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Petteri Niemelä

BIOACTIVE BONE TISSUE ENGINEERING SCAFFOLDS

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TIIVISTELMÄ

Petteri Niemelä: Bioactive bone tissue engineering scaffolds
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Luupuutosten korvaaminen on vielä nykypäivänä hankalaa, sillä luukudos kasvaa hitaasti ja vaatii sopivan alustan soluille. Kudosteknologian tukirakenteilla pyritään antamaan soluille sopiva kasvualusta, sekä mahdollistamaan myös sellaisten vikojen korjaaminen, mitä ihmiskeho ei itse pysty korjaamaan. Tutkimuksen tavoitteena on tarkastella ja luoda yhteenveto sekä nykypäivän että lähitulevaisuuden materiaaleista, joilla voidaan tehostaa luun paranemista ja uuden luun muodostumista.

Tämä tutkielma on kirjallisuuskatsaus, jossa ensiksi tutkitaan, millaisia vaatimuksia näille erilaisille kudosteknologian tukirakenteille on ja mistä nämä vaatimukset johtuvat. Toiseksi tutkimuksessa käsitellään nykypäivänä käytössä olevia yleisimpiä materiaaleja, kuten esimerkiksi kalsiumfosfaatteja ja biolaseja. Kolmanneksi tutkimuksessa tutkitaan sekä tulevaisuuden materiaaleja että nykypäivän materiaalien ongelmia, joiden jälkeen tarkastellaan tukirakenteiden erilaisia valmistusmenetelmiä.

Tämä tutkimus osoittaa, että nykypäivänä käytettävissä olevien kudosteknologisten tukirakenteiden valmistusmateriaalien lukumäärä on melko vähäinen, ja että näillä materiaaleilla on monia niiden käyttöä rajoittavia ongelmia. Todetaan myös, että nykypäivän valmistusmenetelmissä on parantamisen varaa, sillä halutun rakenteen luominen on usein hankalaa. Tukirakenteiden rakenne vaikuttaa sekä sen ominaisuuksiin että solujen toimintaan, jolloin rakenteella on merkittävä vaikutus luun kasvuun. Tulevaisuudessa pitäisi löytää uusia materiaaleja ja valmistusmenetelmiä, joilla voidaan ratkaista sekä mikro- että makrokokoluokan ongelmia tukirakenteissa. Nämä ongelmat rajoittavat nykypäivänä tukirakenteiden käyttöä, minkä seurauksena luusiirteet ovat edelleen paras vaihtoehto luupuutosten korjaamiseen. Tutkielmassa myös todetaan, että vaikka nykypäivän materiaalit eivät ole optimaalisia, ovat ne silti erittäin hyödyllisiä tilanteissa, joissa luusiirteiden avulla luupuutoksen korjaaminen ei ole kannattavaa tai mahdollista.

Avainsanat: Biomateriaalit, Kudosteknologia, Luun paraneminen, bioaktiivisuus, keraamien valmistusmenetelmät

ABSTRACT

Petteri Niemelä: Bioactive bone tissue engineering scaffolds
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Healing bone deficiencies is still challenging in the present day, as bone tissue grows slowly and requires a suitable platform for cells. Tissue engineering scaffolds aim to provide cells with a suitable substrate, and also enabling the repair of defects that the human body itself cannot repair. The goal of this study is to review and create a summary of both today's and near-future materials to enhance bone healing and new bone formation.

This thesis is a literary review which first explores the requirements for these different types of tissue engineering scaffolds, and where these requirements derive from. Secondly, the study reviews the most common materials in use today, such as calcium phosphates and bioglasses for example. Thirdly, the study examines materials of the future and the problems with materials in use today, followed by a review of different manufacturing methods of supporting structures.

This study shows that the number of materials available today for tissue engineering scaffolds is quite low, and that these materials have many problems which limit their use. It is also noted that there is room for improvement in today's manufacturing methods, as creating the desired structure is often difficult. The structure of the scaffolds affects both its properties and cellular functions, whereby the structure has a significant effect on bone growth. In the future, new materials and manufacturing methods should be found to solve both micro- and macro-size problems with tissue engineering scaffolds. These problems limit the use of tissue engineering scaffolds in the present day, as a result, autografts still remain as the best option for correcting bone deficiencies. The thesis also states that while today's materials are not optimal, they are still very useful in situations when using bone grafts to correct bone deficiency is not possible.

Keywords: Biomaterials, Tissue engineering, Bone healing, Bioactivity, Ceramic material manufacturing methods.

PREFACE

This is a thesis about bioactive bone tissue engineering scaffolds, which covers all the basic information needed from material science perspective. It has been written to give a final show of knowledge and comprehension about material science studies in Tampere university. I came up with the idea for this thesis in early 2020 but wrote everything during time between June 2021 and August 2021.

Hardest part of writing this thesis was the start, because there is so much information and so much to write about, but with my supervisor we were able to decide the scope of the thesis and what I should start with, which made this process a lot easier. And so, I would like to thank my thesis supervisor Juha Nykänen for all the help in regards of questions about the thesis, friendly pressure to push me to write and understanding of my personal situation.

I would also like to thank all the professors and teachers who have taught me about this subject and got me hooked to learn more during my courses related to biomaterials. Without that information I most likely would not have been able to write this. One last thank you goes to my family but especially my dad and brother who have helped me tremendously during my studies and thesis writing.

Hopefully you will enjoy reading this and enjoy learning while you do so!

Tampere, 19.8.2021

Petteri Niemelä

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ABBREVIATIONS AND SYMBOLS

2D	two-dimensional
3D	three-dimensional
BCP	biphasic calcium phosphate
BG	bioglass
CaP	calcium phosphate
et. al	lat. et alii or et alieae, and others
etc.	et cetera: and other similar things
FAST	field-assisted sintering technique
FDA	U.S. Food and Drug Administration
FDM	fused depositon modeling
HA	hydroxy apatite
HCA	hydroxy carbonate apatite
I_B	bioactivity index
i.e.	id est, that is
m	meter
Pa	Pascal
SLA	Stereo lithography appearance
SLS	Selective laser sintering
SPS	spark plasma sintering
t	time
TCP	tricalcium phosphate
x	x-coordinate
y	y-coordinate

1. INTRODUCTION

Bone has high potential to regenerate, and it is able to repair itself as long as the wound is not too big. The limit for this is called critical size defect. The bone which forms to repair these wounds is just like the bone which was there before. If the wound is too big then we need to use something which will help the bone to grow. The most common material used in the present day to heal bone deficiencies is autografted bone. [1] Nowadays over four million surgeries are done annually in which bone grafts are used [2]. Using autografts for healing bone deficiency requires multiple surgeries as the bone needs to be collected from some other part of the patient's body before it can be grafted. Multiple surgeries mean that more time is used for the treatment and multiple sites are needed which will hurt and could get inflamed. Because this method is expensive, requires a lot of time and might cause issues even though the material will not cause immune reaction as autografts are perfectly histocompatible, new methods for healing bone deficiencies are desperately being researched to replace autografts. Tissue engineering has shown great promise already in regenerating bone when the body would not be able to heal itself anymore. [1]

Tissue engineering methods for bone healing include scaffolds, which can have cells planted into them before the scaffold is applied to the body or the scaffolds can be free of cells before the implantation. The scaffolds provide 3D structure for the cells to interact in. Properties of the scaffold structure such as interconnectivity, porosity and pore size have been shown to greatly affect the success rate of the treatment and the rate of healing. The right structure design is only one part of the scaffold, other part is the material itself. Choosing the right material for the job is hard because the material choice affects cell proliferation, cell differentiation, healing rate, strength of the scaffold etc. [3]

Nowadays mostly bioactive ceramics are used for bone healing because these promote cell growth and degrade so eventually the material will be completely replaced with real bone [3]. Ceramics are quite obvious choice because natural bone is a composite structure of which inorganic parts mostly consists of calcium phosphates [4]. Even with materials which are only similar to the inorganic parts of the natural bone, we are able to heal some bone defects with success, but those materials are not optimal because those only partly mimic the inorganic parts of the bone and not the whole structure. In order to make

the perfect bone tissue engineering scaffolds, we would need a material which is bio-compatible, mimics the natural bone completely, has great bioactivity, degrades at the same rate as new bone grows and finally has good mechanical properties so it does not get crushed in load bearing applications, but does not provide too much support to cause stress shielding. [3]

The goal of this literary review is to evaluate, compare and compile the most important pieces of information about many kinds of bioactive bone tissue engineering scaffolds to enable the reader to make good material choices for healing bone defects using tissue engineering methods. The main advantages, disadvantages and working principles of the most common materials are the main focuses for this research. But also, because the whole systems properties are partly affected by structure of the scaffolds, we need to investigate manufacturing methods for these scaffolds as well. After the materials and manufacturing methods have been explained these are tied together by looking deeper into applications of these bioactive bone tissue engineering scaffolds.

Most important findings of this review were that there are several materials out there such as Calcium phosphates, Calcium silicates and bio glasses which have shown great promise and have most of the required properties for a good scaffold material, but these are still not perfect materials in any case. Manufacturing methods on the other hand are slowly being developed to give much better results than the traditional manufacturing methods, but expensive and complex equipment required by those are limiting the use of the new and modern manufacturing methods. Currently the results got with scaffolds made with the modern manufacturing methods are not that much better than with the more random and less precisely made scaffolds, so the cost are not really justified by the acquired benefits. But those improvements are going to be essential later when we are trying to squeeze the last performance improvements out of the scaffolds.

2. REQUIREMENTS

Scaffolds for tissue engineering applications have wide variety of properties such as biocompatibility, biodegradability, porosity, mechanical properties, bioactivity etc. [2]. When we are designing a suitable scaffold for any tissue engineering application, we need to take these properties into consideration as in many cases most of these are necessities for having a suitable scaffold.

2.1 Biocompatibility

Biocompatibility is the most important criterion for any tissue engineering scaffold [5]. This is also the very first one tested because if the scaffold material is not biocompatible then it is not suitable for medical use. Biocompatibility means that cells must be able to adhere to the surface of the scaffold, the scaffold will not interfere with normal functions of the cells, cells must be able to proliferate on the surface of the scaffold and that it will not cause a significant immune reaction [5]. After something is implanted the reaction in the recipient's body can vary a lot and it is even affected by age, sex, general health etc. of the person. So basically, every little difference in the material and the host can change the outcome.

When the material is implanted first water and ion layer is formed, then proteins adsorb to it. This is when the inflammation and rejection will happen if it happens. Then the cell adhesion process begins, if inflammation or rejection of the implant did not happen, and cells start to spread and proliferate. And eventually cells start to differentiate and form tissues. The appropriate host response is acquired by having the correct surface properties which attract the correct proteins to attract the right host cells to bind the material to surrounding tissues. [6]

The expression biocompatibility comprehends different properties of materials, such as toxicity, tissues compatibility, blood compatibility, and bio functionality properties. And because no material is truly bioinert, it means that there will always be some beneficial or harmful effects with every material. So basically, when some material has better biocompatibility than some other material the recipient's body will accept it better and show less signs of harmful effects. [2,5]

2.2 Bioactivity

The second very important requirement especially for this thesis is bioactivity. Normally metals and polymers are nearly bioinert so those do not interact with the body in any significant amount, but usually a fibrous capsule forms around bioinert objects inside a body. Human body for example can easily tolerate bioinert materials because there is nothing to tolerate except that they are there. Bioactive materials are also considered to be tolerated materials also, as the way these do interact with the body is in desired and helpful manner. Bioactive materials can for example bind with cells covalently and speed up the healing process. Which means that these materials can be a part of osseointegration process. [3,7]

Bioactive materials can be divided into two classes, A and B. Class A bioactive materials are materials which are both osteoconductive and osteoinductive, these materials do not only help existing bone to bond and grow but also allows bone to grow in places where it normally would not grow [7]. Which means that these materials attract osteoblasts to them even without bone being present. Which leads to that these materials can also be called osteostimulative materials. Class B materials on the other hand are only osteoconductive materials which bond with bone and allow the bone to grow inside the scaffold [7]. Class A materials are considered to be better than class B as class A materials do not need bone contact for bone to grow inside the scaffold, but it allows the bone to grow all over the scaffold from the beginning. The most notable actual difference and one of the ways to determine which class the material belongs to is its ability to bind with soft tissue. Class A materials cause the healing process to be way faster than what it is with class B materials. Which means that only class A materials can bind with soft tissue and class B materials cannot. This leads to the second way to determine the class of some material is with bioactivity index (I_B), it is a comparable value about how long it takes tissues to bond with half of the interface, i.e., $t_{0,5bb}$ [8].

$I_B = 100/t_{t0.5bb}$

Materials which have higher bioactivity index than 8 are considered to be class A materials so the bonding speed is enough for the material to bond with both soft and hard tissues [8].

2.3 Porosity

Porosity is very important part of pretty much every tissue engineering scaffold, porosity and the architecture affect how well the cells bond with the material and how well the

cells spread inside the scaffold. Pores in the scaffold structure should be 100% accessible and interconnected. If the pores are not accessible, those pores will not be filled with cells until the material degrades enough to open a way to the previously blocked pores. Interconnectivity on the other hand is about how many paths there are to any pore. If there is only one path to some pore, it will take longer for cells to get there than when compared to situation where the cells can move freely for pore to pore without any blockages between pores. Accessibility and interconnectivity are not only important for the cells migration but also for nutrient and waste transportation as all cells require nutrients and produce waste from using those nutrients. One more property of porosity is pore size. It is important because cells will not bind well to very big pores but also if the cells will not fit into the pore, then the pore is too small. [4,5,9] Normally good size for pores for bone tissue engineering is in micron scale and more commonly around 50-350 microns, but recently nano sized pores have shown to be beneficial and are required for some types of tissues [9]. So, for multiscale interaction of cells it is important to have nanoscale pores as well in the scaffold.

Porosity does not only affect the cells but also other properties of the scaffold. When there are pores in the scaffold it will be weaker than a solid block. These pores can very quickly decrease the strength of the material to quite low value. But this must be taken into consideration in the design phase already so there will not be any surprises in the end. [10]

Porosity will also affect degradation rate quite significantly as when there are pores in the structure there will be less material in the first place, but it has a lot more surface area which leads to that water or enzymes can penetrate the structure faster and cause it to degrade faster [10]. For example, solid hydroxyapatite blocks can be considered non degradable but porous hydroxyapatite scaffolds are biodegradable class B bioactive scaffolds. [9]

2.4 Additional information

As important as biocompatibility, bioactivity and porosity are those are not the only important things to know about bone tissue engineering when designing the scaffolds. Other things to consider also are the structure, how the body dispose of the material and choosing the right manufacturing method. [3,4,9]

Natural bone is a composite structure with quite thin but dense outer layer and soft spongy bone in the middle to fill the space but still keeping the bones quite light weight [11]. This causes a lot of problems in designing the scaffolds because we would need to

make a composite structured scaffold which makes the two different kinds of bones to grow at the suitable speed so those can interact with each other well. This is not possible in the present day, so we are settling with materials which promote bone growth but do not cause the cells to differentiate to some certain type.[5]

One of the challenges of tissue engineering currently is biomimetic properties of tissue engineering materials and scaffolds. Biomimetic materials are materials which mimic the natural bone well enough that the human body believes that the material which has been implanted is recognized by the body to be its own creation and it will not be rejected. Biomimetic materials are materials which the body can dispose of naturally, which in this case with bones would be that the material gets broken down by osteoclasts and then gets disposed. With biomimetic materials it would also be possible to further reduce the rejection rate of implants therefore increasing success rate and reducing additional costs caused by failures. [9]

Biodegradation processes of bioceramics are still largely unknown [12]. Biodegradation of bioceramics is pretty much solubilizing process so, hydrolysis is a big part of the process. For example, calcium phosphates degrade because calcium and phosphate ions are released from the structure due to them being in acidic environment [13]. These released ions continue to form other materials which will end up degrading into hydroxy apatite in the end which will degrade into ions which human body can naturally dispose of. [7]

Sterilization is also a vital part of any medical product which will be in direct contact with tissues, blood, or other body fluids. Sterilization is a process where we try to kill all the living microorganisms from the product. How this differs from disinfection is that spores will not die here. The sterilization need depends on the chosen material, and the sterilization process must be validated for all the products, this can be done for example by using indicators to prove that the sterilization has been good enough at all parts of the product and not only on the surface. There are plenty of different sterilization methods which all have their own benefits and limitations, but for bioactive ceramics there are only two viable methods gamma irradiation or electron beam sterilization. These both are methods which will be able to penetrate the whole object and effective sterilize the products without causing much damage to the structure as these will not cause much degradation to bioceramics. In gamma irradiation we are using high-energy photos which are emitted from isotope source such as Cobalt 60, and in electron beam sterilization we are using electrons. These cause ionization to happen in all the material, which for living cells and other microorganisms result in damage to the DNA and other structures of the cell, which eventually can lead to death of the cells. [14]

3. MATERIALS

When we are talking about materials, we need to realize that there are no perfect materials around and most likely there will never be, but the materials we have currently are good enough for some basic healing but for more complex situations we would need new materials. But as the materials nowadays can be helpful in over 4 million surgeries every year, we need to take a closer look at the most common and the most promising new materials. [2]

The ideal scaffold would be bioactive, biodegradable, has good enough mechanical properties, is easy to manufacture and allows creating complex shapes. All these requirements are requirements for the ideal biomaterial for bone tissue engineering scaffolds. [3]

When we are choosing the right material, we need think about what kind of properties are needed from the scaffold material. There are two types of bone tissue around the body and both of those have their own natural mechanical properties, which in ideal situation we would want to match. Bones are anisotropic material which has higher strength and tensile/compressive moduli in longitudinal direction compared to radial and circumferential directions. Also, as bones are mostly made of ceramics these have better compressive strength than tensile strength. Cortical bone which is also known as dense bone is the stronger one out of the two. It can be found on the surface of bones, and it has only 5-15 % porosity. In longitudinal direction cortical bone has elastic modulus of about 20 GPa, ultimate tensile strength of about 120 MPa and ultimate compressive strength of about 180 MPa, in Transverse direction all of these drop by over half. For trabecular bone these values are slightly different when the results are corrected in a way as if the test samples were nonporous. Elastic modulus of trabecular bone is about 22 GPa and ultimate tensile strength is about 100 MPa, so roughly 10-15% change between the types of bone. When porosity is involved, trabecular bone's ultimate strength drops down fast all the way to elastic modulus being around 50 MPa and ultimate strength of about 5 MPa. So, from this we can easily see how huge difference porosity makes in the mechanical properties of materials and why it is important to know what kind of properties we are looking for as the difference between the types of bones is quite huge on apparent level even though on the tissue level the difference is not very large. [11,15]

3.1 Calcium phosphates

First major group of bioactive ceramics are Calcium phosphates (CaP) which are the major constituents of the inorganic parts of natural bone. [16] And because the inorganic part is about 70 % of the whole natural bone using CaPs seems like obvious choice for a material to fix damages on bones. It is important to note that not all CaPs are bioactive even though those eventually degrade into hydroxyapatite (HA) which is class B bioactive material. For example, tricalcium phosphate (TCP), which is the second simplest CaP after HA, is not considered to be bioactive material. These two are the most common CaPs which are currently being used, but HA is used quite a bit more than TCP. [3,4,5,7]

Hydroxy apatite has Ca/P molar ratio of around 1.667 and it is much more stable pH wise than other CaPs [4]. It has excellent biocompatibility and because it is very similar to natural bone chemically it is quite good choice for a material for tissue engineering purposes. It can be used in various ways but most important for this thesis it can be used to make porous scaffolds. Porosity affects the degradation rate of HA quite much making it viable as almost nonporous HA degrades withing few years when porous scaffolds can degrade in less than year [4]. HA releases Ca, P and some other less significant ions when in contact with body fluids. Crystallinity of HA also affects the bioactivity because Ca^{2+} ions source is the amorphous calcium phosphate phase in synthetic HAs so, when the crystallinity is high because then there are less Ca^{2+} ions to be released which lowers the bioactivity. The problem with HA is that even though we can get material which bonds with bone well and we can get material which has good mechanical properties and would not degrade too fast either, but we cannot get a material which would have all of these. To tackle these problems substituents for HA have been researched for a long time. In biological apatite there are Na^{+}, Mg^{2+} and CO_3^{2-} ions as substitutes which are looking quite good for synthetic HA also. It must be noted that even though there are huge number of substituents which could work there are as many different outcomes as each change the behavior differently to good or bad direction. [18]

TCP like previously mentioned is the second most used CaP in bone tissue engineering applications and it is also the simplest CaP after HA. The reason why TCP is important to mention here even though it is not bioactive it has properties which are useful. TCP is a less stable than HA but it has faster degradation rate and better mechanical properties, both of which we would want for HA. So, with these pieces of information, we can introduce biphasic calcium phosphate (BCP), which is a mixture of both HA and TCP. BCP is a material which is a class B bioactive material, has excellent biocompatibility, more

optimal degradation rate and better mechanical properties than HA. BCP gets its bioactivity from HA, so the mechanism for that is the same what it was with HA. The reason why BCP has more optimal degradation rate than HA or TCP alone is that HA degrades a little bit too slowly for bone to grow optimally and TCP on the other hand sometimes degrades too fast, so mixing these we can get a degradation rate which is in between the times of HA and TCP. [9]

3.2 Calcium silicates

Calcium silicate-based materials have shown to have good biological and favorable mechanical properties which are close to the human bone, but the challenges with these are controlled dissolution of ions, too fast dissolution rate, low mechanical strength, fabrication methods etc. Plain Calcium silicates have quite poor mechanical properties and too fast dissolution rate which makes these quite bad choice of material as these are factors which may cause an implant failure. To tackle this problem, researches have shown that trace elements such as magnesium, zinc and zirconium all of which promote beneficial biological phenomena in the human body also change the mechanical and chemical properties of calcium silicates. Adding these trace elements can tackle the too fast degradation rate and poor mechanical properties problems but for example magnesium stimulates cell growth and proliferation, zinc plays important role in bone growth and preserving healthy bone and zirconium adds mechanical strength, has great biocompatibility, and exhibit excellent osseointegration. [1]

Important calcium silicates for this thesis are monocalcium silicate, dicalcium silicate and tricalcium silicate as these are materials which have the ability to induce HA when in contact with the body fluids. The mechanism of HA forming happens in four steps. In the first step Ca^{2+} ions are exchanged with H^{+} ions, this ion exchange causes Si-OH rich layer to form on the surface. In the second step Ca^{2+} ions cause pH to increase which leads to supersaturation of the body fluid. Then after this step the freed Ca^{2+} ions in the body fluid are electrostatically attracted to the silica-rich layer which is negatively charged. After these steps PO_4^{3-} and HPO_4^{2-} ions adsorb to the Ca^{2+} ions which then leads to apatite layer formation. [1]

Monocalcium silicate is the most studied calcium silicate for bone repair and regeneration applications. There are two types of monocalcium silicate, low and high temperature wollastonite. Wollastonite has been shown to have faster carbonated HA growth rate than other bioactive materials such bioglasses by Siriphannon P et al. [19]. Their study also showed that this material can also support attachment of osteoblasts and affect their proliferation and differentiation. When Wollastonite is compared to CaPs, it can be seen

that Wollastonite has better mechanical properties, better bioactivity and that it has faster degradation rate. [1]

3.3 Bioglasses

Bioglasses (BGs) are one of the two main groups of materials which are used for bone regeneration with CaPs. These are amorphous materials which are often surface reacting to physiological fluids, bioactive, biodegradable and can be even osteoinductive. Bioglasses often form a hydroxy carbonated apatite (HCA) layer on top of their surface, which promotes bone regeneration [16]. That HCA layer alone would not make BGs as good materials as they are, but because these release ions which promote bone regeneration also. The combination of HCA and the released ions are what makes BGs so good materials and able to be class A bioactive materials. There are many kinds of bioactive glasses, but the most important ones are silicate glasses, borosilicate glasses and phosphate glasses [20].

Bioglasses have couple basic requirements which are that SiO_2 content must be lower than 60%, it has high Na_2O and CaO content and thirdly that it has high CaO/P_2O_5 ratio. When these three requirements are met, we will most likely have a material which has high surface reactivity in physiological fluids. Other requirements of biomaterials also apply but these are the ones that make bioglasses stand out from the rest. [21,22]

3.3.1 Silicate Bioactive glasses

Silicate bioactive glasses are the most common and most known bioactive glasses. these have been around since Larry Hench developed bioglass 45S5, which has composition of 45 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O, and 6.0 wt% P_2O_5. This was the first FDA approved bioglass, and it is still in use in medical devices. Most notable product which used this material is called perioglas, which is just bioglass 45S5 in granule form. [22]

These silicate glasses form HCA layer on top of the surface in following manner. First rapid exchange of Na^+ or K^+ with H^+ or H_3O^+ happens, this causes the loss of soluble silica, which is a result from breaking Si-O-Si bonds and formation of Si-OH. These two stages cause Si-OH bonds to form. Then after that polycondensation of SiOH + SiOH to Si-O-Si takes place. Fourth stage is when Ca^{2+} and PO_4^{3-} groups migrate to the surface forming $CaO\text{-}P_2O_5$-rich layer on top of the SiO_2-rich layer which continues to grow due to soluble calcium and phosphates in the fluid. Finally, the $CaO\text{-}P_2O_5$ layer crystallizes with the help of OH^-, CO_3^{2-} or F^- ions to form mixed layer of hydroxyl, carbonate and fluorapatite which is known also as HCA. After the HCA layer has formed then biological

moieties adsorb onto the HCA and cells can start to attach to those and begin to differentiate to generate a bone matrix. HCA layer formation only takes few hours, but the complete bone matrix formation happens over several hundred hours. This reaction which forms HCA is also the reaction which causes degradation to the material. Degradation of silicate BGs is non congruent process as the composition of the glass changes during the degradation process. [7,23]

Silicate BGs crystallize quite easily when they are hot worked so for example during fiber drawing or sintering porous scaffolds. This makes it hard for us to make scaffolds out of silicate BGs because crystallization reduces bioactivity by slowing down Ca-P layer formation. And one more problem of silicate BGs is that their mechanical properties are not too great, so these are not suitable for load bearing applications. But because these are the most used bioactive glasses out there, we can come to a conclusion that the advantages of silicate BGs are far better than the disadvantages. [23]

3.3.2 Borosilicate bioactive glasses

Because silicate bioactive glasses are so widely used but have few problems other materials have been developed to tackle those problems. Borosilicate glasses are of one those groups of materials which are trying to replace silicate BGs. The most important difference of silicate and borosilicate glasses is that borosilicate glasses have B_2O_3 added to the composition. B_2O_3 is not the only addition to the material composition as MgO and SrO can often be found from borosilicate glasses, because these significantly improve cell attachment, proliferation and causes cell morphology to be more similar than on typical silicate BGs. B_2O_3 on the other hand causes faster and more complete HCA layer formation and this also speeds up degradation of the material. The formed reactive layer on borosilicate BGs is thicker than on typical silicate BGs but it is just on the surface and does not extend to the core of the grains, and if there are Sr or Mg present then those can be found on the surface also. [24] Borosilicate BGs were developed due to the high crystallization tendency of silicate BGs. The addition of B_2O_3, MgO and SrO have made it possible to draw fibers or sinter these into scaffolds, but it has to be noted that without Mg and Sr borosilicate glasses do not show significant sintering [25]. In addition to these benefits of borosilicate BGs are also more antibacterial than typical silicate BGs are. Viability tests of borosilicate BGs have shown that when B_2O_3 content increases the cell count drops but cells become bigger, more roundish, and more spread, these suggest that with low concentration of boron early osteogenic differentiation happens and higher the concentration becomes later the osteogenic differentiation happens [25]. So, overall borosilicate BGs sound great overall but there are still problems with those as well, so these have not gotten as popular as normal typical silicate BGs

are, this is mainly due to most people know silicate glasses better and those are not as complicated as borosilicate glasses are.

3.4 Hybrid and composite materials

Researchers have been looking for a suitable material for tens of years now for bone tissue engineering purposes, but not a single perfect one has been found. And because the natural bone structure is composite structure, researchers have started to look for combination of materials which would be more suitable for these applications. Hybrid materials which have more than two different materials mixed on molecular level. These form bonds with each other with or without help from coupling agents. [3,26]

Most of the current hybrid materials are polymer-ceramic hybrids, with these we have been able to make materials which have more close mechanical properties of natural bone than with just bioceramics as ceramics have quite poor fracture toughness. And by mixing materials together like we saw with HA and TCP we can customize properties far better to be suitable in multiple applications than what we can with changing factors like porosity. There is an issue with polymer-ceramic hybrids though because we mix something non bioactive with bioactive, we will have material which will have worse bioactivity than what we would have had before. And this is why these materials have not shown much else so far except that these have a lot of potential, but with the current materials we cannot benefit from the potential because bioactivity takes such huge hit, and the other properties are not worth it. [26]

Composite materials are materials which consists of two or more distinct phases to produce a material which would have a combination of both materials' chemical, physical, mechanical, and biological properties. These are usually structures which consists of bioceramic bulk which then have polymer coating on top of them. This could work in theory as the polymer coating often increases the toughness of the structure and can even promote bioactivity when we use bioactive natural polymers such as melanin. This is because this is very similar to the natural bones structure where the carbonated apatite provides structural reinforcement and stiffness, and collagen provides flexibility and toughness. With these composite structures we can get compressive modulus to almost match but due to the polymer/bioceramic interface being weak with synthetic composites it often fails to match in the end. Also, because we are coating a bioceramic bulk with polymer we are still relying on the ceramic to provide mechanical support against stress, as the polymer is there basically just to provide little bit more toughness to the mix. But like with hybrid materials this shows promise for the future as we can make materials which have better mechanical properties by combining two materials together. [3,5,26]

4. MANUFACTURING

Manufacturing of tissue engineering scaffolds overall is quite hard and tricky. And when we are trying to replace bone with tissue engineering methods, we are most likely introducing bioactive ceramics to the mix, this is when we get in serious trouble in regards of manufacturing. Ceramics are hard to shape after sintering so the shape must be given to the material before that final hardening. There are plenty of old and new manufacturing methods out there nowadays but all of them have their own advantages and disadvantages. The older and so-called traditional manufacturing methods rely on particles and other substances to make the pores and the new modern manufacturing methods are much more precise and has a lot less randomness to the scaffolds. [3,16]

4.1 Traditional manufacturing methods

Traditional manufacturing methods, such as porogen leaching, gas foaming and freeze drying, are quite easy ways to manufacture scaffolds and are often quite cost-effective methods also, but the problem is that these methods provide us random uncontrollable pores which will have random pore size, porosity, interconnectivity, pore shape and accessibility [2,3,10]. Because these methods have such random pores and variation between the scaffolds, we don't always have the exact same results and sometimes the pores are so wrong that those could end up causing implant failure if the poor-quality scaffold was used. [3,16]

4.1.1 Porogen leaching

Porogen leaching is probably the most common and most known out of these older scaffold manufacturing methods at least for bone tissue engineering. This is a decent method for manufacturing porous scaffolds. This method involves mixing the scaffold material with porogen in liquid or gel form and then letting it to harden to shape. After the scaffold material has hardened, we must remove the porogen from the scaffold by leaching it with a solvent. Commonly the porogen is salt and the solvent is water because these are cheap, nontoxic, and easy to work with. This method may leave some of the porogen inside the structure only to be released later when the scaffold degrades as not all the pores are accessible when the scaffold has been manufactured with this method so the porogen must be nontoxic and the human body will need to be able to dispose of it. What makes this method decent is that this method is quite low-cost method and only uses simple equipment. Even though this method gives us random scaffolds often these are

enough as there are plenty of other variables and the pore architecture is not the worst problem, so scaffold made with porogen leaching are often good enough because of its price and ease of manufacture. [2,16]

4.1.2 Gas foaming

Gas foaming is a method pretty similar to porogen leaching in a way that there is another substance making the pores to the scaffold, but there are few key differences. First of all, the substance what makes the pores is not solid, but it is a gas. And secondly this requires pressurized chamber where the gas is pumped into and saturates the scaffold material, after the material is saturated with the gas then the pressure is released causing the gas to expand and to form pores inside the material. So basically, this gives us similar results as porogen leaching but does not require solvents to be used to remove the substance creating the pores. For example, we do not want to use water if the material starts to degrade very quickly when in contact with water. Typically, the gas in gas foaming is CO_2 as it is cheap to buy and easy to get hold of. Also, CO_2 is nontoxic and found inside the human body in blood and air inside the lungs so we know that it will not cause any problems inside the human if some was trapped in the scaffold. [2,16]

4.1.3 Freeze drying

Freeze drying is basically a combination of these previous methods porogen leaching and gas foaming. In freeze drying the material is being frozen and then moisture forms solid ice crystals inside the material which then will act like porogens but in the end this method does not require us to use solvents to get the porogens out of the material structure. Instead, the ice crystals sublimate into gas when the scaffold is heated, as the water sublimates it leaves the scaffold leaving it much drier than it was originally. This method pretty much has the same drawbacks as the previously mentioned methods in regards of pores, but this method allows us to affect the pore size and shape a little bit better by affecting the freezing rate. Even though this method uses water in the beginning which could cause degradation to the material this can still be viable as the moisture will evaporate soon during the manufacturing process. [2,16]

4.2 Modern manufacturing methods

There are plenty of different methods for manufacturing ceramic scaffolds, some give random results such as previously mentioned traditional methods and then there are the modern and more complex methods which will give us much better controllability over the whole structure. Modern manufacturing methods for bone tissue engineering have shown great promise so far and those can help with some of our issues leading to better

performance out of the scaffolds with the more precise and complex structures, but there are still some issues with these methods [3]. For example, it is difficult to make nanoscale pores or even several micron sized pores, which makes it harder for cells to grow inside the scaffold as the ideal pore size cannot be reached and then the cells will not attach to the scaffold as dense as we would want them to. Many of these methods are novel methods which require experimental set ups, these can be expensive and difficult to make. Most known modern manufacturing methods are rapid prototyping methods such as fused deposition modeling, selective laser sintering and stereo lithography appearance [3,16].

4.2.1 3D printing and fused deposition modeling (FDM)

How 3D printing works is probably something most people in today's world considers to be quite common knowledge. In theory it can be very simple as it just requires flowing material which will harden fast, piston to push the material, nozzle which can be moved and finally a substrate to print on. And even though many thinks that 3D printers are always like this there are also different types of 3D printers such as 3D printers using SLS or SLA process. Normally for medical use the equipment in reality is also much more complicated than what was described above, because we need to control the nozzle or laser placement on the substrate extremely accurately if we want to make tissue engineering scaffolds. Controlling the nozzle or laser often happens in x-y plane with a help of a computer which gets its data from its own calculations via pre-programmed programs and data given to those programs. There can be sensors providing more data such as information about the actual placement of a nozzle if the motors have some errors and do not work as intended. Also, things like eliminating vibration would be necessary for printers for making tissue engineering scaffolds, the problems with things like this is that these are time consuming and money costing tasks, but it would be absolutely necessary for printing something in nanoscale as just a small vibration could mess up the whole print. [2,3,16,28]

Pretty much always we see 3D printers which are printing polymer, and these are 3D printers using process called fused deposition modeling (FDM). FDM is a method where a continuous filament is being used to print objects. This filament is being brought directly to the print from outside and so requires less of printing material than many other methods which need a lot of the material even though those also use the same little amount. One problem with this small amount of printing material is that there are nothing supporting the structure like there is for example with powder bed printers where the left-over powder provides support. [2,27,28]

There are also 3D printers printing other materials than plastics such as metals or even chocolate, but printing ceramics is a little bit more difficult as ceramics will not melt easily, so we cannot get an easily flowing material to push out of the nozzle. The solution to this often is to mix the ceramic particles with molten polymer to make a solution which consists of both and after the printing is done then the polymer is removed by for example pyrolysis while at the same time the ceramic gets sintered and hardens into the printed shape. There is a problem with this is that the prints will have quite low material density and the printed parts will be quite randomly porous after the polymer is removed. Other method to print ceramics is with methods like SLS, which includes a powder bed and a laser which melts and sinters the powder into shape. [2,27,28]

So, with 3D printing we can get custom scaffolds which are accurate in theory. 3D printing also has the issue of having too high scale still as the smallest we can print is in the scale of tens or hundreds of microns when the desired size to print is in nanoscale to several microns. To make the prints small enough we would need to get the printer to be even more accurate as it is and somehow get a nozzle and suitable polymer mix which could give us that small print. If we got to desired size and the prints would look uniform, then I would believe that the structure would be good enough in regards of pores, but the density problem would just get even worse because the volume of polymer compared to ceramic would most likely increase as the polymer would still need to be able to bind the ceramic and flow through the nozzle evenly. Pretty much the only way to get 3D printing to work perfectly would be to somehow make a ceramic 3D printer. [2,3,27,28]

4.2.2 Selective laser sintering (SLS)

SLS is one of the widely used rapid prototyping methods which tackles some of the issues which 3D printing has. It is also classified as additive manufacturing method just like 3D printing is. SLS for example does not require us to have polymer in the mix and we can just print ceramic structures. So, with SLS we get more dense but not as dense as possible due to our raw material being a powder which will not get 100 % sintered with this method so there will be extremely tiny voids inside the scaffold affecting the structural strength. And the other problem which still could not be solved is that also this method is unable to make the pore size small enough due to spot diameter being too big and cannot be made small enough yet. [2,27,28]

SLS is a method where powdered ceramics get sintered into a shape using a laser. This method creates the object slowly layer by layer and the completed part sinks a bit every pass of the laser. There are two places where there is powder, the powder bed where the printing happens and then powder delivery system. After every pass the powder bed

is filled and leveled so there is powder to melt evenly at every place. As the object is being printed the powder bed will have a lot of excess powder there which makes it that making just one object from one material could be expensive as we need quite a lot of powder, but there is also a benefit to this. We do not need to make any supporting structures for the object as the powder provides support to it. So, in short SLS is a method where powder is being sintered by laser layer by layer and between each layer more powder is added while the object sinks lower with the help of a piston. The powder typically ranges from 15 to 100 microns in size so from this alone we can conclude that with powders like this we will not be able to make nano or couple micron sized pores to a scaffold at least in high amounts. [2,27,28]

4.2.3 Stereo lithography appearance (SLA)

SLA is a method which has benefits and similarities of FDM and SLS as it need photochemical polymers in the print and to that polymer melt, we can mix ceramic powder. After the print is done with the printer then it needs to be sintered somehow while also removing the polymer. Other thing is that this requires a lot of printing material even though it uses it less than FDM as the polymer and ceramic suspension supports the object while its being printed. [28,29]

SLA is very fast method of printing as photoactivation is often quite fast process, but the materials and equipment is very costly still even though the prices have come down quite a lot lately. SLA is a method where there is a tank filled with photochemical polymer and the bottom of that tank is transparent. Inside the tank there is a lifting platform where the object is being printed onto. In this method the new layers form to the bottom of the object and it rises up from the liquid unlike in SLS where the object sinks with the powder. These new layers are formed as light is being emitted to the polymer solution which then hardens. This is not too great method for tissue engineering scaffolds as the spot size of light is usually just way too big and the reaction of photoactivation a little bit random as the line between affected and not affected polymers is hazy, currently resolutions of 0.01 mm are possible which means that this method is still quite far from being perfect. To get this to work with tissue engineering scaffolds we would need to figure out how we could get small enough features to the scaffolds made and still have enough ceramic particles so it would not crumble instantly under smallest pressure after the polymer is removed. Also, because these printed scaffolds must be cleaned due to resins being sticky and messy, biodegradable materials might be difficult to handle unless we are using something which will not progress the degradation of the ceramic. [2,27,28,29]

4.2.4 Spark plasma sintering (SPS)

SPS or field-assisted sintering technique (FAST) is a manufacturing method which uses low voltage direct current and pressure to synthesize new compounds or to densify materials. This method can be used for both electrically conductive green bodies and non-electrically conductive green bodies, for non-electrically conductive green bodies we need to use electrically conductive tool through which the current can pass through and where the heat is generated, and the powder being sintered is heated by conduction. When we are working with electrically conductive green body, we do not need the same special tool as the voltage can pass through the material directly and in this case the heat is generated inside the material rather than outside. Overall, this method is quite similar to hot pressing, but the differences are the heat is generated and transferred in different manner. And in fact, even though the name indicates that there will be sparks or plasma neither of those are produced with this manufacturing method. [30]

Using this method to make tissue engineering scaffolds can be quite difficult because this is meant to be used to densify materials, but with low pressure this can give us scaffolds with relative density of around 90 %. The main benefits of this method for tissue engineering are that with this method we can make HA-BG composite scaffold with only CaP phases and bioactive glassy regions. Which means that even though there is less surface area due to reduced porosity we can use the surface more efficiently so the bioactivity does not take as big of a hit as one could expect. Studies have shown that scaffolds made with this method can support bone mineralization and have also shown promising results of proliferation and osteoinductive characteristics. [31]

5. CURRENT SITUATION

As mentioned before there are no perfect materials out there for bone healing, no material is even close to being perfect for any application at the moment. This implicates that there are some challenges with all the materials which are being used currently. Even though there are a lot of problems with the materials we must remember that there are other sources of problems than just the material, other problems come from manufacturing, design, and sterilization. But even though there are a lot of challenges we have been able to make products which have helped a lot of people already. [2,3,4,26]

5.1 Challenges

5.1.1 Materials

Materials side of challenges in this field is heavily focused on the material's poor mechanical properties when other properties such as bioactivity and degradation rate would good. In other words, it is very hard to find a material which would have good bioactivity, suitable biodegradation rate and mechanical properties all at the same time. There are materials which have two of these, such as bioglass 45S5 which has good degradation rate and excellent bioactivity, but with about 90 % porosity scaffolds made out of bioglass 45S5 have compressive strength of 0.3-0.4 MPa. Kaur G et al. has made a great study about mechanical properties of bioactive materials which also lists values about strength, elastic modulus, fracture toughness and much more about these materials. But it is important to note that the main reason why these are considered to have poor mechanical properties is because of the requirement of high porosity in tissue engineering scaffolds. If porosity did not affect the mechanical properties, then for example HA would have mechanical properties very close to natural bone in most aspects. [26]

Biomimetic properties are another issue related to the material properties. It would be very important and beneficial to find a material which would also be biomimetic so it would be degraded by osteoclasts like natural bone is and it would be disposed naturally. Biomimetic properties also would help us tackle the problem of rejection as if the material was biomimetic the human body would think it is natural material and would not have any reason to attack it. It is certainly possible that there are no materials out there which would have all these properties so most likely the solution would be to have a hybrid or composite material like the natural bone is also. [9]

5.1.2 Manufacturing

Manufacturing causes its own problems because the older methods which have been used for quite long time produce random pores to the scaffold and this randomness leads to varying results, this also means that there are no 100 % accessible and inter-connected scaffolds made with these methods. New and modern methods on the other hand produce predictable pores and structure, but the problem with those in addition to costs obviously, is that some are not suitable for just ceramics as for example 3D printing ceramic scaffolds is basically just printing polymer solution with ceramic particles mixed into it in a shape of the scaffold, after this printing process is ready then the polymer must be removed somehow, for example by pyrolysis. This method produces accessible and inter-connected pores, but the scaffold structure is not as good as with other methods as there are material removed from places where it is designed to be. So, this gives us scaffold with worse mechanical properties than what it could be with other methods. To tackle the manufacturing problems, we would need to figure out a method which we could use to precisely manufacture micro-scale ceramic structures without any additional materials such as polymers. We would also need to harden the material somehow at the same time as we manufacture it as shaping it after the hardening would be impossible, but we still do not want the scaffold to collapse on itself. [2,3,16,27,28,29]

5.1.3 Design

Design of the scaffold can cause problems as well in many cases. It is hard to manufacture a scaffold which would be 100 % correct shape to the damage mainly because of the limitations of current manufacturing methods so at the moment the shapes which we can make are quite simple such as blocks and wedges. These are often good enough and are definitely better than nothing but having perfectly shaped scaffold would be ideal. Other problem with the designing part of making new product is how we could design other important features into the scaffold. One of these features is the biggest issue of tissue engineering, vascularity [5,33]. If the scaffolds were vascularized blood would flow through the scaffold and then more nutrients would be distributed to more cells, and we could scale our scaffolds to fix even bigger damages than what we can currently, as right now the range how far nutrients can travel is limited but if the scaffolds could be vascularized then we would not have that issue anymore. So, pretty much the problem with designing is that we would want to make very complex structures which currently are impossible to make with our current manufacturing methods and knowledge. We for example do not even know how vascularity could be achieved in large scale so getting that to ceramic scaffolds will take a long time. [32]

There are plenty of things which can cause challenges with ceramic scaffolds and so every step of the manufacturing and design process must be researched and improved even more in order to make even better bone tissue engineering scaffolds. One thing which many could forget when designing something out of ceramic is that we kind of must use gamma irradiation or electron beam sterilization, but the issue is that it can affect the properties of some bioceramic structures [14].

5.2 Applications

Bioactive ceramics overall have a lot of different applications for tissue engineering even though pretty much all of those are related to bones in some way. Bone is something we cannot really grow in 2D culture to be useful like we can for example grow skin. So, because of that we always need scaffolds when we are growing new bone using tissue engineering methods. [4,9]

Currently the products out on the market are quite simple but versatile, some of these are actually very good at doing what those were intended to do such as Pro Osteon 200R by Zimmer Biomet. Which is a scaffold made out of hydroxyapatite and calcium carbonate. These scaffolds are small granules with interconnected 200-micron pores. Pro Osteon 200R is used to fill voids and gaps in non-load bearing applications, for example in oral maxillofacial procedures. [33]

Currently the most common applications for bioactive bone tissue engineering scaffolds are in non-load bearing applications because of the lack of mechanical strength of the current materials. These can be used in places where the normally would be load to bear but then there has to be supports like metal plates absorbing the forces rather than the scaffold taking the hits. The most common application is the one which Pro Osteon 200R is intended so void filling. Voids are filled with the scaffolds which can be in all kinds of shapes but mostly in blocks, wedges, granules, and putties and then these scaffolds provide a substrate for natural bone to grow while the synthetic material hopefully degrades away at the same rate the bone grows. [3,4,9,33]

5.3 Research focuses

Currently there are plenty of research avenues because of all the challenges we currently have with our current materials and manufacturing methods. So, first of all on the materials side of things we are trying to find a material which even when in highly porous form has good enough mechanical properties to be used in load bearing applications, is very bioactive, has excellent biocompatibility and can be customized for many indications.

Most promising way to achieve this is with hybrid or composite materials, which have already shown promise to be better than other materials would be alone. [2]

Then on the manufacturing side as we are trying to achieve the possibility to manufacture very complex and customizable products with suitable porosity and other features. These would be possible already with current 3D printing methods if it we did not need the details to be so small. So, the actual difficulty is to go from micron scale to nano scale. For this we need to figure out how we can get grain size of powders, filament size or laser spot diameter down to nanoscale. [2,3,16,27,28,29]

And then there are the new things we are trying to incorporate with our scaffolds, these are the ones which are making our products so complex, so first of all ability to scale, shape and modify our designs to fit everyone perfectly, which requires more than just manufacturing side to be solved as we also need something to image the products digitally very accurately for our advanced manufacturing methods. Then second research focus related to design is small features such as vascularity for example. Achieving vascularity is one of the biggest if not the biggest problem of tissue engineering currently, because we do not know how we would be able to get our products to be properly vascularized due to lack of knowledge, and because it is so important feature for all tissues except for cartilage. Third thing regarding to design of scaffolds is to incorporate other parts of medicine to the product such as drugs. If we were able to make drug delivery systems which were also bioactive scaffolds, we could be able decrease healing time, amount of pain, and adverse effects [4]. And at the same time, we would be able to increase success rate of surgeries using synthetic bone grafts. [2]

So, overall there are plenty of things which are being researched, and to some people this does not sound like too long list of issues, but we have to remember that each of these consists of many and difficult smaller hurdles and also that sometimes fixing one problem causes one or more new and completely unexpected problems to rise to the table.

6. CONCLUSIONS

So as a conclusion, bioactive tissue engineering scaffolds have a need in today's world which needs to be filled but so far, we do not have a perfect materials or manufacturing methods to do so. Some great bioactive materials have been found such as HA, BCP and bioglass 45S5 already, and the current potential materials are hybrid and composite materials. Currently the materials usually have two of the following, good biodegradability, good bioactivity, or good mechanical properties. This nowadays means that we are giving up the mechanical properties and only using these materials in non-load bearing applications. In table 1 there are more information about how the current material groups compare with each other and in tables 2 and 3 there are more information about how current manufacturing methods compare with each other. There currently are some products which are being used commercially already, but the commercial products are not very complex yet, and such the applications are quite limited also. Most used commercial products nowadays are fillers. Currently the research interest is towards finding new materials, new or improved manufacturing methods for existing materials and for new potential materials, possibility to customize implants by using rapid prototyping methods to make scaffolds and also adding drugs or other biomolecules to the scaffold to make a product which would be both a drug delivery system and a scaffold at the same time to increase the possibility of successful damage repair or implantation.

	Calcium phosphates	Calcium silicates	Bioglasses	Hybrid and composite materials
Biocompatibility	very good	very good	very good	good or very good
biodegradability	All degrade in varying speeds	Degrades quickly without additives	Varies but overall these have good biodegradability	Customizable
Bioactivity	HA and BCP are class B bioactive materials	A little bit better bioactivity than on calcium phosphates	Excellent (many class A bioactive materials available)	Customizable but often worse than with pure bioceramics
thermal stability	decent thermal stability	Crystallization in high temperatures is an issue	Some bioglasses have quite good thermal stability	may have increased thermal stability
mechanical properties	poor mechanical properties, only suitable for non-load bearing applications	Better mechanical properties than calcium phosphates but not suitable for load bearing applications either	Cannot be used in load bearing applications due to having poor mechanical properties	Mechanical properties are almost always better in scaffolds than what bioceramic scaffolds would have
adverse effects	No significant adverse effects	Without additives these have too fast degradation rate, which may cause implant failure	Ions which are released may cause high pH around the implant	Depends on the materials used but should be less than with plain bioceramics unless natural polymers are used.

Table 1. Comparison of material groups

	Porogen leaching	gas foaming	freeze drying
complexity	simple	simple	quite simple
porosity	Quite random, but dependant by the amount of porogen	Random	can be affected with process parameters but can be used to achieve very high porosity
pore size	dependent on porogen	random sizes	quite even size and it can be affected by process parameters
interconnectivity	random	random	random
accessibility	random	random	random
cost	very cheap	quite cheap	cheap
future potential	low	low	low

Table 2. Comparison of traditional manufacturing methods

	FDM	SLS	SLA	SPS
complexity	quite complex	complex	quite complex	medium complexity
porosity	totally customizable as long as resolution of machines is small enough	totally customizable as long as resolution of machines is small enough	totally customizable as long as resolution of machines is small enough	quite low porosity (about 10 %)
pore size	With current manufacturing methods about 100 microns is the smallest size	With current manufacturing methods about 100 microns is the smallest size	With current manufacturing methods about 0,01mm is the lowest possible resolution and it determines the smallest pore size	poresize is random but quite small due to pressure
interconnectivity	100 % interconnectivity is possible	100% interconnectivity is possible	100% interconnectivity is possible	random
accessibility	100 % accessibility is possible	100 % accessibility is possible	100 % accessibility is possible	random
cost	medium costs in beginning. Becomes high with large batches	Very high initial costs	Very high initial costs	high costs
future potential	High	High	High	medium

Table 3. Comparison of modern manufacturing methods

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