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A class II hybrid, by the Sol-Gel route for tissue engineering.

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Hybrid Material

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Supervisors: Donghong Yu

Group members: Mikkel Sjørslev Galsgaard

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Synopsis:

This report was developed during the period of February to December 2021 by Mikkel. S. Galsgaard at Aalborg The report investigated University. class II hybrids with different ratio of silica and *Poly*(Methacrylic acid), as well as two linking agents GPTMS and TMSPMA. FTIR, TGA, Degradation, SEM and Micro-indentation analysis, were performed on each produced hybrid as well as the produced reference materials. These analysis were all performed to investigate whether the produced reference materials were synthesised correctly. They were also used to investigate, if any of the produced hybrid materials were suitable as tissue engineering materials for bone grafts.

By approving submission in Digital Eksamen, each group member accepts that everyone has participated equally in the project work and that the group is collectively responsible for the contents of the report.

Abbreviation

AIBN	Azobisisobutyronitrile
FTIR	Fourier-transform infrared spectroscopy
GPTMS	(3-Glycidyloxypropyl)trimethoxysilane
HA	Hydroxy appatite
HCA	Hydroxycarbonate appatite
MAA	Methacrylic acid
PMAA	Poly(Methacrylic acid)
SEM	Scanning Electron Microscopy
TEOS	Tetraethyl orthosilicate
TGA	Thermogravimetric Analysis
TMSPMA	3-(Trimethoxysilyl)propyl methacrylate

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Introduction

Bone has become the second most transplanted tissue only surpassed by blood transfusions. The transplants are commonly done with natural bone graft such as autograft and allograft. Both of these types can accelerate the regeneration of bone, but they can also cause infections at the extraction and insertion sites. Furthermore the body might even see the transplant as hostile and the graft material will get rejected by the immune system. Therefore alternate bone graft. The first major breakthrough in tissue engineering was the discovery of 45S5 BioglassTM in 1969. From this discovery, commercial products like NovaBoneTM, BonAliveTM, NovaMinTM and others have been developed based upon the composition of the orignal 45S5 bioglass, however these products are limited in their usage. [1] Pure 45S5 bioglass has shown to form strong bonds with bone, which enable the possibility to accelerate the regeneration of bone and become a suitable graft material for tissue engineering. [2] [3]

For a scaffold to be considered as an ideal bone graft, it must contain properties such as being biocompatible and biodegradable. It should also be able to accelerate the natural regeneration process of bone, while compensating for the missing strength of a broken bone. Even though bioglass has shown promising results in the field of bone graft, because of its ability to bond to bone and accelerate the regeneration process, it is too brittle to handle a normal load without shattering or fracturing of the bioglass. Furthermore pure bioglass have such a strong bond to bone that the removal of bioglass from the bone will result in severe damage. A way to utilize all of the good properties in bioglass, is to combine it with another material, creating a hybrid material, which can compensate for the brittleness and strong bonding of bioglass. A possible material to combine with bioglass could be a biocompatible and biodegradable polymer. The polymer alone does not contain all properties needed, but it is able to add a higher impact strength and ductility compared to that of bioglass alone. Furthermore the polymer degradation rate can specifically be tailored to match the regeneration rate of the fractured bone. [1] In some cases the integration of polymer with bioglass has led to concerns due to difficulties with the bonding strength between the two materials, which can cause an uneven degradation rate. Worst case scenario of a difference in degradation is where the polymer has a faster degradation rate than bioglass, hence causing a change of the hybrid materials ratio and result in the remaining bioglass material to behave differently than intended

for the hybrid material. If the polymer has side chains, those will in most cases degrade faster than the backbone of the polymer, therefore a polymer with few or none side chains is favourable. Some polymers can have an unstable backbone in certain environments which creates a fast degradation of that polymer. As the polymers degradation rate has to match that of the bioglass, a polymer such as Poly(Methacrylic Acid) (PMAA) could be used since it has short side chains, and a stable backbone. Furthermore, the hybrid material must be classified as a class II hybrid, which makes the material appear as one material at a nanoscale range, such that the material appears to be one material instead of multiple materials, which helps to obtain a even degradation rate of the hybrid material. [4]

The aim of this project is to use silica/polymer hybrids in combination with a linking agent to ensure a true hybrid which appears at a nanoscale to be one material, and therefore hold the exact same rate of degradation. This will be investigated using different ratios of the organic/inorganic parts, as well as the linking agent itself. Furthermore the degradation of the hybrids will be investigated in vitro as well as analysing them at a nanoscale range.

1.1 Problem Statement

How will the use of different linking agents and different ratios of Silica and Poly (Methacrylic Acid) affect the characteristics, brittleness and degradation capability of hybrids synthesised by the sol-gel route?

Theory 2

2.1 Bonegraft

The human skeleton system is responsible for six major mechanisms. These are support, production of blood cells, mineral storage, movement, endocrine regulation and protection. For bones to fulfill all of these properties throughout the entire body, it has different sizes, shapes, toughness, ductility, porosity and building blocks. Bones located at different sites of the body, having different tasks to perform for the system and therefore the properties of bone varies. [3]

When designing a graft material, all of the properties must be taken into consideration, with emphasis on the structure and activity of bone. Bones consist primarily of a hydroxyapatite (HA) phase and collagen, where 65-70% are $Ca_5(PO_4)_3OH$ in the form of hydroxyapatite crystals trapped in a matrix of 30-35% collagen. The crystal matrix is considered a composite material at a nanoscale. For the material to be considered as such, at least one dimension of the composite bone has smaller structure size than 100 nm. [2]

Injuries and diseases etc. can cause damage to bone, and in severe cases the bone need a graft material to aid their natural regeneration process. Graft materials must be able to form a connection in the form of a chemical bond, with the crystal matrix of the damaged site. Bone is able to fully regenerate itself over time. However in severe cases it can take years, which excellently justifies the use of graft materials as they would accelerate the natural regeneration of bone, while furthermore adding strength to the damaged bone. There are disadvantages to the use of graft materials, but these depend on which type of material is used. The natural graft materials are autograft and allograft, which is commonly used transplant materials for tissue engineering. [5]

2.1.1 Autograft

Autologous graft also called autograft, is one of the natural bone graft materials. It is an extraction of bone from the patient with the purpose to be used at another site in that patient. When using the body's own material to regenerate an injury. The compatibility of that material insures a minimal rejection rate at the insertion site. The autograft is used as a scaffold which is a template to where the new bone should be created, while promoting the growth of new bone. Autologous graft material is able to stimulate the osteblast within the fractured bone, which then uses material from the graft to repair and create new bone, which is called osteoconduction. Furthermore autograft is also able to be osteoinductive meaning that it can stimulate the osteoprogenitor cells to become osteblast, making it able to start creating new bone. If a graft material possesses both the ability to be osteoconductive and osteoinductive, the graft will be able to promote the creation of new osteblast, which in theory will result in a faster and more effective regeneration of the fractured bone. The osteoinductive process is present if it is a graft from a similar and compatible organism. [5] [6] [7]

Even though autologous graft material is both osteoconductive and osteoinductive, the disadvantage of having two surgeries can, for the patient, result in post operational issues such as pain around the donor site or infections at any of the operational sites. To decrease the issues and risks for the patient associated with autograft, allograft can be used as an alternative. [5]

2.1.2 Allograft

Allotransplant also called allograft, is another natural bone graft material. However this type is an extraction of bone from another human being than the one receiving the graft. This includes donors and bone from corpses. This type of bone graft can be preserved by freezing or freeze drying, whereas autograft can be used directly after harvest, hence no need for storage. When using allograft from corpses the concern of post operational issues at the extraction site is no longer an issue. An allograft material is more likely to be rejected by the body because it is extracted from another donor, which might result in compatability issues, as well as post operational pain and infections a risk. If the allograft manage to form bond with the HA crystal phase of the broken bone, it will only be able to be osteoconductive, which is the stimulation of the osteblast within the broken bone. If the allograft have not been preserved and the donor is compatible, allograft might in some cases be able to have some osteoinductive properties. Overall allograft has a weaker acceleration of the bone regeneration compared to that of autograft. [5]

2.1.3 Alternative graft

Both autograft and allograft are widely used in tissue engineering, but there are risk of post operational issues, especially at the extraction site of autograft. Alternate bone graft materials are therefore highly desired. In order for a material to be categorised as a bone graft, it is required to fulfill six criteria. [4] [8]

Biocompatible: The material is required to be biocompatible, meaning that the body will accept the material as a part of the body. Otherwise the material will be rejected and have no effect on the regeneration process of the bone. In order for the material to be accepted by the body, it is required to be fully sterile and other

precautions must also be taken into consideration in order to obtain a biocompatible scaffold for tissue engineering.

Bioactivity: The material has to be able to create a connection with bone though a chemical bond. The best way to form a connection from the material to the bone is with the creation of a hydroxycarbonate apatite (HCA) layer. This layer can be formed around the material when exposing it to the body fluid. The HCA layer can form a connection with the HA layer of bone.

Osteogenic: The material must contain some kind of osteogenic properties. The most important osteogenic property is the stimulation of osteoblast within the bone matrix. It is the osteoblasts that has the ability to form new bone. Another osteogenic property is the creation of new osteblast. If the material is able to both stimulate osteoblast and create it, the regeneration of bone could be accelerated even further. [6] [7]

Vascularisation: When the bone is trying to regenerate itself, it needs nutrients as new building blocks. These can be transferred with the body fluids, normally by means of the blood. When a material is applied to the surface of a damaged bone, the material must be sufficiently porous that the blood can travel though the material into the bone, where nutrients are needed. For the material to handle the creation of new blood vessels though it, the material must have an interconnected structure that ensures a clean pathway of these blood vessel to the bone. The blood vessels are also useful when waste is transferred away from the graft material and bone.

Mechanical properties: When the material is bonded with bone, it must cooperate with the broken bone to maintain the bones structural strength. The material should therefore contain mechanical properties similar to that of the specific bone to which it is bonded. The task of the material is to compensate for the missing strength of the broken bone.

Degradation: The material must be biodegradable in the human body, which insures that the material segregate and extracts itself from the body. The degradation rate of the material should match the regeneration rate of bone, such that the material is removed from the bone when the regeneration process is completed. The regeneration process of bone highly depend on the size, porosity and severity of the damage. Therefore a tailoring of the materials degradation rate to match the difference in bones regeneration rate, is highly desirable. [8]

If a material can fulfill all of these six criteria it is suitable as a graft material for tissue engineering. If the material is able to fulfill only some of these six criteria, it can still be used as a graft material after some modifications to try and fulfill all criteria. A material that has shown a great potential in tissue engineering is 45S5 BioglassTM. [4]

2.2 Bioglass

An inert glass material, composed of 6.1 mol.% SiO₂, 24.4 mol.% Na₂O, 26.9 mol.% CaO and 2.6 mol.% P_2O_5 , was the first ever synthesised bioglass. This material has the ability to form bonds with natural bone via the creation of a HCA layer. The first synthesised bioglass was produced with the commonly glass synthesis route, called the melt quenched technique. This method is performed by mixing the oxides together followed by heat treatment of the mixture at 1100-1600 °C. At this temperature the oxides melt together and after a cooling period they arrange themselves in a homogeneous crystal. Since the first discovery of bioglass in 1969 by professor Larry. L. Hence, other types of bioglass have been synthesised to develop the best possible material for tissue engineering. This involved different ratios of the original bioglass composition, other oxides such as titanium oxide and different techniques to synthesise the bioglass. Even though the first bioglass was discovered over 50 years ago, it is still a huge research area in present days, as no other material has been proven to be better in the field of artificial bone grafts for tissue engineering. [9] [1]

When the bioglass comes in contact with the body fluid, ions from the bioglass starts to precipitate to the surface, where an ion exchange with the body fluid takes place. It is mostly calcium ions and phosphor ions that precipitate from the bioglass. The precipitation of ions, from the bioglass and the ion exchange at the surface, creates a layer of HCA around the bioglass. When the created HCA layer gets close enough to the crystal matrix HA layer of the bone, it can form bonds. The bonding forms a connection between the bioglass and bone, which allows the bioglass osteogenic properties to stimulate the osteoblasts within the bone and the production of these osteoblasts. If bioglass is bonded closely, the osteoblast can use the minerals of the bioglass as new building blocks when regenerating the bone. When the vascularisation occurs, the bioglass minerals as well as other nutrients can be transported to the osteoblast. [9]

The melt quenched bioglass results in a low porosity bioglass, which inhibits the vascularisation. Without proper vascularisation the bone is not able to retrieve all the minerals it needs from the body, to complete the regeneration process. Furthermore this type of bioglass is very brittle, which might lead to mechanical failure when sharing the load with the bone. This has lead to different alternate techniques being developed, such as foam scaffolds or Sol-gel. Both techniques makes a more porous material, which does inhibit the vascularisation process, hence accelerating the regeneration process, compared to not using a graft material. [9]

Melt quenched Bioglass fulfills some of the six criteria for a suitable graft material for tissue engineering. Though it lacks the ability of vascularisation. Without this ability the broken bone cannot be regenerating as the nutrients have no path to the osteoblasts. To create a bioglass material that has vascularisation ability, another synthesis route is needed. The sol-gel method is a promising technique, as it has shown to create highly porous materials. [9]

2.3 Sol-Gel

The sol-gel technique is a way to synthesise solid materials from small molecules. The technique is mostly used to produce metal oxides, such as the silica oxide that bioglass consist of. The technique is cost efficient as it can be carried out at a relatively low temperature, compared to the melt quenched technique. The properties of the material can easily be tailored during the sol-gel process by varying any of the following three synthesis parameters. [1]

Colloidal solution: The first stage of the sol-gel route is the creation of a colloidal solution, also called a "sol". This is done by mixing one or more precursors with the appropriate solvent. The colloidal solution is a mixture of both liquid and solid phases. [10]

Gelation: When the precursor starts to react with the solvent, it forms networks resulting in a denser solution. As the solution becomes denser and more gelated, the solvent and/or byproducts can be trapped in the network. [10]

Drying: The trapped solvent and byproducts can be removed from the gelated material by heat treatment. If the trapped solvent and byproducts are heated too fast, they will be able to penetrate though the small pores of the material, resulting in the material collapsing, shrinking or leaving cracks in the final products. Therefore the drying process must be able to remove the trapped solvent and byproducts with a small increase in the heat rate. The removal of the remaining solvent is also performed by the heat treatment. When the drying process is completed, a solid single network of the precursors should have been formed. [10]

The environmental influence is a parameter that can be altered, as illustrated in Figure 2.1. The figure illustrates that a higher acidic environment creates smaller particles and obtains a higher porosity of the final product. If the environment becomes more basic it will create denser particles resulting in lower porosity. This depends on H_3O^+ and OH^- influences on the sol-gel system. [10]



Figure 2.1: A acidic environment creates smaller particles resulting in a more porous material. A basic environment creates larger molecules resulting in a denser material. [10]

The catalysts of the sol-gel system is either an acid or a base which depends on how porous or dense the final material should be. The catalyst is needed to start the reaction and to accelerate the gelation process. Another parameter which can be altered is the temperature.

Alterations of the temperature is used to find the perfect balance, creating a material without collapse, shrinkage or cracks while speeding up the drying process. This is a parameter which is useful in mass production as it can decrease the production time. The sol-gel technique can be used, creating bioglass for tissue engineering. [10]

2.3.1 Sol-Gel Bioglass

A common metal precursor for sol-gel synthesis is alkoxide tetraethylorthosilicate (TEOS). This precursor can spontaneously create the sol, though simple hydrolysis with water and catalysed with acid. The hydrolysis mechanism is shown in Figure 2.2. Each TEOS molecule is hydrolysed with four water molecules to produce orthosilicic acid and ethanol. The reaction does not necessarily happen fully or even at the same time throughout the sol, as it is highly dependent on the amount of water present and which kind and amount of catalyst is used. [11]



Figure 2.2: The water molecules attack the silica in TEOS, resulting in the cleavage of ethanol, while TEOS gets hydrolysed. This happens four times for each TEOS molecule in the solution.

While the hydrolysis of TEOS is happening, a condensation reaction occurs simultaneous. When a hydroxyl group on a silica gets in contact with another hydroxyl group they can form siloxane bonds by the removal of water. The condensation reaction can also occur between a hydroxyl group and a carbon chain, which create siloxane bonds. However this will instead result in the removal of an ethanol molecule. The condensation reaction between two fully hydrolysed TEOS molecules is illustrated in Figure 2.3. The condensation reaction could also be described as a polymerisation reaction, where the fully or partially hydrolysed TEOS molecules are the monomers. The monomers bind and connect to each other, to form long chains through siloxane bonds in a continuous reaction. [11]



Figure 2.3: Two fully hydrolysed TEOS molecules react with each other to form a siloxane bonds by the removal of water.

The whole sol-to-gel reaction of TEOS is slow without a catalyst. Both acid or base can catalyse the reaction, but as illustrated in Figure 2.1 the pH have a strong impact on the final shape and size of the product. The acidic environment will yield long chains with a small degree of branching though siloxane bonds, giving a three-dimensional structure. The basic environment creates highly branched and compact material with few particles. One of the criteria to obtain a graft material is for the material to possess the ability of vascularisation. The use of acid creates highly porous structure, which is favorable to create vascularisation though the final SiO₂ glass product. [11]

The sol-gel technique is carried out at a low temperature which is essential to introduce a polymer before the glass solidifies. As most polymers become fully degraded at temperatures above 200 °C [12], they are able to be introduced to the low temperature sol. However the incorporation of other oxides such as CaO cannot be introduces without breaking down the polymer. The reason for this is that these oxides need temperatures of 400 °C to be hydrolysed in the sol. Alternate calcium source is needed in the sol, as the calcium ions are part of the HCA layer formation. [11]

The polymer can be introduced in the sol of TEOS, because the temperature can be regulated below 200 °C. When the polymer and sol are combined, they will start creating a high degree of entanglement between each other. After the drying process they should be simulating a single material at nanoscale.

2.4 Polymer

A polymer is a long linear chain or it can be a branched chain composed of a single or multiple monomers. The monomers are the building blocks which decide the final properties and structure of the polymer. Polymers can be produced in many different ways, such as free radical polymerisation which is one of the mostly used and it is the technique used to synthesise the polymer part in this project. [13]

During free radical polymerisation, the monomers needs a free radical source for the chain reaction to take place, which creates the polymer chain. This is normally created using an initiator molecule. The initiator is able to create radicals when activated. Some initiators can be activated when introduced into a liquid, otherwise it will need energy such as heat. When the initiator is activated it will typically donate a proton to the surroundings in order to become a free radical. The initiator can also decompose and eliminate the group which holds the molecule together, leaving behind two radical molecules. The decomposition reaction of the initiator, Azobisisobutyronitrile (AIBN), is illustrated in Figure 2.4. [13]



Figure 2.4: The decomposition of AIBN creates two free radicals by eliminating a N_2 molecule.

The formed radicals are able to attack a monomer molecule. The attack makes a radical transfer from the initiator to the monomer while creating a covalent bond between the two molecules. The radical on the monomer can then attack another monomer and transfer the radical once more. The transfer of a radical creates a chain reaction, which will continue until all of the monomers are used or until the radicals react with a terminating group. A termination can be caused by two radicals, which connects to create a covalent bond, hence stabilising the radicals. The second radical can come from either a radical on the initiator or on the monomer chain. The transfer of a radical from the initiator to the monomer is illustrated in reaction 2.5. [13]



Figure 2.5: A radical from a decomposed AIBN molecule attacks the carboncarbon double bond and transfers the radical to the tertiary carbon.

The most important properties that the polymer must possess are to be biocompatible and biodegradable, while having a high tensile strength and high ductility. A polymer like methacrylic acid (MAA) has proven to contain these properties, as well as short side chains which makes it a suitable candidate to aid bioglass for tissue engineering as it can match the degradation of bioglass. The polymerisation chain reaction of MAA is illustrated in Figure 2.6: [13]



Figure 2.6: The polymerisation chain reaction of MAA and the termination of the chain reaction is illustrated.

AIBN could be used as the initiator and MAA as the monomer, which creates a polymer backbone of PMAA and AIBN as the end groups. Even though MAA has shown great possibility for tissue engineering, it does not fulfill all six criteria. It must be incorporated into bioglass to form a hybrid material of organic and inorganic phases. When these materials have been synthesised into one material, it should be able to fulfill all six criteria mentioned in section 2.1.2. This makes it suitable as a graft material for tissue engineering. [13]

2.5 Hybrid Material

The combination of bioglass and polymer is a hybrid material, as it consists of both organic and inorganic material. A hybrid material is not a combination only between an organic and inorganic phase, but for bioglass and polymer it is. A hybrid material needs to be indistinguishable between its phases, meaning that it must be one material at a nanoscale and posses the same properties. There are two definitions of a hybrid material, which is class I and class II, the difference between them are there ability to create different chemical bonds as illustrated in Table 2.1.

Type of Chemical bond	Class I	Class II
Molceular entagnlement Van der Waals forces Hydrogen bonds	$\sqrt[n]{\sqrt{1}}$	
Covalent bonds	-	\checkmark

Table 2.1: The difference between Class I and Class II hybrid. [1]

As illustrated in Table 2.1 the only difference between class I and class II are their ability to create covalent bonds between two materials. A class II is more desirable than class I since a hybrid with covalent bonds is more likely to act as one material. For the material to be categorised as a true class II hybrid, all the phases must degrade at the same rate. This is to insure that the material keeps its properties while degrading, since a uneven degradation can change the properties of the graft material. Worst case scenario is that the properties of the material might change unevenly, so that it no longer degrades within the body, and extra surgeries are needed in order to remove the remaining graft material. [4]

For the graft material composed of bioglass and biodegradable polymer to be a true class II hybrid, it needs to create covalent bonds with each other. Either the bioglass or the polymer can be modified to have a group which relatively easy can create the covalent bonds between the organic and inorganic parts. This modification is done with the use of a linking agent. [9]

2.6 Linking agent

A linking agent can be introduced when a covalent bond is desired between two materials. Some normal organic/inorganic linking agents are (3-Glycidyloxypropyl)trime-thoxysilane (GPTMS) and 3-(Trimethoxysilyl)propyl methacrylate (TMSPMA). The structures of these linking agents are illustrated in Figure 2.7 & 2.8. [14]



Figure 2.7: The structure of GPTMS.



Figure 2.8: The structure of TMSPMA.

Both of these linking agents have an inorganic trimethoxysilane which can undergo hydrolysis to form silanotriol, which can bond covalently with hydrolysed TEOS. The organic part of the linking agent is different between the two chemicals. GPTMS need to be within acidic environment to open its epoxy ring which then can participate in free radical polymerization. The organic part of TMSPMA consists of a methacrylate group, similar to the monomer MAA. This part can participate directly in a free radical polymerization with MAA. Once both ends of the linking agent have been covalently bonded with the inorganic and organic part, a true class II hybrid have been produced. [14]

If the linking agent is introduced in the bioglass part first, it will bond with the bioglass and have functionalised the bioglass. Then the TEOS will have to be introduced during the polymer synthesis with multiple aggressive radicals. These radicals will attack the monomer, but they might also attack the bioglass, and even destroy the siloxane bonds Si-O-Si. This would result in the bonds between TEOS and the linking agent to break, which destroys the covalent bond between the organic and inorganic phases. If the linking agent is introduced in the polymer synthesis first, it can participate in the free radical polymerisation without destroying the trimethoxysilane group. After the polymerisation reaction the polymer can be introduced to the sol. The trimethoxysilane group of the functionalised polymer will hydrolyse and create bonds with bioglass. It is therefore desirable to first introduce the linking agent to the polymer synthesis in order to ensure that a true class II hybrid get synthesised. When the linking agent successfully creates a linkage between the organic and inorganic parts, the created material will have a linkage as illustrated in reaction 2.9. [14] [1]



Figure 2.9: The linkage between pMMA and bioglass by the linking agent TMSPMA.

3.1 Class II Hybrid synthesis

In order to produce different class II hybrid graft materials, the following procedures are used. The different hybrid materials are a mixture of silica and MAA which varies in ratios of the organic and inorganic phases. Furthermore the linking agent TMSPMA and GPTMS are both investigated and compared. The used inorganic material is TEOS as the hybrid synthesis are carried out in sol-gel. This synthesis procedure is developed based on studies of hybrid materials.

Polymerisation: The polymer is synthesised by mixing 0.004 mmol Azobisisobutyronitrile, 0.07 mol Methacrylic Acid and 6.72 mmol linking agent, either 3-(Trimethoxysilyl)Propyl Methacrylate or (3-Glycidyloxypropyl)Trimethoxysilane in a beaker with toluene as solvent. Furthermore the polymerisation with GPTMS requires 0.35 mmol hydrochloric acid in order to open the epoxyring of the linking agent. The beaker is stirred until the initiator is dissolved and a homogenious mixture is left. The beaker is then purged with nitrogen and sealed. The mixture is heated to 70 °C or above to initiate the polymerisation. Once the polymerisation is completed the polymer is precipitated in n-hexane to remove pollutants.

Bioglass: In order to produce bioglass through the sol-gel route, 45 mmol Tetraethyl orthosilicate, 160 mmol demineralised water and 0.35 mmol Hydrocloric acid are added to a teflon mold. The mixture is vigorously stirred for 40 min or until the hydrolysis is visually confirmed by turning from cloudy to a clear solution. When the hydrolysis is completed the mixture is left to try in a oven at 40 °C for 21 days followed by 60 °C for 10 days, to obtain a dry bioglass.

Hybrid: In order to produce a hybrid material the precipitated polymer is added to the teflon mold after hydrolysis of TEOS has occurred. The mixture is then stirred for one hour. After stirring, the teflon mold is placed in a oven at 40 °C for 21 days followed by 60 °C for 10 days, to obtain a dry hybrid material.

3.2 Structure analysis

To analyse the structures of the hybrid materials, all different products are crushed into powder and then placed between the detector and infrared source of an Fouriertransformed infrared spectroscopy (FTIR). The FTIR then sends out a beam of infrared light containing many frequencies. This is done several times with different sequences of frequencies within the infrared spectra of 400-4000 cm⁻¹. Hereafter the detector measures how much of the initial frequencies passes through the material, without getting adsorbed by the material. The collected data from the detector is fourier-transformed by the software to create a FTIR spectra with the wavenumber (cm^{-1}) range and the relative intensity of adsorbed light. By comparing the peaks with literature, specific bonds can be verified and potentially the hybrid interaction can be confirmed.

3.3 Thermogravimetric Analysis

Using a thermogravimetic analyser (TGA) the ratio between organic and inorganic is investigated. This analyser uses mass, temperature and time as measurements to calculate different data. Approximately 10 mg of a sample is placed in a crucible which then is transferred to the TGA. The analysis is set to a flow of 50 mL/min N2/O2 in ratios of 20/80, and a protective flow of Argon at 20 mL/min. The analysis start at 50 °C to 800 °C with a heat flow of 10 K/min and then cooled back to 50 °C with a heatflow of 25 K/min. The TGA can measure the mass of the initial sample to each temperature difference, as polymers degrade at 200 °C and bioglass needs higher temperatures then 800 °C. This indicates that the total mass loss comes from the polymer part. However this measurement does not account for any impurities in the hybrid materials.

3.4 Micro-Indentation

To investigate Vickers Hardness the material is polished to obtain 2 flat surfaces. One of the surfaces is polished up to a 4000 grit sandpaper so the surface becomes smooth at a nanometer range. The material is placed in a nanovea CB500 hardness tester and a microscope is used to locate the surface and mark a spot. The software uses this spot to move the material to an indenter and start a sequence of indentations creating around 10-12 impressions in the material. The force and movement speed of the indenter are described in table 3.1.

Maximum force (N)	Force rate (N/min)	Approach Speed $(\mu m/Min)$
2	20	100
3	30	100

 Table 3.1: Indentation Settings.

The maximum force is the maximum resistance measured during the indentation. When the maximum force is achieved the measurement has completed. The force rate is the amount of added force to the indenter over time to leave a indentation mark in the material, whereas the approach speed is how fast the indenter travels from its place in space to the surface of the material. After the 10-12 indentations the material is repositioned under the microscope where the travel length of the impressions can be measured, and used to calculate the Vickers Hardness of the material.

3.5 Degradation

A degradation analysis of each produced material is investigated to analyse their degradation behavior and the difference between each compound. A solution of 55.55 mol demineralised water and 0.12 mol hydrochloric acid is made to create a degradation solution with a pH of 3. The solution is then added to a beaker and heated to 40 °C. Then a solid piece of material is weighed and added to the beaker. After 24 hours the material is extracted from the solution by filtration, then washed with demineralised water to stop the reaction and dried for one hour. When it is dry, it is weighed and inserted back into the beaker with a fresh amount of the hydrochloric solution. This process is repeated seven times for all the produced materials.

3.6 SEM

Each material is sputter coated with gold, to make the surface charged. The material is placed in a scanning electron microscope (SEM) which then fires secondary electrons on the materials, sensors read the data, to create an image of the material at nanoscale. For each sample an area is chosen, and each image are compared to investigate any difference.

4.1 Synthesis products

To produce bioglass, PMAA and hybrid materials, the synthesis routes in section 3.1 are applied. The synthesis of hybrid materials are performed by altering the ratio between the organic and inorganic phases to create different hybrid materials, which might obtain different properties. The appearances of these different hybrid materials were varying from white powder to clear glass pellets. The difference of each material is described in Table 4.1. Some hybrid materials were synthesised multiple times, and the appearances of these were the same, therefore only one of each hybrid material ratio is analysed further.

Table 4.1: Synthesised hybrid materials with	a different organic/inorganic ratios and
linking agents.	

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Sample	$SiO_2 (mass\%)$	MAA (mass%)	Visual comment
PMAA	0	100	White Powder
Pure SiO_2	100	0	Glass Pellet
$30 \mathrm{SiO}_2 70 \mathrm{MAA}$	30	70	Very Solid and White
$50 \mathrm{SiO}_2 50 \mathrm{MAA}$	50	50	Solid Crater
$60 \mathrm{SiO}_2 40 \mathrm{MAA}$	60	40	Solid Crater
$70 SiO_2 30 MAA$	70	30	Orange Glass Pellet
70SiO ₂ 30MAA GPTMS	70	30	Solid Crater

The hybrid materials should change the properties of pure bioglass, to be a material suitable for tissue engineering. It should still appear as a glassy solid material, and the only hybrid that fulfills this criteria is the $70SiO_230MAA$. Even though the appearances of the hybrid materials are not similar to pure bioglass, they can contain properties which may benefit the material in being a graft material for tissue engineering. Further analysis will be able to determine what properties each hybrid material has and if a true class II hybrid has been produced.

4.2 Characterisation

In order to figure out whether the produced materials in Table 4.1 actually contain both the organic and inorganic phases and whether their ratio of each phase remains valid. The two various analysis, which are used to investigate these properties, are FTIR and TGA.

4.2.1 Infrared spectra

In order to confirm that both the organic and inorganic phases appear in each hybrid material, FTIR analysis is applied. The materials, pure SiO_2 and PMAA, are used as reference products as well as literature regarding these compounds. Furthermore, this analysis is also used to investigate whether the linking agents have successfully linked the two phases together, thereby creating a true class II hybrid via covalent linkage. A reference FTIR spectra on SiO_2 from an article by M. Kopani et al. [15] is illustrated in Figure 4.1.



Figure 4.1: An FTIR spectra from 700-1500 cm⁻¹ of a reference SiO₂ produced by M. Kopani et al. [15].

From the FTIR spectrum, the peaks created by Si-O and Si-OH bonding are essential in the analysis of the compound. The Si-C bonding provides information about any potential linkage to a carbon chain. These relevant peaks and their wavenumbers are presented in Table 4.2.

Table 4.2:	The relevant	peaks fi	rom the	SiO_2	FTIR	$\operatorname{spectra}$	$\operatorname{created}$	by M.	Kopani
et al. [15].									

Wavenumber (cm^{-1})	Stretching
740	Si-C
814	Si-O
970	Si-OH
1107	Si-O
1240	Si-O

Reference wavenumber and bonding of PMAA are created from multiple reference works. One of these references is by D.M. Garcia et al. [16] who has illustrated a full FTIR spectra of their PMAA, which is illustrated in Figure 4.2.



Figure 4.2: An FTIR spectra from 500-4000 cm^{-1} of a reference PMAA produced by D.M. Garcia et al. [16]

In the FTIR spectra of PMAA the y-axis is illustrated in transmittance, which is the opposite of absorbance. From the work of D.M. Garcia et al. and others, Table 4.3 has been created in order to illustrate the relevant FTIR peeks of PMAA.

Table 4.3:	The relevant	peaks from	the PMAA	FTIR spectra.	[16] $[17]$	[18]
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Wavenumber (cm^{-1})	Stretching	Bending
1160		C-C
1240	C = O	
1450		C-O-H
1740	COOH	
2850-3000	$\operatorname{CH}_2\operatorname{CH}_3$	$\mathrm{CH}_2 \ \mathrm{CH}_3$

According to the references, the polymer PMAA experiences both stretching and bending vibrations. This is why the carboxylic acid components are illustrated at multiple wavenumbers. The carboxylic acid peak at 1240 and 1740 cm⁻¹ is from stretching vibrations, while the peak at 1450 cm⁻¹ is from bending vibrations. The attributions from CH_2 and CH_3 creates a broad band around 2850-3000 cm⁻¹, which may contain intensity from other molecules. Another peak at 1160 cm⁻¹ comes from the backbone of the polymer, the carbon chain which the polymer is created by.

An FTIR analysis has been performed on both pure SiO_2 and PMAA, their FTIR spectra are compared in one spectra, which is illustrated in Figure 4.3.



Figure 4.3: The spectra of Pure SiO_2 is illustrated in orange and the spectra of PMAA is illustrated in blue.

The orange spectrum illustrates the FTIR spectra of pure SiO₂. It has an intense peak at 1050 cm⁻¹ which overlap any other peaks in the range of 1000-1200 cm⁻¹. From the reference two peaks illustrating Si-O should be visible within this range. A small peak at 800 cm⁻¹ is observed in the spectra which also indicates a Si-O bond. The orange spectra shows another peak at 970 cm⁻¹ which matches a Si-OH bond. The Q value cannot be determined from this spectra as the peak at 970 cm⁻¹ overlaps with that at 1000-1200 cm⁻¹ from the Si-O bond. [15] The blue spectra illustrates the FTIR spectra of PMAA, which matches the relevant peaks found in literature. The intense peak at around 1700 cm⁻¹ indicates the presence and majority of carboxylic acid in the material. The carboxylic acid is also present at peaks of 1450 cm⁻¹ from the C-O-H group and a peak at 1240 cm⁻¹ from the C=O group. Furthermore the peak at 1170 cm⁻¹ confirms that C-C bonding is present in the material and finally the broad band from 2800-3000 cm⁻¹ is observed in the spectra, indicating CH₃ and CH₂ groups. [16] [17] [18]

When combining the organic and inorganic phases, all of the above mentioned peaks should be observed in each spectrum to verify that both phases are present in each hybrid material. The peaks from each individual material, cannot be compared directly to another materials peaks, this is caused by the intensity independence during the measurement. All FTIR spectra from each hybrid material are illustrated in Figure 4.4.



Figure 4.4: A, is the FTIR spectrum of the hybrid $70SiO_230MAA$ GPTMS. B, is the hybrid $70SiO_230MAA$. C, is the hybrid $30SiO_270MAA$. D, is the hybrid $60SiO_240MAA$. E, is the hybrid $50SiO_250MAA$.

From all of these spectra, their peaks have been compared to the reference FTIR spectra, and in Table 4.4 all the peaks have been summarised according to them.

Reference $\rm cm^{-1}$	$30 \mathrm{SiO}_2 70 \mathrm{MAA}$	$50 \mathrm{SiO}_2 50 \mathrm{MAA}$	$60 \mathrm{SiO}_2 40 \mathrm{MAA}$
740 (Si-C)	Inconclusive	Inconclusive	Inconclusive
814 (Si-O)		\checkmark	
970 (Si-OH)	\checkmark	\checkmark	\checkmark
1107 (Si-O)	\checkmark	\checkmark	\checkmark
1160 (C-C)	\checkmark	\checkmark	\checkmark
1240 (C=O Si-O)	\checkmark	\checkmark	\checkmark
1450 (C-O-H)	\checkmark	\checkmark	\checkmark
1740 (COOH)	\checkmark	\checkmark	\checkmark
$2850-3000 (CH_2 CH_3)$	\checkmark	\checkmark	\checkmark
Reference $\rm cm^{-1}$	$70 \text{SiO}_2 30 \text{MAA}$	$70 \text{SiO}_2 30 \text{MAA GPTMS}$	
$\frac{\text{Reference cm}^{-1}}{740 \text{ (Si-C)}}$	70SiO ₂ 30MAA Inconclusive	70SiO ₂ 30MAA GPTMS	
$\frac{\text{Reference cm}^{-1}}{740 \text{ (Si-C)}}$ 814 (Si-O)	$\frac{70 \text{SiO}_2 30 \text{MAA}}{\text{Inconclusive}}$	70SiO ₂ 30MAA GPTMS - -	
$ \frac{\text{Reference cm}^{-1}}{740 \text{ (Si-C)}} \\ $	$\begin{array}{c} 70 \mathrm{SiO}_2 30 \mathrm{MAA} \\ \hline \\ \mathrm{Inconclusive} \\ \\ \end{array}$	70SiO ₂ 30MAA GPTMS - - -	
	$\begin{array}{c} 70 \text{SiO}_2 30 \text{MAA} \\ \hline \text{Inconclusive} \\ \checkmark \\ \checkmark \\ \checkmark \\ \checkmark \end{array}$	70SiO ₂ 30MAA GPTMS - - - -	
	$\begin{array}{c} 70 \text{SiO}_2 30 \text{MAA} \\ \hline \text{Inconclusive} \\ \checkmark \end{array}$	70SiO ₂ 30MAA GPTMS - - - - - -	
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{c} 70 \text{SiO}_2 30 \text{MAA} \\ \hline \text{Inconclusive} \\ \checkmark \\ $	70SiO ₂ 30MAA GPTMS - - - - - - - -	
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$70 \text{SiO}_2 30 \text{MAA}$ Inconclusive $$ $$ $$ $$ $$ $$ $$ $$ $$	70SiO ₂ 30MAA GPTMS - - - - - - - - - - -	
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$70 \text{SiO}_2 30 \text{MAA}$ Inconclusive $\sqrt[]{} \qquad \sqrt[]{} \qquad$	70SiO ₂ 30MAA GPTMS - - - - - - - - - - - -	

 Table 4.4:
 The presense of peaks in each FTIR spectrum.

The FTIR analysis of 70SiO₂30MAA GPTMS had no indication of possessing either the organic or inorganic phase. When preparing the the material for the FTIR analysis, it showed a separation of the phases in the core, after breaking a piece of the material. This separation in the core, could indicate that the phases have not been covalently bonded together, or that the epoxy ring has not been activated during the synthesis. When investigating the spectra of all the hybrids with TMSPMA, it was found that each spectra were quite similar, however the intensity between the characteristic peaks were changing in accordance to the ratio change of the two phases. In the spectra of $70 \text{SiO}_2 30 \text{MAA}$ the intensity at the 1740 cm⁻¹ (COOH) peak is four times smaller than that of the peak in the 1000-1200 cm^{-1} range, but when it is compared to the spectra of $30SiO_270MAA$, the two peaks are almost identical in intensity. The difference of the materials in the two spectra are their ratios, which indicate that in the material $70SiO_230MAA$ the inorganic phase have a majority over the organic phase. The direct comparison between the 1740 $\rm cm^{-1}$ peak and the peak within the 1000-1200 $\rm cm^{-1}$ range does not confirm anything, as this peak range contains intensity from different bonding energies, such as the C=O in the organic phase and the Si-O in the inorganic phase. Therefore the organic phase gives intensity to the peak which is characteristic for the inorganic phase.

This is visualised clearly in the $30 \text{SiO}_270\text{MAA}$ spectra as the peak in the 1000-1200 cm⁻¹ is a doublet, meaning that the peaks overlap each other. The peak of Si-C at 740 cm⁻¹, which indicates a linkage directly between the organic and inorganic phase, is not shown in any of the spectra. This analysis can therefore neither refute nor confirm that a linkage between the two phases has occurred.

4.2.2 Ratio determination

To determine whether the actual ratio of the two phases are valid after synthesis, a TGA analysis is performed on each produced material. This method of analysis measures the weight change in the material when the temperature rises to a maximum of 800 °C. At this temperature, heat should degrade the organic phase without degrading the inorganic phase of each hybrid material. A TGA analysis of $70SiO_230MAA$ is illustrated in Figure 4.5.



Figure 4.5: TGA analysis of 70SiO₂30MAA.

In the figure showing $70 \text{SiO}_2 30 \text{MAA}$ the green curve illustrates the mass loss over time, and the red line indicates the temperature over time. When analysing the green curve, the mass at the beginning compared to the mass at the end has changed 12%. This indicates that the material has lost 12% of its mass after the analysis. This procedure is performed on each material, and their mass loss are summarized in Table 4.5.

Sample	Mass Loss (%)
PMAA	20
Pure SiO_2	3
$30 \mathrm{SiO}_2 70 \mathrm{MAA}$	10
$50 \mathrm{SiO}_2 50 \mathrm{MAA}$	7
$60 \mathrm{SiO}_2 40 \mathrm{MAA}$	5
$70 SiO_2 30 MAA$	12
$70 \mathrm{SiO}_2 30 \mathrm{MAA} \mathrm{GPTMS}$	2

Table 4.5: The mass loss, observed from the TGA analysis.

The organic phase of PMAA experiences a 20% loss of its mass, while the inorganic phase of pure SiO_2 only experiences a 3% loss of its mass. This indicates that the polymer is not capable of losing more than 20% of its mass, while the pure SiO₂ should not be able to lose any of its mass at a temperature of 800 °C. Therefore the 3% mass loss is likely to be from impurities which do get degraded at this temperature. This behavior of the reference products, suggests that materials with higher amount of polymer should experience higher loss of their masses. This is true when comparing the materials of 30SiO₂70MAA, 50SiO₂50MAA and $60SiO_240MAA$, because the hybrid with 70% weight of polymer lost 10% of its initial mass, while the hybrid with 50% and 40% polymer weight lost 7% and 5% of their initial mass, respectively. If these three hybrid materials are compared to the full mass loss of the PMAA, their ratios would have been altered to be 50%, 35% and 25% weight of polymer, respectively if neglecting the 3% mass loss of the pure SiO₂. This indicates that either the material is not able to form a hybrid with all of the organic phase, or that the bonding between the two phases is so strong that it can shield some of the polymer from getting degraded by the temperature. The hybrid $70 \text{SiO}_2 30 \text{MAA}$ which experiences a 12% mass loss, indicates that the ratio of the polymer should have been 60% instead of the 30%. This specific hybrid is different in appearance from all other hybrids, and this analysis indicates that this material is more likely to degrade more of its material, compared to the other hybrids. The exact opposite results happens when analysing the $70SiO_230MAA$ GPTMS, as this hybrid only experiences a 2% loss of its mass, which is smaller than the reference material of pure SiO_2 . This indicates that something might have gone wrong during the synthesis or the linking agent has not been able to form a connection between the phases, resulting in a material which cannot be compared to the reference products.

4.2.3 Micro-Indentation

To investigate whether the surface hardness of Pure SiO_2 has changed, when adding polymer to create the hybrid materials, a micro indentation analysis were performed on both Pure SiO_2 and $70SiO_230MAA$ to determine the vickers hardness. This analysis is only performed on these two materials, since none of the other hybrid materials synthesised could be analysed using this method due to their physical appearance. The impression in both materials from the micro indentation analysis are visualised in Figure 4.6a & 4.6b



(a) Pure SiO₂

(b) 70SiO₂30MAA

Figure 4.6: The figures show the impressions after indentation at 2 newton.

Both of these micro-indentations are done with the same parameters of 2 newton impact force, meaning that the impressions left behind in the material are done with the same force profile. The vickers hardness is determined from the force profile and length of the impression and illustrated in Table 4.6.

 Table 4.6:
 Vickers Hardness analysis.

Material	Vickers Hardness (H_v)
$\begin{array}{c} \text{Pure SiO}_2\\ \text{70SiO}_230\text{MAA} \end{array}$	0.0353 ± 0.0019 GPa 0.0283 ± 0.0012 GPa

In a study by J. Nychka et al, who performed a vickers indentation analysis on 45S5 bioglass, they created a micro indentation profile from 30 mN to 2 N. The vickers hardness obtained in the study for 2 N force, were approximately 5 GPa. This value is significantly higher than that of the bioglass. In the study they produced the bioglass though the melt quench technique, whereas the bioglass in this rapport is produced by the sol-gel technique. Based on this, the bioglass produced by the sol-gel route. Furthermore, the study found out that their impression in the bioglass had a tendency to create radial median cracks after indentation. This is also the case of the impression in the produced bioglass, as this kind of cracks create a half penny shape between two lateral cracks. [19]

The vickers hardness of the hybrid $70 \text{SiO}_2 30 \text{MAA}$ is lower than that of Pure SiO₂. This indicates the need to add more force to crack or fracture the pure SiO₂ than the hybrid $70 \text{SiO}_2 30 \text{MAA}$. Because less force is required to crack or fracture the hybrid $70 \text{SiO}_2 30 \text{MAA}$, it indicates that the material is softer and that the hybrid

contains properties from both the organic and inorganic phase. Therefore the hybrid $70SiO_230MAA$ is considered to be better for tissue engineering compared to the pure SiO₂. However this do not necessarily mean that the hybrid $70SiO_230MAA$ is a perfect scaffold for tissue engineering.

4.3 Degradation

To investigate the degradation ability of all the materials, they were exposed to a hydrochloric acid solution with a pH of 3, followed by heating to 40 °C. Each material were filtrated, dried and weighed after 24 hours for a total of 168 hours. During the degradation analysis, two overall phenomena occurred. Either the material swelled in the solution hereby gaining more weight than from the beginning, or the material cracked and fractured into small pieces or dust. The materials which swelled are illustrated in Figure 4.7 and the materials which fractured are illustrated in Figure 4.8.



Figure 4.7: Figure A is the degradation curve of the polymer PMAA, figure B is the hybrid $30SiO_270MAA$ and figure C is the hybrid $50SiO_250MAA$.



Figure 4.8: Figure A is the degradation curve of the pure SiO₂, figure B is the hybrid $60SiO_240MAA$, figure C is the hybrid $70SiO_230MAA$ and figure D is the hybrid $70SiO_230MAA$ GPTMS.

When the materials were weighed at the beginning, they were completely dry from the synthesis procedure. However when the materials PMAA, $30SiO_270MAA$ and $50SiO_250MAA$ were filtrated and dried for one hour, their weight were increased and the materials with higher ratio of polymer showed a higher weight increase. This indicates that the materials have been swollen, meaning that the solvent had penetrated into the polymer network, and the one hour of drying were not enough to remove all of the solvent. The weight of the PMAA increased 100%, while the hybrids $30SiO_270MAA$ and $50SiO_250MAA$ weights increased 90% and 45%, respectively. Even though the materials have swelled and therefore gained a higher mass, the procedure were the same during the 168 hour of degradation analysis. The polymer MAA showed a small mass loss after each weighing, indicating that the polymer slowly degrades over time in this solution and temperature. For the two hybrids $30SiO_270MAA$ and $50SiO_250MAA$, there were no indication of degradation, as their weight showed a variety of increased and decreased mass after each weighing.

The four other materials consists of Pure SiO₂, $60SiO_240MAA$, $70SiO_230MAA$ and $70SiO_230MAA$ GPTMS. Each of these four materials fractured vigorously after being added to the 40 °C solution with a pH of 3. The hybrid $60SiO_240MAA$ turned into dust after the first encounter with the solution, while the hybrid $70SiO_230MAA$ GPTMS showed the least amount of fracturing with a larger piece of material still connected to some of the fractured pieces. The other two materials, Pure SiO₂ and

 $70SiO_230MAA$, both experienced some degree of fracturing after the first encounter with the solution. After 24 hours and drying they both fractured once more when added to a fresh solution. Due to this fracturing behavior, small amount of materials got removed during the filtration and extraction from and to the beaker, which resulted in a small mass loss during each sample time. This happened primarily with the hybrid $60SiO_240MAA$, where the filtration paper were weighed before filtration and after drying. This weight is compared to the weight when extracting the material from the filtration paper to the beaker. This mass change has been taken into account when creating the degradation curves. The degradation curves indicate that the hybrid materials, $60SiO_240MAA$ and $70SiO_230MAA$ GPTMS, do not experience degradation in the time period of the analysis under these particular conditions, as their masses shifts between gaining and losing mass. However the materials, Pure SiO_2 and $70SiO_230MAA$, have a consistency in losing some of their masses at each time sample. The Pure SiO_2 lost 10% of its initial mass after 168 hours, which indicates that it experiences a degradation under these conditions. The hybrid $70SiO_230MAA$ lost 7% of its initial mass, but at the first 72 hours of analysis, there is no mass change. This indicates that in the first few days the material does not experience any degradation, which could be from swelling of the small amount of polymer present. After the 72 hours, the material starts to lose some of its mass, which indicates that the hybrid is degrading under these particular conditions.

4.4 SEM

To investigate the physical influence of the degradation analysis on both Pure SiO_2 and the hybrid $70SiO_230MAA$, SEM analysis is performed on these materials before and after degradation to confirm any change. This analysis also investigate the hybrid material at a nanoscale to visually confirm the true class II hybrid. Furthermore any degree of porosity, within the analysis range can visually be confirmed, by the SEM analysis. In Figure 4.9, the hybrid material is illustrated with a magnification of 100 nm.



Figure 4.9: The hybrid material $70SiO_230MAA$ with a magnification of 100 nm.

This image is quite unsharp contains a lot of noise, this is caused by the conductivity of electrons in the material. Even though the material is coated with gold, the conductivity still creates problems when analysing the material in a nanometer range. Therefore, to get results which can be analysed and compared to each other all of the SEM images are within 1-100 μ m, as this range does not create any visual problems.

The images created from the SEM analysis on Pure SiO_2 before and after degradation analysis, as well as the hybrid $70SiO_230MAA$ before and after the degradation analysis are shown in Figure 4.10, Figure 4.11, Figure 4.12 and Figure 4.13, respectively.



Figure 4.10: The material Pure SiO_2 before degradation, where figure A is illustrated in 1 µm range, figure B is 10 µm and figure C is 100 µm.



Figure 4.11: The material Pure SiO_2 after degradation, where figure A and C is illustrated in 1 µm range and figure B and D is 10 µm.



Figure 4.12: The material $70SiO_230MAA$ before degradation, where figure A is illustrated in 1 µm range, figure B and D is 10 µm and figure C is 100 µm.



Figure 4.13: The material 70SiO₂30MAA after degradation, where figure A and C is illustrated in 1 µm range and figure B and D is 10 µm.

When sputter coating the materials, the amount of time to create the pressure needed in the sputter coater took longer than expected. When introducing the materials in the first pressure chamber of the SEM machine, the amount of time needed to create the proper pressure took three to four times longer than expected. This slow equilibrium of pressure is due the removal of air from within the materials, when air is trapped inside of a porous material. Higher degree of porosity and smaller pore sizes results in air being excreted more slowly compared to a non porous dense material. Therefore the long pressure equilibrium indicates that one or more of the materials are highly porous with small pore sizes. When analysing the images created by SEM, there are no indications of any of the materials being porous. However from the pressure equilibrium time there must be some degree of porosity in the materials, which suggests that the pores are only visual in a nanometer range, as the interference only allow clear images within a micrometer range. Therefore the porosity and pore sizes could not be determined by this method, but this analysis still suggests that they are present in one or more of the materials.

Figure 4.10 shows three images which are of pure SiO_2 before degradation. These images illustrate that the pure SiO_2 has a visual smooth surface from 100-1 µm. This is a small piece of material which was broken of the larger material, when breaking it the edge started to fracture, and the long crack in the middle is most likely from this breaking. Even though the cracks are from breaking the material, they indicates that the material is brittle as this breaking was performed with human fingers. Figure 4.11 shows four images which are of the pure SiO_2 after degradation. These images shows more impurities on the surface, and the surface is a little rougher compared to the Pure SiO₂ before degradation. The higher amount of impurities in these samples also suggest, that the material has created a rough surface, due to the degradation, where impurities easier bonds with and stay on the surface of the material. Figure 4.12 shows four images which are of the hybrid $70SiO_230MAA$ before degradation. These images illustrate a semi smooth surface with a small degree of impurities on the surface. It seems that the surface is smooth, but underneath are pores within the material, which could be the pores creating a porous material. However this cannot be confirmed as these pores are sealed at the surface, or the entrance to these are smaller than the SEM analysis can visualise in an image. The material do not show any separation of the two phases that the material is created by. This indicates that the material created is a true class II hybrid linked by covalent bondage. Figure 4.13 shows four images which are of the hybrid $70SiO_230MAA$ after degradation. These images does not resemble the previous discussed images. The surface is rough with craters created by the degradation of this particular material. It is especially visual in the images at 1 micrometer range, as these show craters with connecting cracks. However this degradation of the material does not seem to be uniform, indicating that the materials surface does not consist of the exact same components. This still indicates that the material is a true class II hybrid, with a degradation rate where the two phases almost matches each other.

To confirm that all of the hybrid materials as well as the reference materials Pure SiO_2 and PMAA has been synthesised correctly, the analysis method FTIR and TGA were used. Also they were used to confirm that the organic and inorganic phases have been covalently linked, by the linking agents, creating a true class II hybrid. To investigate the properties of different hybrid materials, micro-indentation, degradation and SEM analysis were used. The degradation investigation were performed on each material produced, while micro-indentation and SEM analysis were only applied on Pure SiO_2 and the hybrid $70SiO_230MAA$. The reference materials and the hybrids were compared to investigate which properties the hybrids have received from either the organic or inorganic phases.

The materials Pure SiO₂ and PMAA were expected to be used as reference materials for the hybrids. These two materials were validated in the FTIR analysis, by comparing them to literature. The peaks observed in Figure 4.3 indicates that the two reference materials have been synthesised correctly. However, some of the characteristic peaks of the two materials overlap each other. This overlap creates a broader and more intensive peak at the 1000-1200 cm^{-1} . This makes the distinction of the two phases in the spectrum difficult, when investigating the hybrid materials. When these two materials were exposed to the TGA analysis, the Pure SiO_2 was not expected to develop any degree of mass loss, while the PMAA should develop a high degree of mass loss. The Pure SiO_2 did however experience a mass loss of 3%, which is most likely due to impurities during the synthesis of the material. The PMAA experienced a mass loss of 20%, which is less than expected for the polymer. The 3% and 20% indicate how much of the hybrids mass is expected to be removed during this analysis, according to their ratio of organic and inorganic phases. Both the Pure SiO_2 and PMAA were chosen as materials for the hybrid, as literature described both of these materials to be biodegradable. The degradation analysis in a pH of 3 and 40 °C was expected to degrade both materials to some degree. This analysis indicates that the Pure SiO_2 is degradable, while the PMAA is a polymer, which tend to swell. However it still showed some degree of degradation. The degradation behavior of the PMAA, could not be confirmed as the polymer swelled, indicating a uncontrollable weight change behavior of the material.

Hybrid materials with two different linking agents were produced. These are GPTMS and TMSPMA. These two linking agents were used to investigate differences in the properties of the produced hybrid. One material produced with the GPTMS was the material $70SiO_230MAA$ GPTMS. This material did not have any peaks at the expected values obtained from the reference materials. There were no indications in the spectra, that any of the two reactant materials, were present in the hybrid. Furthermore, the result from the TGA analysis, showed only a loss of material of 2% of its initial mass. This small loss of mass, is even less than the 3% mass loss of Pure SiO₂, which is expected to derive from impurities. The degradation of this material, experienced swelling, and irregular weighing during the whole analysis.

All other produced hybrid materials were synthesised with the linking agent TMSPMA. However their ratios of organic and inorganic phases were varying. Each of these four hybrid materials showed peaks witch could be matched with the reference. Each individual intensity in every spectrum had a different relation between peaks resonating from the organic and inorganic phases. These different characteristic peaks, followed a trend according to their ratios. In the material with 30% SiO₂ the intensity between the peaks of the two different phases, was at the same intensity. Meanwhile, the material with 50%, 60% and 70% SiO₂ showed a more intense peak from the SiO_2 than the peak created by the PMAA. This showed that a higher amount of SiO_2 creates a higher difference in the characteristic peaks of the two phases. This trend is not equivalent to the TGA analysis. The material 30SiO₂70MAA, 50SiO₂50MAA and 40SiO₂60MAA showed the trend, that higher ratio of the PMAA experienced higher loss of their initial mass. The material $70SiO_230MAA$ experienced an even higher mass loss than any of the trend of the other three hybrids. The appearances of the material $70SiO_230MAA$ is also different from that of the three other hybrids. This indicates that something changed in the material from 60% SiO₂ to the 70% SiO. When investigating the degradation analysis, each of the hybrid materials experienced some degree of swelling. The two materials with 70% and 50% PMMA swelled, creating a material, which contained more mass after drying than the initial weight of the material. The other two materials which contained 40% and 30% PMAA, also experienced swelling. After a few cycles, the weight of the material went below the initial mass weight. None of the hybrids showed any specific trend, indicating that the swelling of the polymer caused difference in the weighed mass.

Two other analysis were performed, but only on the materials Pure SiO_2 and the hybrid $70\text{SiO}_230\text{MAA}$. One of these analysis showed which properties the hybrid $70\text{SiO}_230\text{MAA}$ had received from the PMAA. This tests the surface strength of the two materials. They were exposed to the same load, and the surface of the hybrid $70\text{SiO}_230\text{MAA}$ showed that less force was needed to create impression in the material, meaning that the hybrid material is softer than the Pure SiO_2 . A second analysis was performed on the materials Pure SiO_2 and the hybrid $70\text{SiO}_230\text{MAA}$ before degradation and on the same two materials after 168 hours of exposure in the degradation solution. Each of these four materials, were analysed in a SEM at a micrometer range. The Pure SiO_2 showed a rougher surface after degradation than

before degradation, indicated by more impurities on the surface of the material. However the surface of the material was still smooth without pores or high amounts of cracks. The hybrid material before degradation showed a smooth surface, with some degree of pores trapped in the material. When the material was exposed to the solution at pH of 3 and 40 $^{\circ}$ C, there was a substantially rougher surface with many cracks and open pores, which have been exposed by the degradation of the surface. The degradation of the hybrid, has created craters with fracture lines going from pore to pore.

Conclusion 6

The aim of this project was to synthesise a true class II hybrid of silica and *Poly* (Methacrylic acid) though the sol-gel route. The use of these two materials varied in mass ratio, in order to investigate their differences. In order to create covalently bonds between the organic and inorganic phases, both GPTMS and TMSPMA was used as linking agents. The purpose was to obtain a hybrid, useful to assist or replace the graft materials used today for tissue engineering.

When analysing the two reference materials Pure SiO_2 and PMAA, both showed to be synthesised correctly according to the FTIR analysis. The other analysis further validates that these two materials have been synthesised as expected. They are therefore accepted as reference materials to compare to the hybrid materials.

The hybrid material created with the linking agent GPTMS, showed no indication of being synthesised correctly. This material is therefore not suitable for tissue engineering, by the synthesis procedure followed during this project. The other hybrids, created with the linking agent TMSPMA, showed two distinct patterns. The material with 70% mass of SiO₂ was different from the other three materials, which all had smaller mass ratio of SiO₂. The materials with less than 70% mass of SiO₂ showed properties from both the organic and inorganic materials. However they were more brittle than the Pure SiO₂ itself, which is one of the properties that the PMAA should provide to the material. Hence the only material, whose properties were altered for the better, was the hybrid 70SiO₂30MAA. This material showed that it was softer than the Pure SiO₂ and it showed pores within the material. Therefore further work on this specific material or a material with higher amounts of SiO₂ could be suitable for tissue engineering based on this project.

Outlook

If this study should be continued, there would be some changes to the synthesis procedure and some of the analysis performed. A longer study would also take other analysis into consideration, in order to investigate the hybrid materials characteristics and properties.

To mimic the first produced bioglass 45S5, other materials should be introduced to the SiO_2 during the sol-gel. One of the most important materials, to have present in the hybrid, is the addition of calcium. In this work, there were no calcium present in any of the hybrids. As mentioned calcium oxide needs 400 °C to be properly introduced in the sol-gel. In further investigation of this topic, calcium should be added without compromising the other elements. Furthermore the synthesis procedure should be altered, to yield materials that can be used in testing of the materials physical properties.

The materials, synthesised during this project, were to brittle and fragile to perform any compression or stretching tests, as well as a dynamic mechanical analysis. These analysis would be able to give a lot of information about each materials physical properties. Another synthesis route to produce materials suitable for these analysis is therefore favorable in order to investigate the hybrid materials.

Furthermore a solid state nuclear magnetic resonance analysis could be used to investigate the Q values of the silica within the hybrids. However this analysis would also investigate whether any silica has a bond to a carbon atom, which would indicate a linkage between the organic and inorganic phases. This analysis is therefore a great tool to proper investigate the hybrid materials, especially to validate the covalent linkage between the two phases.

This project analysed the degradation behavior of each material over seven days in a solution at a pH of 3 and a temperature of 40 °C. In order to further investigate the degradation behavior, a analysis which simulates body fluid in vitro should be carried out. Eventually if the material showed to be degraded in vitro environment, a degradation analysis in vivo can then be performed. The degradation analysis time period should be altered into months instead of a week, to fully investigate each hybrid materials degradation behavior. The formation of a HCA layer around the material should be created in both of the degradation analysis suggested.

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