many areas according to laboratory studies, but commercial applications are yet to been developed. [15] Despite this, SMPs and other smart materials will undoubtedly have many applications in the future. [17]

Additionally, in the experimental stage but holding a promising future, is conductive polymers. After discovering that many cellular activities can be manipulated with electrical stimulation, electrical properties of conducting polymers have become significantly interested in the biomedical field and they are being researched for devices, such as biosensors, drug delivery systems, neural probes, and TE scaffolds. [4, pp. 75–76] Besides these stimuli-responsive polymers, nano-formulation of polymeric biomaterials and new ways to obtain the necessary polymers are important future research topics. For example, marine collagen has been under attention for many application areas from tissue engineering to tissue regeneration of bone, cartilage, skin etc. due to its similarity to mammalian collagen and many appropriate physicochemical properties [18]. Nanotechnology, on the other hand, is a growing field as a whole, and this is also seen in biomedicine. Nanoparticles and nanofibers offer interesting fabrication possibilities and are currently mostly used in wound healing and drug delivery applications [15].

Important to keep in mind, is that even TE and other advanced therapies are now highly studied, biodegradable polymers have promising future in other areas as well, such as orthopaedics and cardiovascular applications. In many cases, composites and copolymerization offer properties that could not been achieved otherwise and developing them continues. Recent studies have been made with developing, for example fully biodegradable stents and polymeric composites with improved characteristic for orthopaedic fixation. Alternatively, surface moderation, has been found to be successful way to overcome some current limitations. [10] The functional behaviour of an implant can be greatly influenced by chancing its surface properties. In this way, the implant can be made, for example, more hydrophilic or cell friendly. [19] In addition, to improved materials, new fabrication methods, most importantly 3D printing, has revolutionized the use of polymeric biomaterials and presents a clear choice for the future. The layer-by-layer manufacturing process allows anatomically individual designs for single patient and complex geometric shapes that would otherwise be difficult to create. Consequently, 3D printing has promising future already in craniofacial implants, organ printing and tissue models for drug delivery, scaffolds etc. [15]

The future of biodegradable polymers is bright, and development is fast. However, similar challenges seem to remain regardless of the intended use. Optimizing the tissue healing and enhancing functionality of the biomaterial is the definite future goal and to be able to achieve this, regarding the biodegradable applications, is deep understanding of the degradation process. For controlled drug delivery, for example, the release mechanism, is particularly complex and choice of material affects greatly to how drugs are delivered into the system [16]. Thus, the material properties and the degradation rate and style must be well known, and this requires extensive test both *in vitro* and *in vivo*, to ensure that all relevant factors are considered.

3. DEGRADATION

Degradation is the most important property required of from a polymeric biomaterial in temporary use. The term “degradation” can be defined as an irreversible change in the overall properties, formation and shape of the polymer which most commonly is resulted by chemical cleavage of chains [20]. The mechanism of the chain cleavage depends on the degradation mode, and for polymers, there are 4 major modes: photo-, mechanical-, thermal- and chemical degradation which all might affect the biomaterial. Chemical degradation, however, is the most important one after the polymer has been implanted inside the body, while other degradation modes are commonly related to the processing of the polymer. [21] It should be noted though, that the effects of processing, such as sterilization and manufacturing, can also affect the final degradation, and the entire process should be considered when studying the degradation of a particular material. Especially mechanical degradation must be considered in applications where the biomaterial is subjected to high stresses [21]. Nevertheless, in this case as well, the loss of mechanical properties *in vivo* is mostly associated with chemical chain scission [20]. Therefore, the most common forms of degradation in a biomedical *in vivo* environment are hydrolytic and enzymatic degradation.

The term “biodegradation” is often referred to these types of degradation, where also biological processes, such as body fluids and enzymatic reactions have an effect. It is a complex process and the conditions of the environment as well as the properties of the biodegradable polymer have a determining impact on the rate of the degradation. [22] In general, biodegradation can be illustrated with two processes, degradation, and erosion, which complement each other. Whereas the degradation meant cleavage of bonds, which results to polymer breaking down into low molecular weight fractions, erosion refers to mass loss of the polymer matrix, which can occur as result of the dissolution and diffusion of these fractions [21][23].

3.1 Hydrolytic degradation

The degradation of most synthetic polymers takes place passively by hydrolysis, which means that water breaks the bonds in the polymer backbone, leading to the erosion of the polymer. The hydrolytic degradation process is possible with polymers that contain functional groups that become unstable to an aqueous environment, such as esters, orthoesters, amides, urethanes, carbonates, etc. [2][21][23]

The degradation starts with the diffusion of water, which hydrolyses the sensitive functional groups, and causes the chains to start breaking down into oligomers and monomers [21]. The chain scission can occur as chain-end scission meaning that the breakage happens systematically from the end of the chain or as random scission, which means a cleavage of bond anywhere along the chain. The end-chain scission is, however, with rare exceptions, controlled by enzymes and without their presence the scission is random. [24] Nevertheless, enzymes alongside acids, bases and salts can catalyse hydrolysis and induce end-chain scission [25, see 26]. Due to series of cleavage events, the molecular weight of the polymer decreases, and erosion starts as the transportation of degradation products causes loss of material, which in turn eventually leads to a loss of mechanical stability [20][22]. During this process the polymeric biomaterial undergoes extensive changes in its microstructure, including swelling, increases in porosity, surface cracking and so on [21]. Therefore, for degradation testing, it is important to remember, that the complete erosion of the polymer takes considerably longer than the loss of mechanical properties. The erosion can occur as a surface erosion or as bulk erosion depending on the rate of the diffusion of water compared to rate of hydrolysis. [20] Figure 3 shows a schematic illustration of both erosion modes.

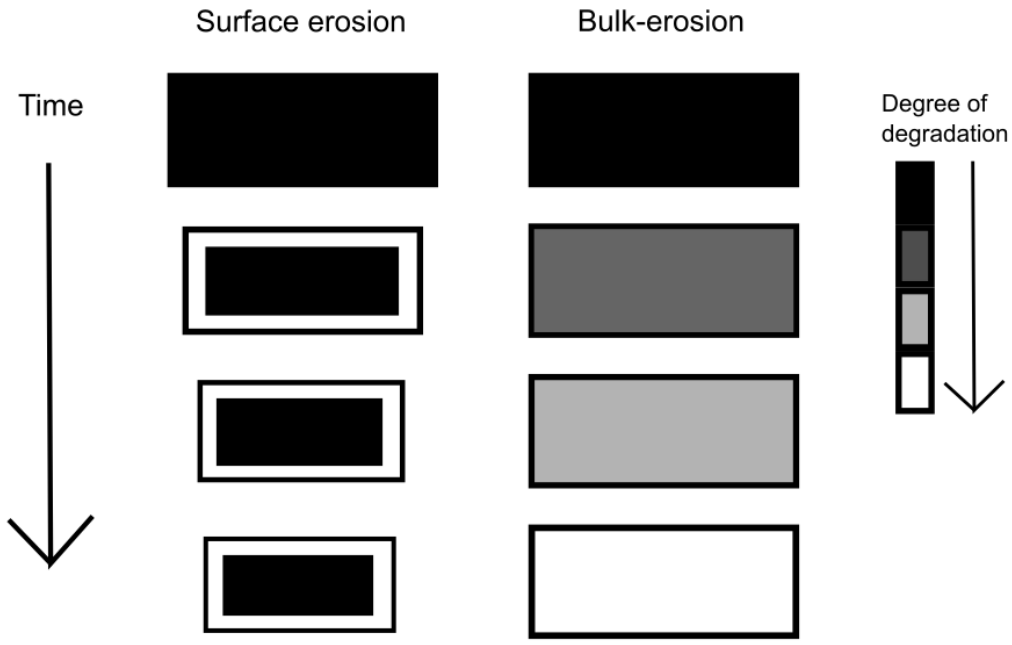

Figure 3. *Illustrative demonstration of surface and bulk erosion. Modified from [27].*

In the bulk erosion the water is able to penetrate into the bulk faster than the hydrolysis is occurring and results in degradation through the thickness of the polymer. Uniform degradation is enhanced by the presence of hydrolysis catalysts whose rate of diffusion is also faster than the reaction rate. This can happen, for example, with polyesters such

as PGA and PLA, which degrade to acidic end groups that autocatalysis the process. Thus, the bulk degradation is the controlling mode of degradation for polyesters, as well as for polyamides. [20] In bulk erosion, even before observing any mass-loss, the molecular weight of the polymer undergoes changes. This is then followed by increased water content and loss of material, leading to dramatic changes in mechanical properties [20][22].

In contrast, surface erosion occurs when the rate of hydrolysis is faster than the rate of water diffusion. As a result, the polymer decreases in thickness and the material is released from the surface. Surface erosion can occur when the hydrolysis rate is very fast or when the properties of the polymer, like hydrophobicity or high crystallinity, slows down the water diffusion. From hydrolytically degrading polymers, some of the polyanhydrides, polycarbonates and poly(ortho esters), tend to degrade by surface erosion. Unlike, in bulk erosion, surface eroding polymers can maintain their high molecular weight and mechanical properties relatively unchanged in their core until the end of the process. Additionally, the load bearing capability decreases more steadily compared to rapid changes in bulk degradation. [20]

Erosion mechanism is one extremely important factor in determining the suitable material for a specific application. For example, bulk eroding polymers are preferred in some applications that require permeable membranes that allows necessary diffusion through the material bulk, whereas surface eroding polymers are attractive, for example, for controlled drug delivery. [12] That is because in surface erosion its release rate is independent of diffusion and payload kinetics can be more easily maintained and tailored resulting in detailed rate of degradation [12][22]. However, it should be emphasized, that surface and bulk erosion presents the two extreme degrees of erosion and that, in general, features of both modes can be seen simultaneously in a polymer [20].

3.2 Enzymatic degradation

Enzymatic degradation, which is typical to natural polymers, is caused by enzymatic reactions that result in the cleavage of bonds [2][22]. Enzymes are proteins which have specific active site regions that interact with their substrates [20]. They are secreted by the micro-organisms of the body and the degradation of the polymer can occur via several enzymes either by hydrolysis or oxidation. Enzymatic degradation is therefore also crucial for hydrolytic degradation by accelerating the rate of degradation. [28] In this case both mechanisms compete each other, but the overall degradation type is categorized by the faster process, which controls the process [21]. The mode of degradation, which

can be either surface or bulk erosion as well, is determined by the kinetics of the enzymatic reactions and the availability of the required enzymes at the point of use. Overall, it comes down to if the enzyme can access the inside of the polymer. [22] For example, proteins main erosion mechanism is bulk erosion, but enzymatic hydrolysis occurs via surface erosion. [20].

The enzymatic degradation process begins with the enzymes diffusing from the solution to the surface of the biomaterial. Here, the enzymes are adsorbed, and they catalyse the degradation reaction resulting in cleavage of bonds. Finally, the small fractions of the polymer or in other terms the degradation products are released into the solution. Some of the products can even be integrated by the micro-organisms and result in new organic compounds. [28] Different enzymes have different operating mechanisms depending on the environment and availability of specific reagents. For example, exoenzymes induce end-chain scission, while endozymes cause random chain scission. Some enzymes that are commonly associated with biodegradation are lipases, amylolytic enzymes and lysozymes which, for example, attacks chitin. [20]

3.3 Factors affecting degradation

The rate of the degradation depends on multiple factors including the dynamic biological environment as well as physical, chemical, and morphological characteristics of the biodegradable polymer [22]. Altering the degradation rate is one of the biggest challenges for scientists and an opportunity to better mimic the healing time and make more efficient solutions for temporary biomedical applications and therefore extent understanding of the interaction between the material and the host environment is crucial.

The biological environment, such as temperature, pH, cell infiltration capability and enzyme concentrations all have significant impact on the rate of degradation. Additionally, the environment is dynamic and therefore changes can alter the rate to great extent. For instance, the degradation rate can increase dramatically in a highly inflammatory environment. [22] pH is also an important factor, especially for hydrolytically degradable polymers, due to its possibility to catalyse the reaction [21]. In hydrolytic degradation, the rate of the process is low at neutral pH, and for example poly(ortho esters) are resistant to basic pH. However, the hydrolysis occurs much faster when the pH decreases in the presence of acids. Temperature of the environment also affects the rate of degradation, as higher temperatures increase the mobility of the polymers and sometimes lead to swelling, which increases the permeability of the polymer and further accelerates degradation. Consequently, in modelling the degradation in theory, as well as in vitro testing

should take particular account of the effects of temperature and pH. Additionally, mechanical forces, both static and dynamic, such as compression, shear and tension, affect the lifetime of the polymer and testing must also take into account the possibility of mechanical degradation. [20]

The degradation mode and also the rate is greatly affected by the properties of the polymeric biomaterial, and hence is one of the key factors for controlling the degradation rate. For hydrolytically degradable polymers, the highest impact on the degradation rate has the functional groups in the backbone of the polymer but additionally water uptake, crystallinity, swellability and molecular weight have their contribution to the velocity of the degradation. For instance, the water uptake can be controlled by affecting the lipophilicity of the polymer, which means that more hydrophobic polymers degrade significantly slower than more hydrophilic ones because they limit water accessibility. Further, amorphous polymers have faster degradation rates compared to organized crystalline regions that have increased resistance against degradation. Polymers with lower glass transition temperature T_g degrade also faster due to easier mobility of the polymer. [21][22] The effect of molecular weight is, however, more complicated because it works both in direct and indirect way. For example, higher molecular weight correlates to higher T_g which results in slower rate of degradation but the change is not linear. On the other hand, higher molecular weight also means a higher number of bonds, which in turn means that in order to erosion occur more cleavage of bonds is necessary, and the process takes longer, which can be considered to be direct influence. Finally, it is important to note that copolymers do not have the same properties as the homopolymers it contains, and therefore copolymerization can change the rate of degradation enormously. [21]

For enzymatically degradable polymers, the rate of degradation varies remarkably depending on the site of degradation and the concentration of relevant enzymes present at that site [4, p. 79]. In addition to concentration, other characteristics of the enzymes, such as composition, conformation, stability, and activity, can affect the rate of absorption. The biological environment, like for instance temperature, pH, oxygen levels and microbial population, also influences the velocity of the process. The main way in which this degradation can be influenced is through chemical modification of the enzymatically degradable polymer. For example, crosslinking, removal, or addition of chemical groups etc. affects to the ability of the enzyme to identify the biomaterial and therefore, affects to degradation rate. [20] Additionally, materials with higher permeability degrade faster, because the enzymes can more easily interact with the material [28].

In addition, to polymers properties, the design of the material, such as geometry and shape, can affect the rate of degradation. Porous structures allow faster water penetration, and for example, high surface area of the polymer has an increasing effect on the degradation rate [4, p. 80]. This information is particularly used in controlled drug delivery, where speed control is often achieved by altering the geometry and shape of the polymer. When estimating the degradation rate, it is also worth noting that the process is, as established, dynamic, and the rate can change as the process moves forward. For example, the semicrystalline polymers degrade faster in the beginning due to their amorphous regions but decreases when the crystallinity increases after the amorphous parts are eliminated. Another example of this is autocatalytic degradation of acidic monomers. [22] The assessment must also consider the fact that each body is an individual and can affect the results.

4. *IN VITRO* DEGRADATION TESTING

As stated at the previous chapter, degradation is a complex, multi-step process, which is affected by many dynamic factors from the biological environment to the properties of the polymeric biomaterial. However, in order to create biomaterials that are suitable for particular applications, an extensive evaluation and comprehension of the degradation behaviour is critical [29]. To meet the demands for polymers with controlled, predictable degradation kinetics, active efforts are constantly made to both model the degradation process of different materials in theory as well as carry out tests both *in vitro* and *in vivo*. Even a slight change in conditions can significantly affect the behaviour of a biomaterial and change the rate of the process during degradation and the process itself may affect a range of events, such as cell growth and host response. This makes the biodegradation a key element when choosing the material for specific application and requires extensive characterization under a range of conditions. [20][29]

To this end, many mathematical models have been created to predict the rate of degradation, but currently the understanding of the interactions that take place in a natural environment and the degradation mechanisms, is not advanced enough to achieve a combined theory that would work for different applications and materials [20]. Similarly, in experimental studies *in vitro* taking all controlling variables into account is challenging and therefore there are often no uniform methods for degradation testing. Instead, various techniques are used to simulate the biodegradation *in vitro* [30]. Simulation depends largely on the location of the implantation as well as contact time with the body. Thus, testing should, for example, take into account duration of the study, mass/volume ratio and choice of solution, which should mirror the effects of natural body fluids. Many solutions are used for this purpose, such as phosphate buffer solutions and enzyme solutions depending on the material and its mode of degradation. [29][30] *In vitro* degradation tests are important aspect of predicting the degradation rate and can act as a guideline for *in vivo* tests, but it should be remembered that the results do not necessarily correlate between *in vitro* and *in vivo* due to complexity of the *in vivo* conditions and should not be assumed so [29][31].

Given the variety of applications for biodegradable polymers in the biomedical field, a targeted characterization is necessary and many inherent properties of the polymer, as well as its surface, morphology, and biological compatibility need to be characterized in advance using application-dependent methods for a successful clinical outcome. The

characterization of mechanical properties is particularly important because the biomaterial must above all remain its mechanical stability over a period of time appropriate for tissue healing. The matching of mechanical properties with host tissue properties is also a key factor for biocompatibility and can influence cell fate. [32, p. 177] However, despite the extensive characterization required of the polymer, the degradation test itself, in its simplicity, can be, for example, recording the mass loss and molecular weight during *in vitro* degradation in a solution that mimics the conditions of the body [30][33]. Nonetheless, on their own they provide very little information for predicting the rate *in vivo* and therefore other characterization is important. In this thesis as well, the focus is on the physical aspects of the degradation process and presents methods that can be used for evaluating the loss of sample mass, molecular weight, and relevant mechanical properties.

Despite the scope of this work, it should be noted that during degradation many other parameters are monitored, such as test conditions and other material properties, which should not be overlooked in order to ensure that the test results are as close as possible to the degradation rate that would occur under real *in vivo* conditions. In many related studies, in addition to monitoring loss of mass, molecular weight and mechanical properties, morphology, surface chemistry, thermal properties, water absorption and changes in conditions are monitored carefully [34−37]. In these studies, many characterization methods were used, such as scanning electron microscopy (SEM) for morphological analysis, differential scanning calorimetry (DSC) and thermogravimetry (TGA) for thermal analyses, X-ray diffraction (XRD) for determining crystallinity, X-ray photoelectron spectroscopy (XPS) for surface chemistry and Fourier transform infrared spectroscopy (FTIR) for analysing functional groups. Chosen methods depended on the application and materials.

Due to diversity of *in vitro* degradation studies, standardisation for degradation testing is difficult as well, and only a few standards can be found that address general requirements for tests. The ISO standards 10993-9 [38] and 10093-13 [39] are part of the biological evaluation of medical devices standard series and they provide general principles for identification and quantification of potential degradation products, which can be done with *in vitro* degradation studies. The part 9 provides the framework and part 13 continues with specific requirements for polymeric medical devices. Although the focus is on the degradation products, the degradation test methods described can also be used as a guideline for general testing. [38][39] The part 13 provides the general requirements for samples, appropriate test conditions and test procedures for measuring initial mass

in different environments. In addition, different characterization methods are proposed to determine the different properties of the polymeric biomaterial, if deemed appropriate, but a more detailed assessment is outside the scope of the standard. [39]

For a more detailed description, a standard ISO 13781 [31] can be found for *in vitro* degradation characterization of poly(lactide)-based homopolymers, copolymer and blends describing the basic evaluation needed for the characterization. Specific standard for poly(lactic acid)-based polymeric systems is foreseeable, as they are the most extensively investigated class of biodegradable polymers and their degradation behaviour have been under extensive investigation. [2][29]. According to this standard, appropriate *in vitro* characterization of the degradation of a biodegradable polymer can be achieved by monitoring the progressive loss of both mass and molecular weight as well as the loss of device-relevant mechanical properties. Test samples should be continuously exposed to a physiologically relevant environment and the testing should include at least one relevant mechanical property related to the intended use of the device. The standard also emphasizes that the results need to be considered carefully and they cannot be used to definitely predict behaviour *in vivo*. [31] A few most common characterization methods and techniques, which are also mentioned in ISO 13781, are discussed in the following sections.

4.1 Evaluation of the loss of mass and molecular weight

The general *in vitro* degradation test, which often refers to evaluation of the progressive loss of mass and molar mass during degradation, is not as application-dependent as other relevant characterization methods of the biomaterial. However, the testing conditions do vary depending on many factors, such as the location of implantation, duration of the study, temperature, pH, choice of solution etc. Thorough review of the general testing conditions is outside of the scope of this thesis, but a few especially important condition aspects for evaluations for mass loss and molecular weight loss will be discussed shortly.

Key factor for successful degradation testing is the choice of solution. Most of the degradation studies found in literature [29][36][37][40] are performed in phosphate buffer saline (PBS) solution, which also mentioned in ISO13781 [31]. However, with use of PBS it is only expected that only occurring degradation is by normal hydrolysis. Therefore, other degradation solutions are used as well, such as ionic solutions, enzyme buffers and simulated body fluid solutions. [29] Another important thing to consider is the sample dimensions and other parameters. Because the shape and structure of the sample has

a strong effect on the degradation rate, the test samples should be manufactured to match the intended product as close as possible. For example, if the finished product will be a plate so should be the sample. In addition, the possible *in vivo* loading, strong fluid flow etc. needs to be taken into account an added to the testing procedure if the device is to withstand these conditions *in vivo*. [31] In this case, further guidance is available in ASTM F1635-16 standard test method for *in vitro* degradation testing of hydrolytically degradable polymer resins and fabricated forms [31, see 41] and in ASTM F2902-16 standard guide for assessment of absorbable polymeric implants [31, see 42].

4.1.1 Mass loss and water absorption

Measuring the mass loss of the biomaterial is one important factor when studying the degradation of biodegradable polymers *in vitro* and commonly used to evaluate the extent of degradation. The procedure includes measuring the initial mass of the sample and comparing it to measured mass values during degradation. [29] According to ISO 13781 [31], appropriate testing requires a calibrated weighing device, at least three test samples in separate containers and systems for drying the samples and separating debris and fragments. The standard states that the measurement of initial mass should be determined from vacuum dried samples within a relative standard uncertainty of 0,1 % of its total mass. [31] Drying the initial mass to constant is important for removing any residual moisture. However, the drying must be carried out carefully, without exceeding temperatures that could irreversibly affect the material. [29]

After degradation, degraded mass should be measured with same uncertainty after thorough rinsing of the samples. Rinsing should be done with distilled or deionized water to remove any impurities, loose particles or residual buffer solution and dried for constant weight. [29][31] *In vitro* studies found in the literature [34][36][37][40][43][44] followed similar procedure, which included regular measurements for masses during degradation. The overall weight loss was calculated in these studies by using the following equation

$$Weight\ loss\ (\%) = \frac{(W_0 - W_1)}{W_0} * 100, \quad (1)$$

where W_0 is the initial weight before degradation and W_1 is the weight after degradation. Results can be presented as a graph such as in the study of Yoshioka *et al.* [40], shown in Figure 4.

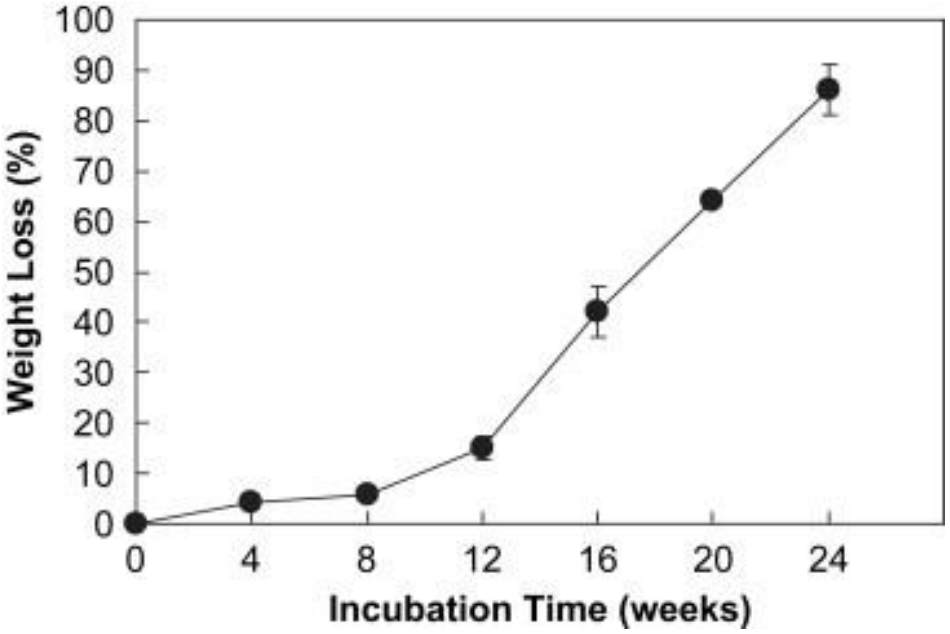

Figure 4. *Weight loss (%) of PLGA sponges as function of incubation time [40].*

When determining the loss of weight, it is important to consider if the separated fragments or debris is considered to be a part of the test sample. In ISO 13781 [31] it is stated that it should be considered depending on the application and if not, careful rinsing and drying is enough between measuring. If, however, recovery and quantification for debris and separated fragments are needed, they should be accumulated by means of a filter or a centrifuge [31]. Same evaluation procedure with same principles for mass loss is also described in ISO 10093-13 [39] standard, but it also states further evaluation considerations, depending on the results obtained.

Water absorption is generally assessed alongside mass loss. After all, the first step in the biodegradation process is the absorption of water when the material interacts with the surrounding fluids. This can, for example, make the material more flexible, cause dimensional changes or accelerate hydrolysis process, if the water absorption is high enough. [44] Hence, water absorption is one key parameter, when studying the degradation of the polymeric biomaterial. From the studies found in literature [37[44−46], the water absorption values can be measured by measuring additionally the wet weight of the sample. The wet weight should be measured instantly after wiping the surface with a filter paper to remove excess surface water. After the measurements, the water absorption value was calculated in these studies with the following equation:

$$Water\ absorption\ (\%) = \frac{(W_d - W_{1)}}{W_1} * 100 \qquad (2)$$

where W_d is the wet weight of the degrading sample and W_1 is the dried weight. [37[44−46] The characterization of water absorption is particularly important for hydrogels, as their use often is dependent on their ability to absorb substantial amounts of water.

4.1.2 Evaluation of molecular weight

Molecular weight (M_w) and its distribution is one of the most important parameters of polymers, which define a lot of their behaviour and physicochemical properties. The changes in molecular weight have direct effect on many properties of the polymer like crystallinity, flexibility, resistance to deformation etc. Especially known example of their interdependence is the relationship between M_w and mechanical strength. This relationship needs to be considered when choosing a suitable biomaterial because for high strength applications, polymers with higher molecular weight are required. This is because the mechanical strength increases with increasing molecular weight up to a certain value. On the other hand, it should be remembered that the biodegradability rate decreases with increase of M_w, which should be taken into account as well when designing a polymeric biomaterial for specific application. [32, pp. 101−106]

During the degradation, changes in the molecular weight can be determined either via inherent viscosity or gel permeation chromatography (GPC)/size exclusion chromatography (SEC) according to standard ISO 13781 [31]. In these techniques various parameters are important for the characterization and should be analysed. The main ones are the number-average (M_n) and the weight-average (M_w) molecular weights as well as their ratio, which is known as the polydispersity index. The polydispersity index is a crucial factor because it tells the width of the molecular weight distribution. A high index means that both small and large chains are present, and low index that the length of the chains is fairly uniform. [29]

For evaluating the molecular weight parameters via inherent viscosity ISO 13781 [31] refers to specific standard ISO 1628-1 [47], which describes general conditions for determining the viscosity of a polymer in dilute solution. According to this standard determination of the viscosity requires capillary viscometer and its holder, thermostatic bath, and systems for measuring temperature and time. Viscosity can then be determined by measuring the efflux times for the solution and the solvent in the same viscometer. As a result, e.g., the inherent viscosity can be determined, which is the ratio of the natural logarithm of the relative viscosity to the concentration of the polymer in the solution and the intrinsic viscosity, which is the limited value of the inherent viscosity at infinite dilution. [47] Viscosity, in general, can then be connected to molecular weight by the fact that

polymers increase the viscosity of the liquid they are dissolved. This is due to large macromolecules of the polymer, which slows down the flow of the solvent. Hence, increase of viscosity is dependent to molecular weight and the average molecular weight can be calculated from intrinsic viscosity with the Mark-Houwink equation

$$[\eta] = K(M_v)^{\alpha} \tag{3}$$

where $[\eta]$ is the intrinsic viscosity, M_v is the viscosimetric molecular weight and K and α are polymer, solvent, and temperature dependent constants. [32, pp. 110−111]

Another common approach for evaluating the initial and degraded molecular weight distributions is called either gel permeation chromatography (GPC) or size exclusion chromatography (SEC). Two terms are used, because the method is based on size exclusion, but the term GPC is more used in the polymer field. The idea of GPC is to separate dissoluted molecules in the sample according to their size. The GPC instrument includes a sample injector, solvent pump, refractive index detector and thermostated column compartments. The process is initiated by dissolving the polymer sample in an appropriate solvent. Now when the sample goes through the porous filler, the largest polymer size goes only through a short flow path due to its inability to access the deep are of the porous filler. In contrast, the smallest size can access deeper into the filler and therefore is eluted later from the chromatographic system. As a result, the different sized chains are separated in their own compartments and according to elution times, different molecular weight parameters can be evaluated from the collected data at the same time. [32, pp. 107−110][48] Schematic presentation of the separation process by GPC is illustrated in Figure 5.

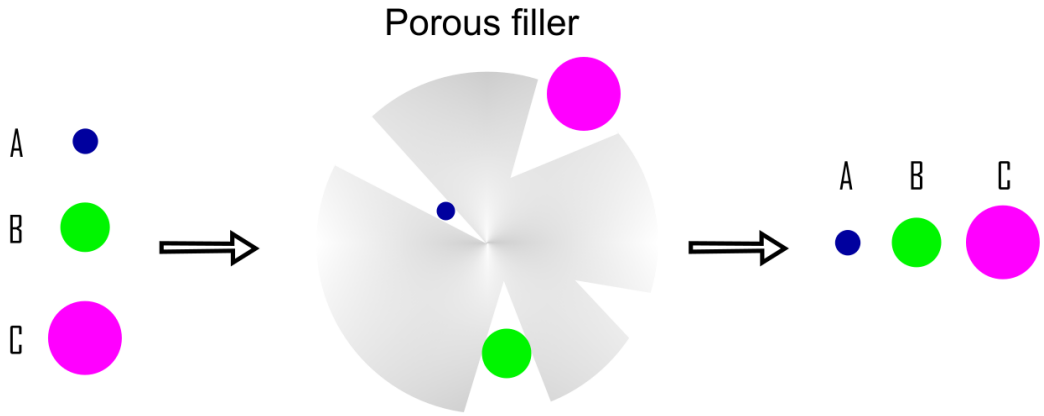

Figure 5. *Schematic presentation of the separation process by GPC. Modified from [48].*

GPC is widely used for the determination of the molecular weight due to its fast and feasible measurements, which unlike other methods can be done in a few hours, depending on the elution rate. However, it should be noted that the results can be influenced by the conditions, such as test temperature, flow rate, solvent, injection volume and other parameters. [32, pp. 107−110][48] Hence, conducting the test according to standards is important. ISO 13781 [31] addresses some conditions for testing with polylactic based polymers, but more thorough directions for conducting GPC/SEC test can be found in a standard ISO 16014 (all parts) [49].

4.2 Mechanical characterization

As discussed previously, mechanical characterization is a crucial part of the study of degradation mechanisms regardless of the application or material in question. However, different mechanical properties are required from different materials and applications and not all tests are suitable for all materials. It is therefore important that there are several different ways to characterize mechanical properties and that existing methods are developed as the range of applications and materials grows.

Mechanical characterization is needed both before and during degradation. Material should be degrading according to tissue healing time while sustaining a specified minimum mechanical strength, which will support the formation of the new tissue. Hence, evaluation of the mechanical performance of the polymeric biomaterial during their degradation is essential. In addition, mechanical properties, especially in hard tissue replacement, should be similar in magnitude as the tissue it replaces. [29] Testing, however, is fairly complicated compared to other materials due to viscoelastic nature of the polymers. This means that the polymers response to forces depends critically on time and temperature, and this should be taken into account when performing mechanical testing. [32, p. 177]

ISO 13781 [31] gives some general instructions for conducting successful mechanical tests before and during degradation. First of all, the tests shall be performed for undried samples, which have not been altered by previous testing. Second of all, the test methods and parameters should be relevant for the device and its application. Table 1 lists possible mechanical test method references for consideration, depending on the form of the polymeric biomaterial. The standard also states that the tests should be terminated either when a predetermined time has been reached or the degradation is so advanced that testing is not possible or meaningful. [31]

Table 1. *Recommendations for mechanical tests depending on the material. Modified from [31].*

Form	Test method reference
Rigid material	ISO 178: Plastics – Determination of flexural properties
	ISO 180: Plastics – Determination of Izod impact strength
	ISO 527-1, ISO 527-2: Plastics – Determination of tensile properties. Part 1: General principles, Part 2: Test conditions for moulding and extrusion plastics
	ISO 6721-2: Plastics – Determination of dynamic mechanical properties. Part 2: Torsion-pendulum method
	ISO 604: Plastics – Determination of compressive properties
	ISO 14130: Fibre-reinforced plastic composites — Determination of apparent interlaminar shear strength by short-beam method
	ASTM D2990: Standard test methods for tensile, compressive, and flexural creep and creep-rupture of plastics
Film, foil, sheet	ISO 527-3: Plastics – Determination of tensile properties. Part 3: Test conditions for films and sheets
Fibre, textile	ISO 2062: Textiles – Yarns from packages. Determination of single-end breaking force of netting yarns
	ISO 1805: Fishing nets – Determination of breaking force and knot breaking force of netting yarns
	ISO 13934-1: Textiles – Tensile properties of fabrics. Determination of maximum force and elongation at maximum force using the strip method

When performing mechanical tests, significant attention needs to be given for designing and controlling the test during execution. All the tests as well as the conditioning and sample preparation should be done according to standards in order to obtain reliable results. Important is to choose correct equipment for the testing because, unlike for example metals, polymers require lower forces, but larger extensions and the machine range should be appropriate for them. [32, p. 191] For accurate test results, it is also necessary to know what kind of load the material is subjected to *in vivo*.

4.2.1 Mechanical tests for more traditional and dense materials

Biodegradable polymers can be categorized in many ways from their origin to mode of degradation. However, for the purpose of evaluating appropriate mechanical test methods for these materials, the polymers have been divided in this thesis to more traditional, rigid, and semi-rigid materials and again for more porous structures. The first group described in this section refers to more traditional applications such as sutures, screws, plates, films etc. which have been one of the first applications of polymeric biomaterials. In addition, this group refers to more solid materials, with non-porous structures. Mechanical tests for these materials vary depending on their form and application. Both static and dynamic testing is required for thorough comprehension of the mechanical properties and predicting in-use behaviour [32, p. 178]. Dynamic mechanical analysis (DMA) is covered in section 4.2.3.

Static stress strain tests are one of the most common ways to characterize mechanical properties where the effects of cyclic stresses and polymer response to time are ignored. These tests include tensile, compression, shear, flexing, hardness, tear, and impact tests. [50, p. 1] All of these tests can tell a great deal about the polymer properties, but it cannot be overemphasized that many variables can affect the results significantly. For example, if the test conditions do not match the *in vivo* conditions or if the geometry of the sample differs from the final product, this may affect the testing results. In addition, timeframe has an impact, as the viscoelastic behaviour of polymers makes the material stiffer at fast test speeds and more flexible at slower test speeds. Therefore, all results should be carefully assessed. [50, p. 10][51, p. 548]

One of the most common versions of the stress-strain tests is performed in tension and are called tensile tests. In tensile test, the specimen is deformed, usually to fracture, with increasing tensile load. Commonly the load is applied uniaxially along the long axis of the specimen, which is usually shaped in a “dogbone” kind of formation. This allows the deformation to occur mainly on the narrow centre region. [51, pp. 156−158] Specific shape and dimensions for the sample are presented in various parts of ISO 527 [52] depending on the form of the polymer and the size of the sample. For some special samples, the standard also refers to other standards for further information. Figure 6 shows an example of typical dogbone specimen, which is designed for injection- and compression-moulded tests specimens. [52]

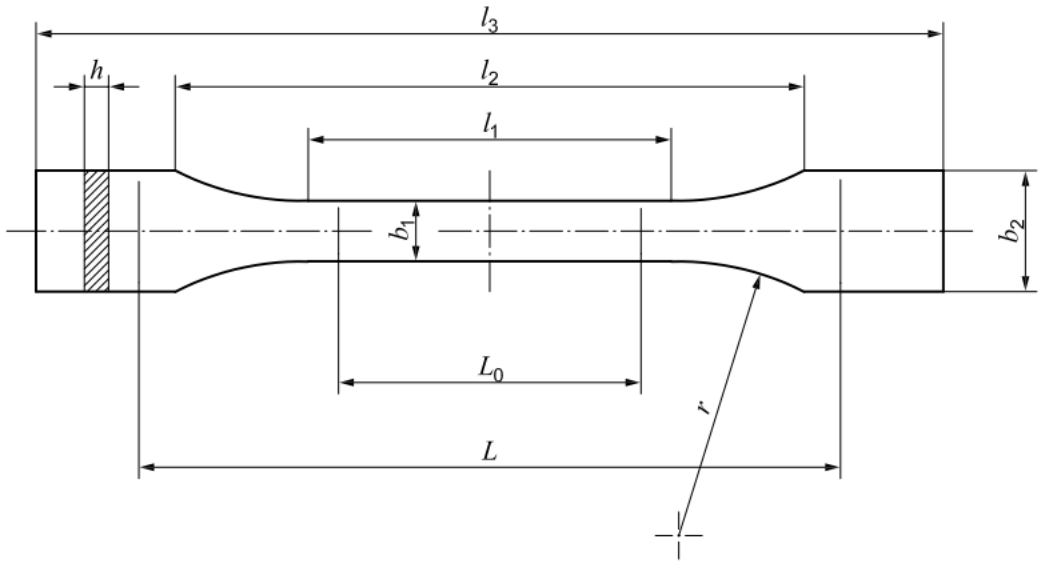

Figure 6. *Typical design of the specimen for tensile testing [52].*

The tensile test machine is then designed to elongate the specimen at constant rate while measuring both applied load and elongation. Applied load can be measured using a load cell and the elongation with an extensometer. [51, pp. 156−158] By plotting the test results, a stress-strain curve is obtained. In the curve, the stress presents the applied load divided with the cross-section area of the specimen and the strain presents the change in length of the specimen divided with the initial length. [32, pp. 181−182] Different appearances of the stress-strain curves are illustrated in Figure 7.

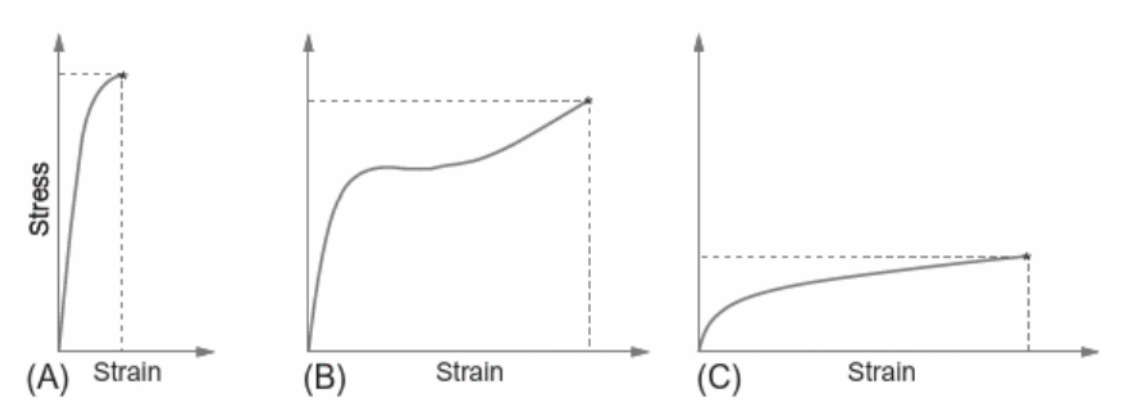

Figure 7. *Common types of stress-strain curves. (A) Highly stiff material, low deformation capability. (B) Stiff material but can sustain large deformations. (C) Soft material with remarkably high deformation capability. [32, p. 182]*

These common types of stress-strain curves in Figure 7 can additionally be the result of changes in temperature or test rate. For example, as polymers react to a decrease in test rate a curve of a type A polymer can look like type B if the test is performed slower or at elevated temperature. [51, p. 548]

The stress-strain curve can tell a lot about the load-carrying capacity of a material and common parameters evaluated form it is the stress values needed for fracture and deformation to occur, stiffness, and how much deformation the material can hold before break [32, pp. 181−182]. As seen in Figure 7, for stiffer materials there is first a linear-elastic region. The slope of stress-strain curve in this region is called modulus of elasticity or Young's modulus (E), which corresponds to material's stiffness. The greater the modulus, the stiffer the material is. However, for many polymers the elastic portion is not linear and determining the modulus as described is not possible. Instead, either the tangent or secant modulus is normally used. Other important tensile properties are yield strength, which can be determined from the maximum of the curve occurring after the elastic region and tensile strength, which is the stress at which fracture occurs and many times referred as the strength of rigid polymer. [51, pp. 160−161, 547] For most of polymers their tensile properties can be conducted according to ISO 527 (1−5) [52], which provides general principles and more detailed instructions for different forms of polymers. In addition, for fibres and textiles in medical applications, the tensile properties can be determined as a breaking force and elongation at break. Table 1 describes the standards that could be adapted for this purpose.

Tensile properties are important for many applications, but one good example is their importance in the use of sutures. Quality of the suture is often determined by its tensile strength in order to avoid unexpected breakage before healing is complete and therefore understanding the rate of mechanical strength loss is important [53]. For example, Khiste et al. [54] studied the degradation of the PGA sutures *in vitro* in a saliva serum solution and found out that the tensile strength remained the same for 3 days and then started to decrease rapidly over time.

In a similar manner to the tensile test, a compression test can be conducted, expect that the force is compressive and cause the specimen to contract in the direction of the applied stress [51, p. 159]. Rigid polymers are often subjected to compressive stresses, but in most applications, it is still more meaningful to determine tensile or shear stresses [50, p. 127]. However, in some applications compressive tests can be conducted like in the *in vitro* study of Schwach and Vert [55], in which compressive tests were conducted for PLA-based interference screws to simulate their work as a cruciate ligament autograft fixation. For softer and porous materials, the compression tests provide a more straightforward description of the mechanical properties and are therefore discussed in more detail in the next section [50, p. 127].

At least as common method as tensile tests to characterise the mechanical properties of polymers is the measurement of flexural properties. This mainly due the fact that normally the material will be under a mixture of loading modes and flexing or bending can often occur even without intention. In general, flexural tests are easier to perform for different types of polymeric materials and do not have, for example, same gripping problems that might occur in tensile tests. [50, p. 143] Bending test, in particular, is especially preferred for brittle and defect-sensitive polymers and composite materials, where it allows to isolate the influence between the matrix and interface to mechanical properties [32, p. 186]. For rigid and semi-rigid materials ISO 178 [56] describes the method for determining flexural properties such as flexural strength and modulus as well as other aspects of the flexural stress/strain relationship. According to the standard the test shall be conducted as a three-point bending test where the specimen is resting on two supports and then loaded midway between the supports. During the test, a maximum tensile force is occurring on the other side of the specimen and a compressive force on the other. [56] Figure 8 illustrates the placements for the three-point bending test.

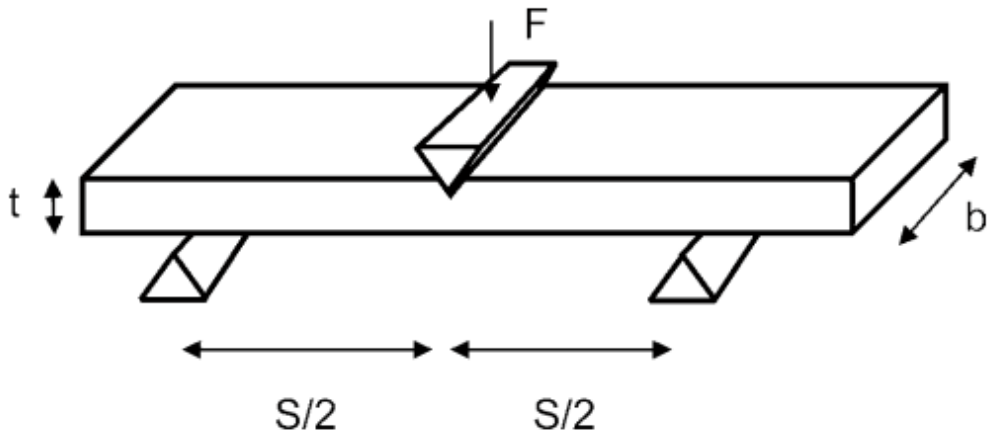

Figure 8. *Demonstration of the arrangement of the three-point bending test [50, p. 7].*

For obtaining results, both the force required to bend the specimen and the extent of bending is monitored [50, p.143]. The study of Liuyn et al. [57] provides a great example of bending tests as a part of the degradation testing. In their study, a bending strength reduction of pure PLGA and two HA/PLGA composites were measured in order to find out the effect of the composite compound to its mechanical properties. The *in vitro* degradation was conducted by soaking the specimens in simulated body fluid for different periods of time and compared to initial bending strength which is an important indicator for bone fracture internal fixation material. [57]

Other common mechanical parameters that are evaluated for biodegradable polymers are toughness and shear strength. The toughness of the material refers to its ability to absorb energy before fracture. In other words, this means the capability of the material to sustain load before failure. Material toughness and sensitivity to defects can be analysed with impact tests. Most common standard methods for this are the Izod and Charpy tests. In both of them, the specimen is placed on a support and then hit with a pendulum. This is done at extremely high loading rates and the results are calculated from the height that pendulum reaches after hitting the specimen. [32, pp. 188−189] ISO 180 [58] describes the instructions for Izod impact test. In the study of Tams et al. [59] the Izod impact test were performed as a part of *in vitro* study for high-impact poly(L/D-lactide) for fracture fixation. In their study the mechanical properties were monitored up to 45 weeks in phosphate buffer solution and even at 17 weeks the specimens were tough and did not break during the impact test.

Shear strength on the other hand is calculated from the forces that are parallel to the plane whereas the tensile and compressive forces were normal to the plane. [50, p. 135] For fibre reinforced materials, ISO 14130 [60] provides general instructions for shear tests and these could be used as a guideline for other polymer designs as well. According to this standard, the general testing procedure is quite similar to 3 point bending test, but with a smaller test span/specimen thickness ratio in order to increase the level of shear stress relative to flexural and to encourage interlaminar shear failure. [60] The study of Niiranen et al. [61] gives a fitting example of monitoring the loss of shear properties during *in vitro* degradation. In their study, the *in vitro* degradation behaviour of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites were monitored for 24 weeks in phosphate-buffered saline. Their results showed, for example, that the shear strength decreased faster with increase in glass content and that all specimens lost their mechanical properties more rapidly *in vivo* than *in vitro*. [61]

In should be noted that methods described in here are not necessarily the only ones needed for accurate monitoring of the mechanical properties during degradation, but other methods might be useful as well depending on the application. For example, hardness or tear properties could be considered as well as the possible results of long-term exposure, such as creep and creep-rupture. Further tests are also presented in the following sections, which all can be used for more traditional and dense materials as well.

4.2.2 Mechanical tests for porous 3D structures and hydrogels

For porous 3D structures like scaffolds and softer polymers like hydrogels and foams, a quite different characterization can be required than for more dense materials. However, it should be noted that all test methods described above and later in this chapter can be useful for several types of materials and for example tensile tests and other static test methods are conducted for porous and softer structures as well. At all times, the decisions on the correct test methods should be done uniquely for specific application.

For porous structures that do require some load-bearing capacity, compressive tests are commonly used to determine mechanical properties. A good example of porous structures in load-bearing applications, are scaffolds for bone tissue engineering. According to the study of Jiang et al. [62] an ideal scaffold for bone tissue engineering should be biocompatible, biodegradable, porous, and mechanically competent to maintain its pore structure in local stress. This is important in order to allow cell and tissue in-growth and thus, the production of bone matrix. In their study, chitosan/PLGA scaffold and control PLGA scaffold were tested in PBS over a 12-week period for the loss of their compressive modulus and compressive strength and found that although the chitosan/PLGA scaffold degraded more slowly, its mechanical properties decreased gradually over the 12-week period whereas the control PLGA scaffolds did not. [62] The procedure of the compressive test is quite similar to tensile tests as discussed. Generally, they are also easier to perform, but careful consideration must be given for the geometry of the specimen, as shape factors have a major influence in compression tests. Important is to design the ratio of height to area in a way that buckling cannot occur, which is why standard tests for rubbers and foams, for example, are usually done on disk- or short cylinder-shaped specimens. [50, p.127] Standard ISO 604 [63] describes general instructions for conducting compressive tests on rigid and semi-rigid materials to determine compressive strength, modulus, and other aspects of compressive stress/strain curve. The basic principle of the test is to compress specimen along its major axis at a constant speed until fracture or when a predetermined value of load or decreased length has been reached [63]. In addition to traditional compression tests, a modified version called confined compression, can be conducted on highly hydrated tissues and hydrogel biomaterials. In the procedure, a cylindrical shaped sample is placed in a close-fitting well and then loaded through a plate that is permeable to fluid flow. [32, p. 198]

Particularly for hydrogels and soft polymeric biomaterials, such as polymer solutions and suspensions, a rheological characterization is a effective method to evaluate their structure and use in various applications. Rheology itself is the study of the flow properties of

the biomaterial and its deformation behaviour which cannot be explained alone by the elasticity theory and fluid dynamics. This means that some complex systems show mechanical responses which fall between liquid and solid and therefore cannot be explained without the other. Viscoelastic and nonlinear characteristics depend on various parameters, such as the duration of the test and the magnitude and type of stress or strain to which the material is subjected. [32, p. 233] Various rheological tests exist but the two most used and simplest methods are stress relaxation test which measures the time-dependent response and oscillatory rheology, which measures frequency-dependent response. The tests can be performed with various instruments called rheometers. The most common ones are rotational rheometers, in which the sample is placed between two parallel, usually circular plates in a rotationally symmetric measuring geometry. A torque or angular displacement is then applied to one of the plates, causing local shear deformation of the sample. The control of the test can then be dependent either from stress or strain. [64]

In a stress relaxation test, the test specimen is rapidly strained to tension to apply an instantaneous strain deformation. The stress response to maintain this strain is then measured over time in a constant temperature. As a result, the stress has been found to decrease with time due to nature of the polymers and the relaxation modulus (E_r) can be defined as ratio of time-dependent stress and constant rate. [51, p. 550][64] When the test is performed in reverse, meaning that the stress is kept constant and the strain is measured over time, another time-dependent deformation, common for some polymers, can be seen. This phenomenon is called viscoelastic creep. The results are again presented as a time-dependent ratio of stress and strain called creep modulus (E_c). [51, p. 553] Both stress relaxation and creep are important characteristics to consider when designing the polymeric biomaterial for specific application.

Another common way to measure rheological properties of soft polymers is oscillatory measurements. With this method, instead of measuring the relaxation of stresses as a response to time, the behaviour is probed by applying sinusoidal shear deformation at a characteristic frequency and measuring the stress response. The time-dependency can then be determined by the correlation between frequency and time. From the results of stress responses, two important parameters can be determined. As the idealistic shear modulus of the material (G) can determined as a ratio of shear stress and strain, with viscoelastic material the modulus can be split to two parts, the in-phase response and out-of-phase response. In this way, the in-phase response is determined as a real part of the stress response and out-of-phase as imaginary part of the response, as

$$G* = G' + i * G'' \quad (4)$$

where $G*$ is the complex shear modulus, G' is shear storage modulus, and G'' is shear loss modulus. G' provides information about the elasticity and energy stored in the polymer during deformation and G'', on the other hand, describes the viscous element and tells information of the energy dissipated as heat during the process. Additionally, the loss angle (tan δ) is determined as a ratio of loss modulus to storage modulus, where delta is the phase difference of in- and out-phase. Material can be considered liquid-like if the loss modulus is higher and solid-like if the storage modulus is higher at given frequency. With frequency sweep tests, in which the moduli are monitored in various frequencies, the linear viscoelastic behaviour of a material can be fully characterized. [32, pp.236−239][64] General measuring principles for rotational and oscillatory rheometric tests are provided in ISO 3219-2 [65]. For determining the same modulus values ISO 6721-2 [66] can be used as well, for determining dynamic mechanical properties using the torsion-pendulum method. More information on dynamic mechanical analysis is given in the next section.

G', G'' and δ are all important factors when considering important properties of hydrogels. By monitoring the evolution of these factors', gelation point can be observed as well as the behaviour of the hydrogel at short vs. long time scales. For example, gelation kinetics and final gel stiffness highly affect hydrogels use in biomedical applications. The rheology of hydrogels is often measured with small amplitude oscillatory shear (SAOS) measurements and creep tests. Variety of tests are important because mechanical properties are important criteria in determining suitability for a specific application. For example, suitable flow properties make hydrogels ideal choice for injectable drug delivery vehicles, and on the other hand, the gel must be rigid enough to perform as a scaffold and maintain its structure. [67] Shear thinning is also a characteristic of hydrogels as well as some polymer solutions, which needs to be considered. As a phenomenon, it means that at low shear rates the viscosity of the material is higher, but after a certain increase in shear rate it decreases rapidly. To characterize this behaviour, shear rate ramp tests can be performed to produce a flow curve. [32, p. 241] For injectable applications, shear thinning can be used as an advantage because material behaves more fluid-like when injected but gains back its viscosity afterwards [67]. In addition, shear thinning is especially essential property of bioinks, which determines the final printability of the ink by reducing shear stress and preventing clogging during 3D bioprinting [68].

4.2.3 Dynamic mechanical analysis

Dynamic mechanical analysis is one of the most common tools for the characterization of viscoelastic properties of polymers. The system allows determining the mechanical properties of the polymers while taking into consideration the response of the material to time, temperature, and frequency. DMA can be used for thermomechanical characterization of a range of materials from rigid polymers to viscoelastic liquids and many parameters can be determined such as storage and loss modulus, glass transitions, extent of polymer curing, damping properties and interactions between polymeric components. The principle of DMA is based on oscillatory stresses as well and thus, DMA and oscillatory shear rheometer share same method for determining the shear moduli. However, in DMA, shear is not the only load mode and provides a lot of additional information compared to rheometer. [32, pp. 203−211] In DMA, the mode of deformation can be either tension, bending, shear or compression. When the sample is then subjected to a periodic stress, the mode of analysis determines which type of modulus is evaluated. Thus, DMA can, in addition to shear moduli, calculate the equivalent storage modulus (E') and loss modulus (E'') for Young's modulus (E), if subjected to stress normal to plane, such as tension or compression. Moreover, the ratio of these, called damping coefficient (tan Δ) can be measured. [69]

Tests, where frequency is kept constant and the temperature changes, are called temperature scans. During the test, for each temperature, storage modulus, loss modulus and tan Δ are determined and plotted in logarithmic scale. The most important part in this test, however, is to determine the used frequency. Standard frequency used is 1 Hz but else can be used as well depending on the application. However, meaningful, and efficient collection of data limits the frequency area. Additionally, appropriate temperature ramp should be selected for the test. The choice depends on frequency, sample size and wanted accuracy in transitions. [32, pp. 211−214] Figure 7 shows the results of typical DMA thermogram.

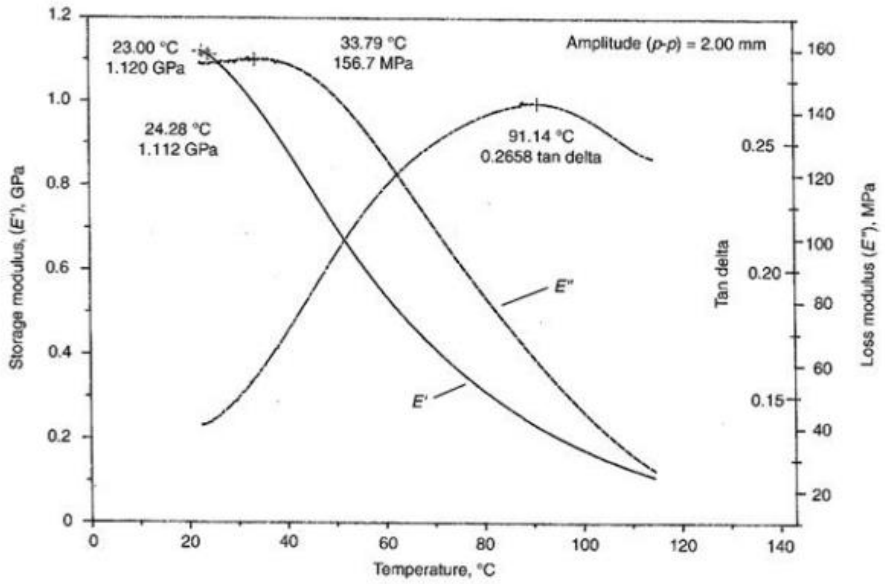

Figure 9. *Results of a temperature scan conducted with DMA [69].*

In comparison to temperature scans, also frequency-dependent measurements can be conducted and are important for practical application of materials [69]. In addition, several time-dependent tests can be performed with DMA as well, such as creep and stress relaxation tests. [32, pp. 218–219]. In general, ISO 6721-1 [70] provides principles for conducting dynamic mechanical analysis and refers to its later parts for more specific test-dependent instructions.

Dynamic mechanical analysis has a lot of potential for characterizing biomaterials. To hydrogels, for example, dynamic mechanical analysis, can be conducted to gain information from different modes of load than shear. Usually, these tests are conducted in the compression mode, due to hydrogels weak mechanical properties. [32, pp. 218–219]. Moreover, in the study of Atkinson and Vyazovkin [71], the dynamic mechanical analysis was conducted as a part of the study of degradation behaviour *in vitro* and found out that the polymers studied had similar storage and loss modulus values at room and body temperature. DMA is also important in the use of shape-memory polymers because a shape recovery test cannot be conducted until actual phase transitions are observed in a temperature ramp test. Afterwards, the shape recovery test can be conducted with DMA as well, either in tensile or compression mode. In a recovery test, the sample is heated above the transition temperature and stretched. Then, the temperature is cooled down while maintaining the strain constant. After cooling, the stress is released, and the sample is heated again and recovered deformation as a function of temperature can be recorded. To mimic the shape recovery under *in vivo* conditions the heating can be performed only up to body temperature. [32, pp. 224–225]

5. CONCLUSIONS

The purpose of this thesis was to present and evaluate the current state of *in vitro* degradation testing in the light of expanding range of materials and applications. The main focus was on testing physical and mechanical properties, and it should be remembered that the methods presented here do not necessarily describe the degradation sufficiently enough alone, but that many other characteristics of the polymer must also be taken into account. It should also be noted that none of the test methods are meaningful if the conditions do not adequately mimic the environment of use, and that even if the tests are performed appropriately, the results may still not correlate with *in vivo* results and those tests are needed as well.

The range of biodegradable polymers is wider than ever, and new materials and applications are being developed rapidly. The fast development of new technologies such as tissue engineering, gene therapy and controlled drug delivery is undoubtedly one of the main factors contributing to this expanding growth and will be continuing to do so in the future. However, as established, similar challenges remain regardless of the application, often heavily related to understanding the degradation kinetics and predicting the rate of degradation. The degradation process itself is already very complex, as there are so many factors to take into account and the ever-expanding range of materials and applications adds to the challenge. Nonetheless, degradation testing should not stand in the way of progress and therefore should be developed in equal measure. The methods currently in use show that creating uniform and consistent procedures is a challenge, and many of the currently available methods need to be adapted to suit the application and material in question.

The scope of this thesis also included an assessment of the current state of standardization of *in vitro* degradation testing and how existing standards can be used for this purpose. The difficulties posed by the diversity and tailorability of each application are also reflected in the development of guidelines for degradation testing, and this can clearly be seen in the availability of standards. Only a few standards can be found that at least somehow present general guidelines for conducting *in vitro* tests. However, these standards leave a lot to the discretion of the researcher and offer more application suggestions through other standards. Specific standards are especially mentioned for each testing method, which do provide important guidelines for testing, but also leave a lot of

scope for application in order to be fit for the purpose. All the standards mentioned in this thesis are listed in Annex A according to their intended use.

Based on current test methods and available standards, it is clear that the challenge for current *in vitro* degradation testing is creating uniform and consistent guidelines that would make the *in vitro* tests more united and comparable with each other. This also includes further standardization of different methods for different applications and materials. Given the complexity of the process, however, the development is not easy and deeper understanding of biological processes as well as technological advances are required to make it possible in the future.

REFERENCES

[1] L. S. Nair, C. T. Laurencin, Polymers as Biomaterials for Tissue Engineering and Controlled Drug Delivery, In: K. Lee, D. Kaplan (Eds.), Tissue Engineering I. Advances in Biochemical Engineering/Biotechnology, Springer, Berlin, Heidelberg, Vol. 102, 2005, pp. 47−90.

[2] L. S. Nair, C. T. Laurencin, Biodegradable polymers as biomaterials, Progress in Polymer Science, Vol. 32, Iss. 8−9, 2007, pp. 762−798.

[3] P. A. Gunatillake, R. Adhikari, Biodegradable synthetic polymer for tissue engineering, European Cells and Materials, Vol. 5, 2003, pp. 1−16.

[4] V. Hasirci, N. Hasirci, Fundamentals of Biomaterials, Springer, New York, NY, 2018, pp. 1−14, 65−82.

[5] D. F. Williams, The Williams Dictionary of Biomaterials, Liverpool University Press, 1999.

[6] A. J.T. Teo, A. Mishra, I. Park, Y. Kim, W. Park, Y. Yoon, Polymeric Biomaterials for Medical Implants and Devices, ACS Biomaterials Science & Engineering, Vol. 2, Iss. 4, 2016, pp. 454−706.

[7] S. V. Madihally, Principles of Biomedical Engineering, Second Edition, Artech House, 2019, pp. 243−288.

[8] Y. Liu, Y. Zheng, B. Hayes, Degradable, absorbable or resorbable—what is the best grammatical modifier for an implant that is eventually absorbed by the body? Science China Materials, Vol. 60, 2017, pp. 377−391.

[9] P. Balakrishnan, V.G. Geethamma, M. S. Sreekala, S. Thomas, Fundamental Biomaterials – Polymers, Elsevier, 2018, pp. 1−20.

[10] Z. Morsada, M. Hossain, T. Islam, A. Mobin, S. Saha, Recent progress in biodegradable and bioresorbable materials: From passive implants to active electronics, Applied Materials Today, Vol. 25, 2021.

[11] W. He, R. Benson, Handbook of Polymer Applications in Medicine and Medical Devices, William Andrew Publishing, 2014, pp. 55−76.

[12] B. D. Ulery, L. S. Nair, C. T. Laurencin, Biomedical Applications of Biodegradable Polymers, Journal of polymer science. Part B, Polymer physics, Vol. 49, Iss. 12, 2011, pp. 832−864.

[13] S. Fakirov, Biodegradable Polyesters, John Wiley & Sons, 2015.

[14] E. Vey, C. Rodger, J. Booth, M. Claybourn, A. F. Miller, A. Saiani, Degradation kinetics of poly(lactic-co-glycolic) acid block copolymer cast fims in phosphate buffer solution as revealed by infrared and Raman spectroscopies, In: Polymer Degradation and Stability, Elsevier, 2011.

[15] C. Kalirajan, A. Dukle, A. J. Nathanael, T.-H. Oh, G. Manivasagam, A Critical Review on Polymeric Biomaterials for Biomedical Applications, Polymers, Vol. 13, Iss. 17, 2021.

[16] D. Radulescu, I. A. Neacsu, Alexandru-Mihai Grumezescu, E. Andronescu, New Insights of Scaffolds Based on Hydrogels in Tissue Engineering, Polymers, Vol. 14, Iss. 4, 2022.

[17] F. Khan, M. Tanaka, Designing Smart Biomaterials for Tissue Engineering, International Journal of Molecular Sciences, Vol. 19, Iss. 1, 2018.

[18] Ye-Seon Lim, O. Ye-Jin, S. Hwang, K. Jong-Young, S. Yoon, Marine collagen as a promising biomaterial for biomedical applications, Marine Drugs, Vol. 17, Iss. 8, 2019.

[19] P. Sengupta, B. L. V. Prasad, Surface Modification of Polymeric Scaffolds for Tissue Engineering Applications, Regenerative Engineering and Translational Medicine, Vol. 4, Iss. 2, 2018, pp. 75−91.

[20] B. Laycock, M. Nikolić, J. M. Colwell, E. Gauthier, P. Halley, S. Bottle, G. George, Lifetime prediction of biodegradable polymers, Progress in Polymer Science, Vol. 71, 2017, pp. 144−189.

[21] A. Göpferich, Mechanisms of Polymer Degradation and Elimination, In: A.J. Domb, J. Kost, D.M. Wiseman (Eds.), Handbook of Biodegradable Polymers, CRC Press, Boca Raton, 1997, pp. 451−473.

[22] C. Lin, K. S. Anseth, The Biodegradation of the Biodegradable Polymeric Biomaterials, In: B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons (Eds.) Biomaterials Science: An Introduction to Materials in Medicine, Elsevier Science & Technology, 2012, pp. 716−728.

[23] D. S. Katti, S. Lakshmi, R. Langer, C. T. Laurencin, Toxicity, biodegradation and elimination of polyanhydrides, Advanced Drug Delivery Reviews, Vol. 54, Iss. 7, 2002, pp. 933−961.

[24] V. Hasirci, K. Lewanfrowski, J. D. Gresser, D. L. Wise, D. J. Trantolo, Versatility of biodegradable biopolymers: degradability and an in vivo application, Journal of Biotechnology, Vol. 86, Iss. 2, 2001, pp. 135−150.

[25] A. J. Coury, Chemical and biochemical degradation of polymers intended to be biostable, In: B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons (Eds.) Biomaterials Science: An Introduction to Materials in Medicine, Elsevier Science & Technology, 2012, 696 p.

[26] W. Schnabel, Polymer Degradation Principles and Practical Applications, Macmillan, 1981.

[27] F. von Burkersroda, L. Schedl, A. Göpferich, Why degradable polymers undergo surface erosion or bulk erosion, Biomaterials, Vol. 23, Iss. 21, pp. 4221−4231.

[28] A. Banerjee, K. Chatterjee, G. Madras, Enzymatic degradation of polymers: a bried, review, Materials Science and Technology, Vol. 30, Iss. 5, 2014, pp. 567−573.

[29] H. S. Azevedo, R. L. Reis, Understanding the Enzymatic Degradation of Biodegradable Polymers and Strategies to Control Their Degradation Rate, In: R. L. Reis, J. San Román (Eds.), Biodegradable Systems in Tissue Engineering and Regenerative Medicine, CRC Press, 2004, pp. 177−201.

[30] M. N. Egorikhina, I. I. Bronnikova, Y. P. Rubtsova, I. N. Charykova, M. L. Bugrova, D. D. Linkova, D. Y. Aleynik, Aspects of in vitro biodegradation of hybrid Fibrin-Collagen scaffolds, Polymers, Vol. 13, Iss. 20, 2021.

[31] Implants for surgery − Homopolymers, copolymers and blends on poly(lactide) − In vitro degradation testing, Finnish Standard Association, SFS-EN ISO 13781:2017, Helsinki, 2017.

[32] M.-C. Tanzi, S. Fare, Characterization of Polymeric Biomaterials, Elsevier Science & Technology, 2017.

[33] Q. Liu, L. Jiang, R. Shi, L. Zhang, Synthesis, preparation, in vitro degradation, and application of novel degradable bioelastomers − A review, Progress in Polymer Science, Vol. 37, Iss. 5, 2012, pp. 715−765.

[34] P. F. McDonald, J. G. Lyons, L. M. Geever, C. L. Higginbotham, In vitro degradation and drug release form polymer blends based on poly(dl-lactide), poly(l-lactide-glycolide) and poly(ε-caprolactone), Journal of Materials Science, Vol. 45, Iss. 5, 2010, pp. 1284−1292.

[35] C.-C. Chen J.-Y. Chueh, H. Tseng, H.-M. Huang, Preparation and characterization of biodegradable PLA polymeric blends, Biomaterials, Vol. 24, Iss. 7, 2003, pp. 1167−1173.

[36] P. Alamán-Díez, E. García-Gareta, P. F. Napal, M. Arruebo, M. A. Pérez, In vitro Hydrolytic Degradation of Polyester-Based Scaffolds under Static and Dynamic Conditions in a Customized Perfusion Bioreactor, Materials, Vol. 15, Iss. 7, 2022.

[37] R. A. Rebia, S. Rozet, Y. Tamada, T. Tanaka, Biodegradable PHBH/PVA blend nanofibers: Fabrication, characterization, in vitro degradation, and in vitro biocompatibility, Polymer Degradation and Stability, Vol. 154, 2018, pp. 124−136.

[38] Biological evaluation of medical devices. Part 9: Framework for identification and quantification of potential degradation products (ISO 10993-9:2019), Finnish Standard Association, SFS-EN ISO 10993-9:2021, Helsinki, 2021.

[39] Biological evaluation of medical devices. Part 13: Identification and quantification of potential degradation products from polymeric medical devices, Finnish Standard Association, SFS-EN ISO 10993-13:2010, Helsinki, 2010.

[40] T. Yoshioka, N. Kawazoe, T. Tateishi, G. Chen, In vitro evaluation of biodegradation of poly(lactic-co-glycolic acid) sponges, Biomaterials, Vol. 29, Iss. 24−25, 2008, pp. 3438−3443.

[41] Standard test method for in vitro degradation testing of hydrolytically degradable polymer resins and fabricated forms for surgical implants, ASTM International, ASTM F1635-16, 2016.

[42] Standard guide for assessment of absorbable polymeric implants, ASTM International, ASTM F2902-16, 2016.

[43] S. Liu, G. Wu, X. Chen, X. Zhang, J. Yu, Degradation Behaviour In Vitro of Carbon Nanotubes (CNTs)/Poly/lactic acid) (PLA) Composite Suture, Polymers, Vol. 11, Iss. 6, 2019.

[44] H. S. Azevedo, F. M. Gama, R. L. Reis, *In Vitro* Assessment of the Enzymatic Degradation of Several Starch Based Biomaterials, Biomacromolecules, Vol. 4, 2003, pp. 1703—1712.

[45] S. Zeng, L. Liu, Y. Shi, J. Giu, W. Fang, M. Rong, … W. Gao, Characterization of silk Fibroin/Chitosan 3D porous scaffold and in vitro cytology, PLoS One, Vol. 10, Iss. 6, 2015.

[46] J. Fernández, A. Etxeberria, J. R. Sarasua, In vitro degradation of poly(lactide/δ-valerolactone) copolymer, Polymer Degradation and Stability, Vol. 112, 2015, pp. 104−116.

[47] Plastics. Determination of the viscosity of polymers in dilute solution using capillary viscometers. Part 1: General principles, Finnish Standard Association, SFS-EN ISO 16628-1:2021, Helsinki, 2021.

[48] S.-I. Yusa, Polymer characterization, In: R. Narain (Ed.), Polymer Science and Nanotechnology, Elsevier, 2020, pp. 105−124.

[49] Plastics − Determination of average molecular weight and molecular weight distribution of polymers using size-exclusion chromatography. Parts 1−5, Finnish Standard Association, SFS-EN ISO 16014:2019, Helsinki, 2019.

[50] R. Brown, Handbook of Polymer Testing: Short-term Mechanical Tests, iSmithers Rapra Publishing, 2002.

[51] W. D. Callister Jr, D. G. Rethwisch, Callister's Materials Science and Engineering, Wiley-Blackwell, 2020, pp. 154−195, 545−599.

[52] Plastics − Determination of tensile properties. Parts 1−5, Finnish Standard Association, SFS-EN ISO 527:2019, Helsinki, 2019.

[53] J. Blessy, G. Anne, G. Sreeraj, K. Nandakumar, T. Sabu, Polymer sutures for simultaneous wound healing and drug delivery − A review, International Journal of Pharmaceutics, Vol. 524, Iss. 1−2, 2017, pp. 454−466.

[54] S. V. Khiste, V. Ranganath, A. S. Nichani, Evaluation of tensile strength of surgical synthetic absorbable suture materials: an in vitro study, Journal of periodontal & implant science, Vol. 43, Iss. 3, 2013, pp. 130−135.

[55] G. Schwach, M. Vert, In vitro and in vivo degradation of lactic acid-based interference screws used in cruciate ligament reconstruction, International Journal of Biological Macromolecules, Vol. 25, Iss. 1−3, 1999, pp. 283−291.

[56] Plastics − Determination of flexural properties, Finnish Standard Association, SFS-EN ISO 178:2019, Helsinki, 2019.

[57] J. Liuyun, X. Chengdong, J. Lixin, X. Lijuan, Effect of hydroxyapatite with different morphology on the crystallization behaviour, mechanical property, and in vitro degradation of hydroxyapatite/poly(lactic-co-glycolic) composite, Composites Science and Technology, Vol. 93, Iss. 18, 2014, pp. 61−67.

[58] Plastics − Determination of Izod impact strength, Finnish Standard Association, SFS-EN ISO 180:2019, Helsinki, 2019.

[59] J. Tams, C. A.P. Joziasse, R. R.M Bos. F. R. Rozema, D. W. Grijpma, A. J. Pennings, High-impact poly(L/D-lactide) for fracture fixation: in vitro degradation and animal pilot study, Biomaterials, Vol. 16, Iss. 18, 1995, pp. 1409−1415.

[60] Fibre-reinforced plastic composites − Determination of apparent interlaminar shear strength by short-beam method, Finnish Standard Association, SFS-EN ISO 14130:1997, Helsinki, 1997.

[61] H. Niiranen, T. Pyhältö, P. Rokkanen, M. Kellomäki, P. Törmälä, In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composite, Journal of Biomedical Materials Research Part A, Vol. 69A, Iss. 4, pp. 699−708.

[62] T. Jiang, S. P. Nukavarapu, M. Deng, E. Jabbarzadeh, M. D. Kofron, S. B. Doty, W. I. Abdel-Fattah, C. T. Laurencin, Chitosan-poly(lactide-co-glycolide) microsphere-based scaffolds for bone tissue engineering: In vitro degradation and in vivo bone regeneration studies, Acta Biomaterialia, Vol. 6, Iss. 9, 2010, pp. 3457−3470.

[63] Plastics − Determination of compressive properties, Finnish Standard Association, SFS-EN ISO 604:2002, Helsinki, 2002.

[64] H. M. Wyss, Rheology of Soft Materials, In: A. Fernandez-Nieves, A. M. Puertas (Eds.), Fluids, Colloids and Soft Materials: An Introduction to Soft Matter Physics, John Wiley & Sons, 2016, pp. 317−345.

[65] Rheology. Part 2: General principles of rotational and oscillatory rheometry, Finnish Standard Association, SFS-EN ISO 3219:2021, Helsinki, 2021.

[66] Plastics − Determination of dynamic mechanical properties. Part 2: Torsion-pendulum method, Finnish Standard Association, SFS-EN ISO 6721-2:2019, Helsinki, 2019.

[67] C. Yan, D. J. Pochan, Rheological properties of peptide-based hydrogels for biomedical and other applications, Chemical Society Reviews, Iss. 39, pp. 3528−3540.

[68] Y. Wu, A. Wenger, H. Golzar, X. S. Tang, 3D bioprinting of bicellular liver lobule-mimetic structures via microextrusion of cellulose nanocrystal-incorporated shear-thinning bioink, Scientific Reports (Nature Publisher Group), Vol. 10, Iss. 1, 2020.

[69] V. Burt, Dynamic Mechanical Analysis, Materials Characterization, ASM Handbook, ASM international, Vol. 10, 2019, pp. 319−323.

[70] Plastics – Determination of dynamic mechanical properties. Part 1: General principles, Finnish Standard Association, SFS-EN ISO 6721-1:2019, Helsinki, 2019.

[71] J. L. Atkinson, S. Vyazovkin, Dynamic Mechanical Analysis and Hydrolytic Degradation Behavior of Linear and Branched Poly(L-lactide)s and Poly(L-lactide-co-glycolide)s, Macromolecular Chemistry and Physics, Vol. 214, Iss. 7, pp. 835–843.

ANNEX A: SUMMARY OF STANDARDS

Type	Number	Name
General guidance for *in vitro* degradation tests	ISO 10993-9, ISO 10993-13	Biological evaluation of medical devices. Part 9: Framework for identification and quantification of potential degradation products, Part 13: Identification and quantification of potential degradation products from polymeric medical devices
	ISO 13781	Implants for surgery — Homopolymers, copolymers and blends on poly(lactide) — In vitro degradation testing
	ASTM F1635-16	Standard test method for in vitro degradation testing of hydrolytically degradable polymer resins and fabricated forms for surgical implants
	ASTM F2902-16	Standard guide for assessment of absorbable polymeric implants
Evaluation of molecular weight	ISO 16014	Plastics. Determination of average molecular weight and molecular weight distribution of polymers using size-exclusion chromatography. Parts 1—5
	ISO 16628-1	Plastics. Determination of the viscosity of polymers in dilute solution using capillary viscometers. Part 1: General principles
Mechanical characterization	ISO 178	Plastics — Determination of flexural properties
	ISO 180	Plastics — Determination of Izod impact strength

	ISO 527-1, ISO 527-2, ISO 527-3	Plastics — Determination of tensile properties. Part 1: General principles, Part 2: Test conditions for moulding and extrusion plastics, Part 3: Test conditions for films and sheets
	ISO 604	Plastics—Determination of compressive properties
	ISO 1805	Fishing nets — Determination of breaking force and knot breaking force of netting yarns
	ISO 2062	Textiles — Yarns from packages. Determination of single-end breaking force of netting yarns
	ISO 3219-2	Rheology. Part 2: General principles of rotational and oscillatory rheometry
	ISO 6721-2	Plastics—Determination of dynamic mechanical properties. Part 2: Torsion-pendulum method
	ISO 13934-1	Textiles — Tensile properties of fabrics. Determination of maximum force and elongation at maximum force using the strip method
	ISO 14130	Fibre-reinforced plastic composites — Determination of apparent interlaminar shear strength by short-beam method
	ASTM D2990	Standard test methods for tensile, compressive, and flexural creep and creep-rupture of plastics