HYDROXYAPATITE/POLYMER COMPOSITES FOR BONE REPLACEMENT

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PROEFSCHRIFT

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To Hongbo
Our parents

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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Cover photo:
A SEM photo shows the fracture surface of EMa-HA composite. Better contact between such EMa modified HA particles and polymer matrix can be seen.

This thesis is based on the following publications:

1 Q. Liu, J.R. de Wijn and C.A. van Blitterswijk, "Composite biomaterials with chemical bonding between hydroxyapatite filler particles and PEG/PBT block copolymer matrix", (submitted to J. Biomed. Mater. Res.).

2 Q. Liu, J.R. de Wijn and C.A. van Blitterswijk, "A study on the grafting reaction of isocyanates with hydroxyapatite particles", (submitted to J. Biomed. Mater. Res.).

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6 Q. Liu, J.R. de Wijn, D. Bakker and C.A. van Blitterswijk, "Surface modification of hydroxyapatite to introduce interfacial bonding with Polyactive^{TM} 70/30 in a biodegradable composite", J. Mater. Sci.: Mater. in Med. 7:551-557, 1996

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10 J.R. de Wijn, Q. Liu and C.A. van Blitterswijk, "Grafting PMMA on hydroxyapatite powder particles using isocyanatoethylmethacrylate", Trans. Fifth World Biomaterials Congress, I-633 (May 29 -June 2, 1996, Toronto, Canada).

CONTENTS

Chapter 1

General Introduction

1. Biomaterials for bone replacement

Much effort has been invested in the development of biomaterials for the repair or replacement of hard tissue. Besides the general consideration of biocompatibility, the specific consideration for bone replacement materials is of biomechanical nature: the biomaterials should possess the mechanical properties necessary for a proper performance in their function. Other properties such as biodegradation, the ability to bond to bone or so called "bone-bonding" property are some additional favourable assets. The bone bonding property can be defined as [Williams et al. 1992]: ***"the establishment by physicochemical processes of a continuity between implant and bone matrix".*** Bone bonding properties - often called "bioactivity" - have been proved to be of great benefit for bone replacement materials.

1.1. Bone derived materials- autografts and allografts

The conventional method for the reconstruction of surgical osseous defects is dependent on an adequate supply of autogenous (host) or allogenic (donor) bone. Bone autograft is widely considered to be the best implant for repairing bone defects [Damien and Parsons 1991, Brown and Cruess 1982]. Common donor sites include the iliac crest, tibia, fibula and greater trochanter. However, the amount of autogenous bone available for transplantation is limited, particularly in children. Also, the harvesting operation carries the risk of post-operative complications [Damien and Parsons 1991, Prolo and Rodrigo 1985]. Cortical bone is selected for strength and mechanical support, while cancellous bone autografts are used to promote lattice formation and rapid bone regeneration.

Allogenic bone has been successfully used in osseous reconstruction [Damien and Parsons 1991, Glowacki et al. 1981, Kaban et al, 1982] and offers several advantages over autogenous bone, including the avoidance of a harvesting operation, ease of manipulation and potentially unlimited material in bank form. Nevertheless, the possibility of transmission of disease from the donor to the recipient raises doubts about its future. Long times needed for the resorption and the replacement of allografts by new bone and the antigenic activity of banked bone are the serious disadvantages when compared with autografts. As a consequence, the search for suitable alternatives to autogenous and allogenic bone has intensified over the past decade.

1.2. Synthetic materials-metals, ceramics and polymers and composites

1.2.1. Metals

Metallic implants have been used either as permanent prostheses such as the hip prosthesis, dental implants, etc, or as temporary implants such as plates, pins, screws and rods

for the fixation of bone fractures. Currently, stainless steel [Small and Misiek 1986], cobalt-chrome alloys [Albrektsson et al, 1986], Titanium and its alloys [Albrektsson et al, 1986, Steflik et al. 1993] have been used to fabricate the implants. These implants are usually not integrated by the bone tissue or only after extended implantation periods. Improvement of implant integration in bone can either be accomplished by cement fixation, the use of a porous bead implant surface to allow bone ingrowth and thus mechanical fixation or the application of bioactive ceramic coatings. Hydroxyapatite coatings applied by various methods [de Groot et al. 1987, Ducheyne et al. 1980, Lacefield 1988, van Raemdonck et al. 1984] are in clinical use today and improve implant performance. In some occasions, the high mechanical stiffness of metallic implants may result in stress-shielding and bone resorption due to the mismatch of elastic modulus of metals with that of bone [Daniels et al. 1990, Terjesen and Apalset 1988]. Other disadvantages of using metallic implants include the need for the second operation to remove temporary implants and the negative tissue response caused by the ions released from permanently implanted devices [Black 1981, Sinibaldik et al. 1976].

1.2.2. Ceramics

The use of ceramics can be dated back to 19th century when calcium sulphate (Plaster of Paris) was first used by Dreesmaanas as a plaster for the fixation of bone. Nowadays, the commonly used bioceramics are metallic oxides (e.g. Al_2O_3, MgO) [Black 1981], calcium phosphate (e.g. hydroxyapatite (HA), tricalcium phosphate (TCP), octacalcium phosphate (OCP)) [de Groot 1981, LeGeros, 1991], and glass ceramics (e.g. Bioglass, Ceravital) [Hench et al.1971, Kokubo 1992]. The metallic oxides are considered to be nearly bioinert in biological environments, while calcium phosphate and glass ceramics can bond to bone in bony sites when implanted. Because of the good biocompatibility and bioactivity of the bioceramics, they have been successfully used for hard tissue replacement.

Among the bioactive ceramics, synthetic hydroxyapatite with a chemical composition $Ca_{10}(PO_4)_6(OH)_2$ has been extensively studied as a bone replacement material. As a bulk material, HA lacks sufficient tensile strength and is too brittle to be used in most load bearing applications [Denissen et al. 1980, Lemons 1988]. Therefore, when hydroxyapatite has to be applied in load bearing situations, the material is coated onto a metal core [de Groot et al. 1987], or is incorporated into polymers as composites [Bonfield et al. 1986, Doyle et al. 1991, Knowles et al. 1992, Verheyen et al. 1993].

1.2.3. Polymers

Although polymers are widely used as implants, such as vascular prostheses, sutures etc. only limited number of polymers have been used for bone replacement purposes due to the limitation of their mechanical properties. These polymers include ultra-high molecular weight polyethylene (UHMWPE)[Park and Lakes 1992], poly(methyl methacrylate) (PMMA)[Saha and

Pal 1984, Pilliar et al 1976], polylactide (PLA), polyglycolide (PGA) and polyhydroxybutyrate [Daniels et al. 1990, Knowles et al. 1992, Coombes and Meikle 1994, Agrawal et al. 1995]. The choice of polymer as bone replacement material largely depends on the following factors (besides the general requirement of biocompatibility): (1). The mechanical properties of the polymer if the polymer is going to be used as load bearing material; (2). The biodegradation behaviour of the polymer if the implant has to be eliminated after certain period. (3). The ability to bond with bone or to induce bone ingrowth.

Generally speaking, polymers have poorer mechanical properties than bone. But the possibility to be mechanically strengthened and to be biodegradable makes polymers very promising as candidates for bone replacement. The improvement of the mechanical properties of polymer can be achieved by either the modification of the structure of the polymer, or the strengthening of the polymer with fibre and/or filler as it will be discussed later.

The structure modification of polymers includes crosslinking, copolymerization more than one type of monomer, using new type of monomers to synthesize new polymers, etc. Some researchers have synthesized polyimminocarbonates by incorporating tyrosine, tyroamine or desamino-tyrosine groups to the polymer chain [Pulapura et al. 1990, 1992]. They found that the synthesized poly(desaminotyrosyl-tyrosine hexylester iminocarbonate) had a tensile strength of 40 MPa, and a tensile modulus of 1.6 GPa which is relatively high among the biodegradable polymers. Attawia et al [1995] incorporated aromatic imide groups to poly(anhydrides) to improve the mechanical properties of poly(anhydrides).

Although the biodegradation ability of the polymer can be carefully designed by introducing ester bonds, imino bonds etc. to the polymer structure, it is rather difficult to obtain a polymer both biodegradable and mechanically strong to the level of cortical bone. Poly(lactide)(PLA) and poly(glycolic acid, PGA) seem still to be the strongest biodegradable polymers available for medical applications; they can have a tensile strength up to 72 MPa for PLLA, 57 MPa for PGA and a Young's modulus of 4 GPa for PLLA and 6.5 GPa for PGA [Daniels et al. 1990]. Compared with the mechanical properties of cortical bone, these polymers are still weaker and reinforcement of the materials is still necessary.

The bone bonding property of a polymer is another issue of concern. Up to now, only a polyethylene/polybutylene terephthalate (PEG/PBT) block copolymer (Polyactive^{TM}) has been identified as a "bone bonding polymer" [Bakker et al. 1989, 1990a, 1990b, 1990c, van Blitterswijk et al, 1993a]. It is explained that the PEG segments of the material can complex calcium ions and thus cause calcification of the polymer. The calcification of the polymer is believed to be a prerequisite for the following bone-bonding to occur [van Blitterswijk et al. 1992]. Although some other polymers were also found to be calcified in vivo, such as polyetherurethane [Coleman 1981, Wisman et al.1982, Schoen et al 1988, Thoma 1987] or poly(HEMA) hydrogel [Swart 1976], none of the above polymers has been studied for bone bonding properties.

The design of polymers with bone-bonding property has not been progressed very much. Since the calcification of material is the common feature shared by bone-bonding biomaterials such as Bioglass [Hench et al. 1971, 1981], AW glass ceramics [Kokubo et al. 1990, 1991, 1993], hydroxyapatite [Jarcho 1981, LeGeros et al. 1992, Ducheyne and Cuckler 1992, van Blitterswijk 1995], and PEG/PBT block copolymer [van Blitterswijk et al. 1992, Radder et al. 1993], the introduction of certain functional groups to the polymer chain or the surface may give the polymer the ability to induce calcification and thus to have bone-bonding abilities. Dalas et al [1991a, 1991b] have shown that introduction of functional groups like the phosphinyl group, the carboxylic acid group, the sulphonate group, fluoride et al, may induce the nucleation of hydroxyapatite on the polymer. Tretinnikov et al [1994] showed that introduction of phosphate groups to the surface of high density polyethylene can induce the formation of a firmly bonded carbonated apatite layer on the surface upon immersion in simulated physiologic solution. When the surface modified polyethylene rod was implanted in the rat femur, they found a high percentage of bone contact for the experimental samples as compared to the control untreated polyethylene rod [Kamei et al. 1996]. Li et al [1996, 1997a, 1997b, 1997c] modified natural bamboo by various methods, including grafting of PEG and introduction of phosphate groups to cellulose of bamboo. The modified bamboo has the ability to induce apatite formation in vitro.

1.2.4. Polymer Matrix Composites

The use of polymer matrix composites for bone replacement may offer the advantages of avoiding the problem of stress shielding, eliminating the need for a second surgical procedure to remove the implants if the implants can be made biodegradable and also the elimination of the ion release problem of metal implants. The possibility to make the composites as strong as cortical bone and to improve the material's bioactivity or bone bonding activity by adding of a secondary reinforcing phase makes the composites very attractive. Fibers and mineral filler particles have been used to reinforce the polymer materials as well as to improve the bone-bonding properties of the composites.

2. Polymer Matrix Composite

2.1. Fiber reinforced composites (FRC)

Carbon fiber, aramid (Kevelar), glass fiber, usually posses very high strength and stiffness [Hancox 1983] and therefore have been frequently used to reinforce polymers like epoxy resin, polyetheretherketone (PEEK), polysulfone (PS), polymethyl methacrylate (PMMA), poly(lactide), poly(glycolide), polycaprolactone, etc. (table 1).

By proper choice of the type of polymer matrix and of the fiber, the composites can be made totally biodegradable [Casper et al. 1985, Kelly et al. 1987, 1988, Andriano and Daniels 1992a,1992b,], partially degradable [Zimmerman et al. 1987], or non-biodegradable [Latour

and Black 1992, 1993]. Also the mechanical properties can be tailored by combining different polymer matrices and fibers. As an example, 30 % carbon fiber reinforced PEEK composites has an elastic modulus of 17 GPa (bone 7 -20 GPa), a flexural strength of 320 MPa (bone 150-250 MPa) [Hastings et al. 1987].

Bone bonding can be improved by using certain bioactive fibers. Marcolongo et al. [1995, 1996] showed that when bioactive glass reinforced polysulfone composites were implanted for 6 weeks, direct apposition of bone tissue with bioactive glass fibers could be observed. Bone tissue was also observed in direct apposition to polymers surrounding the glass fibers.

When making fiber reinforced composites, the mechanical properties of the polymer matrix and the fiber are certainly important for the mechanical properties of the composites. However, the interfacial bonding strength between fibers and polymer matrix is usually weaker than the polymer matrix [Pigott et al. 1985]. Therefore the fatigue fractures usually occur at the interface of fiber and polymer.

Table 1. Examples of fiber reinforced composites for bone replacement

Carbon fiber	
	Epoxy resin [Bradley 1980, Tatton et al. 1982]
	PMMA [Schreiber 1971, Wylegala 1973, Pilliar et al 1976, Ekstrand et al 1987]
	polysulfone [Huettner et al. 1984, Claes et al 1986, Wenz et al.1990, Latour and Black 1992, 1993]
	polycarbonate [[Latour and Black 1992]
	polyetheretherketone [Hastings et al. 1987, William et al. 1987,Wenz et al.1990]
	Polylactide [Zimmerman et al. 1987, Alexander et al. 1981]
Aramid (Kevelar)	
	PMMA [Berrong et al. 1990, Pourdeyhimi et al, 1986]
	polysulfone [Latour and Black 1992, 1993]
	polycarbonate [[Latour and Black 1992]
Polyethylene fiber (high-performance)	
	PMMA [Cheng et al, 1993], poly(DL-lactic acid [Fenner et al. 1996]
Bioactive glass fiber	
	polysulfone [Marcolongo et al. 1995, 1996]
Calcium metaphosphate glass fiber	
	polylactide [Casper et al. 1985, Kelly et al. 1987, 1988]
Calcium phosphate glass fiber	
	poly(L-lactide) [Lin, 1986], polycaprolactone [Foy et al.1996],
	poly-(desamino-tyrosyl-tyrosine ethyl ester) [Perez et al. 1996]
Calcium-sodium-metaphosphate glass fiber	

Poly(ortho ester) [Andriano and Daniels, 1992a,b]

Titanium fiber

PMMA [Topoleski and Ducheyne 1992]

When the composite is exposed to an in vivo environment, the interface of fiber and polymer can be further deteriorated. Several studies have shown the effect of water and simulated in vivo environments on the interfacial bonding strength of ceramic or glass fiber and polymer [Ekstrand, et al. 1987, Foy et al. 1996, Latour and Black, 1992, 1993, Kelly at al. 1988, Andriano et al,1992a, 1992b, Jancar at al. 1993, Slivika et al. 1996]. There is clearly a need for the improvement of the interface of fiber/polymer matrix to improve both the mechanical properties of the composites and the wet stability of the interfacial bond.

2.2. Filler Reinforced Composites

The use of particulate fillers to reinforce polymeric biomaterials is quite important and quite successful in clinical applications, like dental restorative resins and bone cement [Soltese, 1988].

The purpose of using filler particles in the polymer matrix is to improve the mechanical properties such as the elastic modulus [Guida et al. 1984, Castaldini et al. 1984, Bonfield et al. 1984, 1986], fatigue behaviour [Castaldini et al. 1987] and to improve the bioactivity or bone-bonding properties [Bonfield et al. 1986, Doyle et al. 1991, Knowles et al. 1992, Verheyen et al. 1993]. Some other benefits may also obtained by using fillers, such as to diminish the creep of the composites [Castaldini et al. 1986] and to decrease the temperature rise during the polymerization of bone cements [Guida et al. 1984].

The use of a bioactive filler such as hydroxyapatite (HA), AW ceramic or Bioglass particles to reinforce a polymer may improve both the mechanical properties and the bone bonding properties. As indicated by Bonfield et al [1983, 1986, 1988], the elastic modulus of polyethylene can be increased from 1 GPa to about 8 GPa, which is in the low band of the value for bone, retaining a fracture toughness comparable to bone. When implanted in vivo, the HA/PE composites can induce bone apposition and thus create a secure bond between the natural bone and the implant. Inspired by this work, researches have been extended to the biodegradable polymer matrix. When implanted in vivo, such composites will induce bone formation or bone ingrowth and as the biodegradable polymer matrix degrades the implant will finally be replaced by bone tissue. The load thus can be gradually transferred to the newly formed bone. Based upon this idea, several hydroxyapatite reinforced biodegradable polymer composites have been developed, such as HA/polyhydroxybutyrate [Doyle et al, Knowles et al. 1991, 1992, Boeree et al. 1993], HA/polylactide [Verheyen et al. 1992,1993].

The use of a filler to reinforce a biodegradable polymer matrix offers another advantage:

the possibility to control the biodegradation rate. It has been shown that by addition of basic fillers, such as HA and magnesium oxide, the degradation rate as well as the degradation mechanism of poly (DL-lactide) can be changed [van der Meer et al.1996] Jones and Williams [1996] also showed that the degradation pattern of poly(L-lactide) was affected by the addition of ceramic filler.

Although the mechanical properties of the composites can be improved to a certain extent by the addition of bioactive filler particles, it is stated by several researchers [Verheyen et al. 1992, Wang et al.1994] that there is still a need to improve the bonding between filler and matrix since there is clearly no other bonding force between the two phases than mechanical interlock. The use of certain coupling agents was suggested [Verheyen et al. 1992, Wang et al 1994].

3. Method to improve the interfacial bonding of the phases in composites

3.1. Self-reinforcement of fiber/polymer composites (SRC)

Polymer fibers usually possess much better mechanical properties, due to the molecular orientation, when compared to its bulk materials. Use of polymer fibers to reinforce a polymer matrix of the same chemical structure thus will result in a composite without a real interface between fibers and polymer matrix. Such self-reinforced composites have been made by using PLA [Vainionpaa et al. 1988], PGA [Laiho et al. 1988, Pellinen et al. 1988] and PMMA [Gilbert et al. 1995]. The mechanical properties of the composites were significantly improved by using this method. However, the polymer fibers used are still pliable, therefore the Young's modulus of SRC's can not be as high as the glass fiber and carbon fiber reinforced composites. On the other hand, the bone bonding properties of the composites can not be improved by this method.

3.2. Plasma treatment of fibers

Gas plasma treatment has been proven to be effective in enhancing the bond strength between fibers and polymer matrix. The gas used for the treatment of fiber can be argon gas, O_2, methane or CO_2 [Friis et al. 1996, Perez et al. 1996, Hild and Schwartz]. These methods have been used to treat fibers like polyethylene, calcium phosphate glass fiber and PET (table 2)

Table 2. Examples of using gas plasma treatment for the improvement of fiber/matrix interface

Fiber	Gas	Matrix
polyethylene	air	poly(DL-lactic acid) [Fenner et al. 1996]
	N_2, Ar, CO_2,	PMMA [Hild et al. 1996]
PET	Ar, O_2,	PMMA [Friis et al, 1996]

Calcium-phosphate glass fiber	CH_4	poly(DTE carbonate) [Perez et al. 1996]

During the gas plasma treatment, functional groups are generated on the surface of the fiber, therefore the wettability of the fibers by the polymer matrix and thus the bond strength between them is improved.

3.3. Silane coupling agents

A coupling agent is an additive which promotes the development of a strong bond between the filler(fiber) surface and the polymer. Silane coupling agents have been widely used to improve the bonding strength of the two phases and have a general formula as:

$$X_3SiRY$$

X represents a hydrolysable group, Y is a organofunctional group. The organofunctional groups are chosen for reactivity or compatibility with the polymer, while the hydrolysable groups are merely intermediate in the formation of a bond with the filler or fiber surface. The exact behaviour of coupling agent is a matter of some controversy.

The use of coupling agent has two purposes. The first one is to improve the mechanical properties of composites by improving the interfacial bonding strength. The second one is to prevent the surface dissolution of fibers. When using the silane coupling agent [3-(n-styrylmethyl-2-aminoethylamino)-propyltrimethoxysilane hydrochloride] to make calcium phosphate glass fibers reinforced polycaprolactone composites [Foy et al. 1996], the ultimate bond strength was improved both in dry and wet environments, but the ultimate bond strength remained still far below desirable working levels. Andriano et al [1992a, 1992b] used N, beta-aminoethyl-gamma-aminopropyl-trimethoxysilane to treat the surface of calcium-sodium-metaphosphate (CSM) mineral microfibers, which were used to reinforce poly(ortho esters) prepared by a condensation reaction of 3,9-bis(ethylene 2,4,8,10-tetraoxaspiro[5,5]-undecane) and a 60:40 and 90:10 mole ratio of flexible diol, 1,6-hexanediol, respectively. The choice of such a diamine silane coupling agent was also intended to neutralize the CSM fiber's acidity which may cause a fast degradation of the composites. The initial mechanical properties of the composites were modestly improved, and the wet resistance of the composites during in vitro exposure to Tris-buffered saline was markedly improved .

Other research showed that the use of silane coupling agents may still offer hydrolytic problems of the interface. Jancar et al [1993] used 4 types of silane coupling agents to treat the E-glass fibers: amino-propyltriethoxylsilane, glycidoxy propyltrethyoxysilane, methacryloxypropyltrethoxy-silane, amiophenyltriethoxysilane. Although an improved adhesion was observed for some composites, the coupling agents did not prevent the deterioration of the interface under extreme conditions of stress and moisture (100 hrs, 85 ^{o}C in water). Applying a

silane layer by plasma polymerization on PET fiber surface did not change the fracture toughness of bone cement [Friis, et al. 1996].

In all these cases, it was noted that silane coupling agents did not form covalent bonds with the polymer matrix. The interface improvement is most probably due to the result of better wetting of the fiber surface by the polymer matrix.

The use of silane coupling agents in mineral filled dental resins has been employed since the 1960's [Bowen 1963, Venhoven 1994, Jones and Rizkalla 1996] as a method to improve the bonding of the filler to the resin. It was reported that appropriate silane coupling agents were chosen for a variety of mineral fillers to improve the mechanical properties of composites [Pluedemann 1991]. The greatest improvement was observed with silica, alumina, glass, silicon carbide, and aluminum needles. A good but somewhat lesser response was observed with talc, wollastonite, iron powder, clay, and hydrated aluminum oxide, only a slight improvement was imparted to asbestine, hydroxyapatite, titanium dioxide, and zinc oxide. Surfaces that showed little or no apparent response to silane coupling agents included calcium carbonate, graphite and boron. Those results suggest that the coupling activity of silanes is not universal to all mineral surfaces.

The latest results showed that silane [3-trimethoxysilyl)-propyl methacrylate](MPS) treated glass filler can drastically decrease wear rates [Venhoven et al. 1994] in a dental resin based on BisGMA systems. Also, Jones et al [1996] reported that a silane treated bioactive glass filler was found to have a significant effect on the elastic modulus of Bis-GMA based composites. Generally speaking, silane treated glass-ceramic, quartz, or silica particles may have a positive effect on the strength, stiffness and wear resistance and other aspects of the composites [Soltesz 1988, Chowdhury et al. 1995].

The effect of the use of silane coupling agents on hydroxyapatite (HA) seems to have somewhat controversary results. It has been shown that silane treated HA particles have a positive effect on the mechanical properties of a composite. Behiri et al [1991] showed that applying methacryloxypropyltrimethoxysilane (MPS) to the surface of HA particles may enhance the tensile modulus, yield stress and elongation to fracture of polyethylmethacrylate cements. Labella et al [1994] found that by using MPS, the hardness, flexural strength and diametral tensile strength of dental composites were significantly improved.

However, silane coupling agents also have been found to have different effect on the mechanical properties of other composites. Deb et al [1996] and Nazhat et al [1996] showed that MPS treated HA showed decreased tensile strength and Young's modulus of polyethylene composite. Since in both cases there was no chemical bonding between the silane and the polymer matrix, the decreased strength and modulus was explained by a plasticizing effect of the coupling agent. Surface treatment of β-crystalline metaphosphate with silane coupling agent MPS did not have effect on the tensile strength of dental resin composites [Antonucci et al. 1991].

In our opinion, the different results of silane treatment probably depend on the covalent bonding between the silane coupling agent and the polymer matrix. Once a bonding was established between HA and silane, the bonding between silane coupling agent and polymer matrix did determine the interfacial strengths. In dental resin systems, when MPS was used, double bonds were introduced by the treatment of filler. In the following curing process, the bound MPS copolymerized with Bis-GMA monomer, so that a chemical linkage was introduced, and the mechanical properties were improved. In the use of HA/PE system, the introducing of MPS can not lead to a chemical bonding between MPS and PE matrix, therefore no improvement can be observed.

Apparently more efforts should be put in the selection of right silane coupling agents for different polymer matrix. Also, the effect of silane coupling agents on the bioactivity of HA particles has to be studied. Dupraz et al [1996] studied different silane coupling agents on HA powder and showed by a XPS study that a few monolayers of silane film were present on the surface of HA particles. The silane thin film was "transparent" for ionic transport, although aminosilane coatings delayed the release of calcium and phosphate ions during the first few days of immersion of the treated HA powders in Gomori's buffer. In this sense, the bioactivity of the HA particle might be affected by the silane treatment, because the dissolution behaviour of HA plays an important role in its bone bonding activity [LeGeros et al. 1992,van Blitterswijk et al.1995].

3.4. Other methods

Ishhara et al [1989, 1992] found that 4-methacryloyloxyethyl trimellitic anhydride (4-META) containing MMA bone cement could adhere to bone, metals, HA and a composite of HA and fluoroapatite (FAP) with a improved tensile bond strength. The bonding of such cement to dentin was explained by the ability of 4-META to promote the interpenetration of monomers into dentin tissue. No explanation was given to the adhesiveness of such cements to HA and metals. We speculate that the formation of 4-methacryloyloxyethyl trimellitic acid (4-MET) was the reason of adhesion to HA. According to the authors [Ishhara et al. 1989], the anhydride moiety of 4-META can be easily converted to 4-MET by the reaction with water, thus the real mechanism might either be that 4-MET is firmly absorbed to the surface of HA followed by the copolymerization of 4-MET with MMA monomer, or the copolymerization takes place first and is followed by the adsorption of 4-MET moieties onto HA. In both cases, the interface of HA and polymer may be improved.

Misra [1985] found that when the HA surface had been surface treated with zirconyl methacrylate, the diametral tensile strength of dental composites was increased by 50%. Antonucci et al [1991] also found that treatment of fillers by zirconyl methacrylate resulted in a modest enhancement of the tensile strength of the experimental composites.

Introduction of covalently bonded hydroxyethylmethacrylate (HEMA) to a

nonstoichiometric apatitic calcium phosphate was realized by a co-precipitation of apatitic octacalcium phosphate (AOCP) in the presence of hydroxyethylmethacrylate phosphate [Delpech and Lebugle 1990, Dandurand et al. 1990]. The bond of the organic HEMA to AOCP was realized by the ionized phosphate groups which partially replace the OH^- ions located at the tunnel end in the apatite crystal structure. The obtained so called phosphoHEMA apatite then can be used to copolymerize with either HEMA or methyl methacrylate (MMA) to form chemical bonds between the mineral filler and the polymer matrix. It is claimed that such filler could stiffen the PMMA bone cement.

3.5. The nature of the bonding between silane coupling agent and hydroxyapatite

Although silane coupling agents have been used for the surface treatment of calcium phosphate mineral fibers or filler particles, the actual nature of the bond between silanes and the hydroxyapatite is still not clear. For the reaction of silanes with other minerals, two possible mechanisms may be involved in the treatment [Plueddemann 1991].

Due to absorption (hydrogen bonding), all the minerals and metal oxides are covered with at least a monolayer of water at ambient conditions. Alkoxysilanes are capable of reacting with surface moisture to generate silanol groups which also may form strong hydrogen bonds with the hydroxylated surface. This mechanism was supported by experiment [Nishiyama et al. 1987]. A chemical bonding mechanism presumes that water is adsorbed on the nonhygroscopic oxides as hydroxyl groups; Alkoxysilanes are capable of reacting with surface hydroxyl groups to form covalent oxane bonds with the mineral surface .

As for HA, little work has been done except for the work done by Nishizawa et al [1995]. They used silane coupling agents to treat calcium phosphate ceramics. By using thermal analysis, infra-red and mass spectra, they found that the surface hydroxy groups of the ceramics formed covalent bonds with silane coupling agents.

4. PEG/PBT copolymer (Polyactive^{TM})

The bone-bonding properties of poly(ethylene glycol)/poly(butylene terephthalate) (PEG/PBT) block copolymers was first reported by Bakker et al [1989, 1990a, 1990b, 1990c]. It has been found that when the PEG/PBT ratio is higher than 55/45, the copolymer has bone-bonding properties. By varying the ratio of PEG and PBT, as well as the molecular weight of PEG, a series of PEG/PBT copolymers can be synthesized with different mechanical properties, as well as different biological characteristics [van Blitterswijk et al. 1992, 1993, Radder et al. 1994], such as biodegradation rate and bone bonding properties. Concerning the bone-bonding property, it has been proposed [van Blitterswijk et al. 1992, 1993] that the PEG segment of polyactive can complex calcium ions from the environment by a similar mechanism proposed for the calcification of polyetherurethane [Thoma 1987]. The calcification in

Polyactive™ is at least composed of carbonated apatite, and thus similar to the apatite layer formed on other bioactive ceramics and to the mineral phase of bone [Radder et al 1993]. It is hypothesized that following calcification of the PEO/PBT copolymer surface, bone apposition can take place in a similar manner as on the surface apatite layer of other bioactive biomaterials which initially contain calcium and phosphorus ions.

Since the bonding of bone to Polyactive only occurs after the calcification of the polymer, Gaillard [1995] used bioactive fillers like HA and AW glass particles to accelerate the calcification process of Polyactive™, and to promote the bone formation on the implant surface. It was found that the in vitro calcification of both composites was increased by adding AW glass and HA filler particles. Also by precalcification of Polyactive™, the bone bonding rate of the materials can be accelerated [Gaillard et al. 1994].

5. Objective and methods

The objective of this study is to develop a suitable method to improve the interface of hydroxyapatite filler particles/Polyactive™ composites. As we discussed before, the interface of filler and polymer plays an important role in determining the ultimate mechanical properties of composites.

Two methods were developed to improve the interface of HA and /Polyactive™. The first method is to introduce hydrogen bond interaction and/or ionic dipole interaction between the filler and polymer matrix by pretreatment of HA filler particles with polyacrylic acid and ethylene-maleic acid copolymer.

The second method was to introduce covalent bonding between HA and the polymer matrix by using isocyanate group containing coupling agents to graft the same polymers as the matrix to the particle surface. An acrylic resin system was chosen as a model system for the purpose. Also, Polyactive™ 70/30 was grafted to the surface of HA via hexamethylene diisocyanate.

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Chapter 2

Surface Modification of Hydroxyapatite to Introduce Interfacial Bonding With Polyactive™ 70/30 in A Biodegradable Composite

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Abstract

A method was developed to improve the interfacial bonding between hydroxyapatite and a biodegradable copolymer Polyactive™ 70/30. Hydroxyapatite was first surface modified by the polyelectrolytes polyacrylic acid or poly(ethylene-co-maleic acid) in aqueous solutions. Subsequently the surface modified hydroxyapatite was used as filler in composites with Polyactive™ 70/30. The strength, elongation at break and elastic modulus of the composite in aqueous environment were significantly improved by this method. Based on these experimental results, we believe that the interface improvement is due to the hydrogen bonding and/or dipole interactions formed between polyelectrolyte molecules and polyethylene glycol segments in the polymer matrix. Due to the introduction of interfacial bonding by using such method, a new biodegradable bone-bonding composite can be made.

Introduction

In recent years, several kinds of polymer-hydroxyapatite composites have been developed as bone substitute materials [1,2,3]. The purpose of making such composites is to reinforce the polymer and improve the bone bonding properties of the material, since it has been found that adding hydroxyapatite (HA) into a polymer matrix may turn an initially non-bioactive polymer into a bone bonding composite, and might simultaneously improve the mechanical properties [1,2,3], especially the elastic modulus and hardness.

In making HA/polymer composites, the lack of interfacial bonding between HA and the polymer matrix still remains an issue of concern [3,4]. The interfacial bonding between inorganic and organic phase plays an important role in determining the ultimate mechanical properties of the composites. A strong interfacial bonding between the two phases usually is necessary for the composites to achieve better mechanical properties. For example, bone, a natural biocomposite, is mainly composed of inorganic bone mineral (hydroxyapatite-like material), organic matrix of type I collagen and noncollagenous proteins [5]. Bone mineral is not directly bound to collagen, but bound to collagen by these non-collagenous proteins [6]. These interfacial bonding forces are mainly ionic bonds, hydrogen bonds and hydrophobic interactions [5]. They give bone unique composite behaviour.

The polymer, Polyactive^{TM,} used in this study is a block copolymer from polyethylene glycol (PEG) and poly(butylene terephthalate) (PBT). When the weight ratio of PEG/PBT is 55/45 or higher (the molecular weight of PEG is 1000 Dalton), it is a biodegradable polymer and calcifies postoperatively , thereby inducing bone bonding [7,8]. Polyactive^{TM} has been already used in making composites with HA. Such composites showed promising results in guided tissue regeneration applications [9]. However, due to the larger amount of PEG present in the structure of Polyactive^{TM} 70/30, it is a rubber like polymer with low elastic modulus. In an effort to strengthen the polymer, we chose HA as filler to make HA/polymer composites.

Since in contrast to bone mineral and its collagen matrix, there are no strong bonding forces between HA and Polyactive^{TM}, it is necessary to introduce some kind of interaction between the two phases by surface modification of HA Such an approach mimics the role of non-collagenous protein in bone.

In this study, a method was developed to improve the interface between HA and Polyactive^{TM} by using water soluble polyelectrolytes such as polyacrylic acid and poly(ethylene-co-maleic acid). This was based on the principle that polyacrylic acid and the copolymer of maleic acid have the ability to both form complexes with PEG [10], and be firmly adsorbed onto the surface of HA [11,12].

Materials and Methods

Polyacrylic acid (Mw = 5000, 50% water solution) and poly(ethylene-co-maleic anhydride) were obtained from Aldrich. Poly(ethylene-co-maleic acid) was then obtained by dissolving poly(ethylene-co-maleic anhydride) in distilled water (figure 1). Hydroxyapatite was synthesized and sintered in our laboratory. It was milled and sieved to powder with a particle size less than 45 μ . Polyactive^{TM} 70/30 was obtained from HC Implants bv, the Netherlands. The molecular weight was about $1x10^5$.

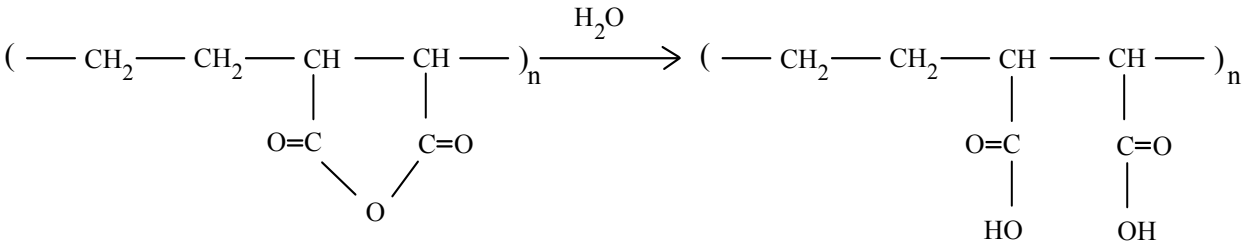


Figure 1 Preparation of EMa

Coating of HA Particles

PAA and EMa solutions were prepared and used for coating purposes. The pH of the PAA and EMa solutions was adjusted to pH 7 by using 10% NaOH solution, and the final concentrations of PAA and EMa were 2.5% and 1.5% respectively.

HA particles were put into the PAA or EMa solution, and the suspension was stirred for 20 hours at room temperature. Then the particles were separated by centrifuging. After the particles were re-suspended and washed for three times in distilled water, the particles were first exposed to 110 ^{0}C overnight and then dried in a vacuum oven at 80 ^{o}C for at least 72 hours. Control HA particles underwent the same procedure but with NaCl solution (pH=7, 1.8 % concentration, the same molar concentration as NaOH in PAA solution.) in stead of PAA or EMa.

Characterization of HA particles

The surface area of HA particles was analysed by using BET methods (Quantachrome Nova 1200 Adsorption Analyzer). N_2 was used as adsorption gas.

The size and size distribution of HA particles were measured both by scanning

electron microscopy (SEM) (Phillips 525) and by Coulter Particle Counter before and after surface modification of the HA particles.

In order to measure the surface property changes of HA particles modification by PAA or EMa, a semi-quantitative sedimentation method was used : 0.5 g particles were put into a test tube of a diameter 1.4 cm containing 10 ml distilled water. After shaking, the time needed for the supernant to become clear was recorded.

The amount of surface adsorbed EMa and PAA was quantitatively measured by using a Total Organic Carbon Analyzer (TOC). An amount of 0.54 grams of coated HA particles was first dissolved in 100 ml hydrochloride acid solution of pH 1, then 10 ml of such solution was used for analysing the carbon content.

Preparation of Composites

Surface modified and control HA particles were premixed with Polyactive^{TM} 70/30 granules at 25% weight percentage and then blended twice at 150 ^{o}C using a single screw extruder (Colin 15 x 25) . The obtained granulated materials were hot pressed into 2 mm thick and 50 X 50 cm^2 sheets at a temperature of 190 ^{o}C and 20 ton pressure . Standard dumb-bell specimens were cut from the sheet by using an ISO R37 type 1 die, and were then used for mechanical and other testing. All the specimens were kept at room temperature for 4 days before mechanical testing was performed.

Mechanical Testing

In order to evaluate the effectiveness of the surface treatment of HA, we determined the tensile strength, elongation at break and the elastic modulus of composites both in dry and wet state (after swelling in distilled water). The wet state testing was carried out after the specimens had been immersed in distilled water for 24 hours. Then the specimens were taken out of the distilled water and kept wet during the testing process. A Hounsfield HN200 testing machine was used. The crosshead speed was 50mm/min., and the gauge length was 25 mm. In order to determine the elastic modulus, an Instron extensometer was used to measure the specimen extension. Ten specimens were used for each testing.

Swelling Degree of the Composites

Rectangular specimens with size 2 x 6 x 20 mm^3 were used for swelling tests in distilled water at room temperature. For each composite , two specimens were used.

At certain time intervals, the specimens were taken out and the water at the surface was quickly removed with tissue paper. The swelling degree at different time intervals was calculated according to following equation

Error!

where S_w stands for swelling degree and W_t is the weight of the sample at time t, W_0 is the weight of sample in dry state at the beginning of testing.

Fracture surface study

The fracture part of the mechanical testing specimens after mechanical testing was cut off from the specimen by using a sharp knife. The fracture surface was first observed by light microscope and then by a Phillips Scanning Electron Microscope. All the samples for SEM observation were sputter coated with gold.

Results

Characterization of HA particles

The surface area of HA particles as measured by BET method was 1.75 m^2/gram.

SEM study showed PAA and EMa modification caused nearly no change in particle size and surface morphology of particles. Particle sizes in scanning electron microscopy were measured between 1-50 microns, the larger particles were of a porous structure (figure 2) .

Figure 2. SEM pictures of HA particles. A. The porous structure of HA particles. B. HA surface modified by PAA.. Note that the typical rhombic crystals of HA can be seen.

The particle size distribution patterns of the HA particles were nearly of the same before and after surface modification by PAA or EMa (figure 3) as measured by Coulter Particle Counter.

The change in surface properties of HA particles after being modified by EMA or PAA can be seen from the sedimentation time changes of HA particles. Surface modification significantly increased the sedimentation time of particles. EMa and PAA have different effects on the sedimentation time of HA particles (table 1). The PAA coating had more distinct effect on the sedimentation time of the particles. More PAA was adsorbed on HA as compared to EMa (table 1).

Figure 3. Particle size distribution of surface modified and unmodified HA particles. (a) HA particles; (b) EMa modified HA particles; (c) PAA modified HA particles.

Table 1. Some of the characteristics of surface modified HA particles

Charicterist ic	Particle		
	HA	EMa-H A	PAA-H A
Sedimentat ion time	50 min.	3 hours	20 hours
Adsorption (mg C/g)	As control	0.57	2.29

Figure 4. Swelling degree of the composites.

Swelling Behaviour of Composites

Figure 4 shows the swelling degree vs. time curve of the composites . After 24 hours of swelling in water at room temperature, the swelling gradually reached equilibrium. It can be seen that the swelling degree of PAA-HA and EMa-HA composites is somewhat lower than that of HA composites.

Tensile Strength

The tensile strengths of the composites, both in dry and wet state, are given in figure 5. In dry state, there are no significant differences between the strengths of HA, EMa-HA and PAA-HA composites. The general level of strengths of composites was lower than that of Polyactive^{TM} 70/30. All the strengths were decreased after the specimens had been immersed in water. In wet state, however, the strength of the composites with surface modified HA particles is higher than that of control HA composites whereas the strength of PAA-HA composite is higher than that of EMa-HA composite and comparable to the strength of pure Polyactive^{TM} 70/30 in wet state.

Figure 5. Tensile strengths of composite.

Elastic Modulus

Figure 6 gives the elastic modulus of the composites. In the dry state, all the composites had much higher elastic moduli than pure polymer. In wet state, although the elastic moduli were decreased for all the materials, all the composites still had higher elastic

moduli as compared to pure Polyactive^{TM}. However, the control HA composite had lower elastic moduli as compared to EMA-HA and PAA-HA composites. EMa-HA and PAA-HA composites had nearly the same elastic modulus in wet state.

Figure 6. Elastic modulus of composites both in dry and wet state.

Elongation at break

Incorporating of filler into the polymer significantly decreased the elongation at break both in dry and wet state. however composites with surface modified HA particles had a higher elongation at break both in dry and in wet state as compared to control HA composites. (figure 7). It also clear that the PAA-HA composite has a higher elongation at break than EMa-HA.. The general level of elongation at break was lower after the specimens had been immersed in water.

Figure 7. Elongation at break of composites both in dry and wet state. The elongation at break of Polyactive^{TM} 70/30 are 432 ± 83 % in dry state and 219 ± 35 % in wet state.

Fracture Surface Study

By using scanning electron microscopy, It could be seen that usually voids existed between the control HA particle and polymer matrix after the samples were broken (figure 8). Sometimes mechanical interlocks were observed between the larger HA particles and polymer matrix due to the infiltration of polymer into the pores of HA. For EMa and PAA surface modified HA particles, a better contact and more mechanical interlocks with polymer matrix were observed.

Figure 8. SEM pictures of fracture surfaces of composites. (a) HA composite; (b) EMa-HA composite; (c) PAA-HA composite. Note the voids between HA particles and polymer matrix in (a) and the better contact still remained in (b) and (c).

Discussion

One of the reasons why Polyactive^{TM} 70/30 is used as bone filler is its bone

bonding ability. The bone bonding ability is closely related to the calcium ion complexing ability of PEG segments [7,8]. Therefore, any strengthening of the polymer should not interfere with the calcium ion complexing ability of the PEG segments. Crosslinking of polymer, which usually is an effective way to strengthen the polymer, is not applicable in this case. Therefore, an ideal way to strengthen the polymer is to make composites by using fibbers or fillers.

However, Due to the high swelling ability of Polyactive^{TM} in water, the interfacial problem may be critical since the composite is intended to be used in vivo. lack of interfacial bonding between HA and polymer matrix may cause loose contact between HA particles and polymer matrix and therefore result in early failure (disintegration) of implants. It can be seen that the mechanical strength, elongation at break and elastic modules of HA composites decreased drastically after being immersed in water for 24 hours. Hence it is necessary to modify the surface of HA to introduce interfacial interaction between HA and polymer matrix and to maintain the necessary strength and the structure integrity of the composites.

Surface modification of HA particles

It is well documented that PAA and EMa can be firmly adsorbed to the surface of HA [11,12]. In this study, the amount of adsorption was not very high (Table 1) due to the relatively larger size particles used in our study. After the surface modification of HA, no obvious surface morphological changes was observed. The surface properties of HA particles were significantly changed after surface modification. One of the drastic changes is their sedimentation rate in distilled water. The surface modified HA particles can suspend very well in water and thus have longer sedimentation times. We also noticed that the sedimentation time for PAA-HA was much longer than that of EMa-HA particles. This difference may come from the lower adsorption amount of EMa on the surface (Table 1).

Mechanical properties of composites

Although the total amount of surface adsorption of PAA or EMa is low due to the small surface area of HA particles in our study, we can see the effect of surface modification on the mechanical properties of composites. The increase in the elongation at break for EMa-HA and PAA-HA composites as compared to HA composites in dry state is the evidence of interfacial improvement. In addition, the introduction of interfacial interaction had a more distinct effect on the mechanical properties of the investigated composites in wet state.

Generally speaking, water can decrease the mechanical properties in two ways. first, water can cause polymer swelling and thereby increase the cross section area and

this decrease the strength and elastic modulus. Second, water can act as plasticizer to decrease the intermolecular chain interaction and thereby decrease the strength and elastic modulus of the polymer. So it is not surprising to find that mechanical properties for all composites decreased after the composites had been immersed in water for 24 hours. But the EMa-HA composite and PAA-HA composite could still maintain relatively higher mechanical properties. In our experiment, in addition to the effect of higher swelling ability of composites in water and the plasticization effect of water molecules, we also consider that the decrease of the mechanical properties was caused by the deterioration of the interface between the inorganic and the organic phases of the composites as we can see from the changes in tensile strength (figure 5). We may see that the swelling degrees of all the composites are about 50%. That means swelling in water will cause about 30% increase in the cross section area of the sample and hence, in principle, 30% decrease in strength (if only consider the effect of swelling or the increase in cross section area). But the decrease in strength was far more than 30% for HA composites and less than 30% for PAA-HA composites. Since the surface modification of the HA particles only change the swelling degree of the composites slightly, we believe that the improvement of the mechanical properties is largely due to the interface improvement of the composites.

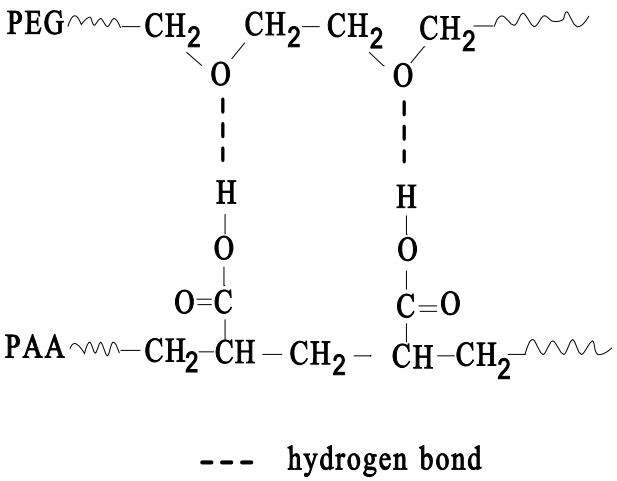
The amount of the adsorption of the EMa and PAA on HA seems to have some effect on the mechanical properties of the resulting composites. Since there was less amount of EMa adsorbed on the surface of HA compared with the adsorption of PAA, so the thinner EMa coating on the HA has less effect on the mechanical properties of the resulting composites.

Fractography of Composites

SEM studies of the fracture surface suggest that there indeed exist mechanical interactions between HA and polymer matrix. Due to the rough surface and porous structure of HA particles, the mechanical interlocks between HA (both surface modified and unmodified) particles and polymer matrix could be observed at the fracture surfaces. But for surface modified HA particles , more such mechanical interlocks could be observed, some of the particles were even largely covered by polymer (Figure 8).

The mechanism of interfacial interactions

The introduction of EMa and PAA onto the surface of HA improved the interface between HA and polymer matrix. It is considered that such improvement is caused by hydrogen bond formation and/or dipole interactions between the surface coated polymer molecular chains and PEG segments of Polyactive^{TM}, Since EMa and PAA may form stable hydrogen bond complexes with PEG both in an aqueous mixed system and in a heated blending system (figure 9) [10,13], the mechanical properties of the composites both in dry and in wet state can be improved. Although the complex is easily formed in aqueous mixed system at lower pH, at a slightly basic condition, complexation may also occur [13], but the interaction may contribute to the dipole interaction similar to ionic bonding . An indication of such complexation between the EMa or PAA and PEG segments is that the swelling degree of EMa-HA and PAA-HA composites is lower than HA composites. Hydrogen bond or ionic complexes formation may cause a slight decrease in swelling degree which is mainly from the hydrophilic properties of PEG segments.



Effect of the coating on the bioactivity of HA

HA has similarity to bone mineral both in structure and composition. It is also considered to be a bioactive material with bone bonding ability. It is thought that the bone bonding process involves a process of HA dissolution followed by repreciptation and formation of calcium phosphate microcrystals [14,15,16]. Therefore the solubility and dissolution rate of HA in a physiological environment are believed to have a close relation to the formation of bone-like apatite mineral both in vitro and in vivo. Although the EMa and PAA on the surface of HA may prevent the dissolution of the HA in some extent, both EMa and PAA are water soluble polymers, and the attachment of such water soluble molecules to the surface of HA may not change the hydrophilic characteristics of HA. Furthermore, it is thought that PAA has the ability to nucleate the formation of HA due to the carboxylic groups it has [17,18]. This may be one of the advantages of using water soluble polymer to modify the surface of HA..

Conclusion

By introducing water soluble polymer, i.e. polyacrylic acid and poly(ethylene-co-maleic acid), onto the surface of HA, we could significantly improve the interface of HA particles with polymer Polyactive^{TM}, thus allowing a better load transfer throughout the material. As a result, the strength, elongation at break and elastic modulus of HA-Polyactive^{TM} 70/30 composites in wet state can be significantly improved. By using 25% PAA-HA filler , a composite with a higher elastic modulus and a tensile strength comparable to the polymer can be made.

Acknowledgement

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Chapter 3

Polyacids as Bonding Agents in Hydroxyapatite/Polyester-ether (Polyactive™ 30/70) Composites

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Abstract

A method had been developed to improve the interface of hydroxyapatite and a polyester-ether (Polyactive™ 70/30) by using polyacrylic acid or poly(ethylene-co-maleic acid) in our previous work. In this paper, we studied the suitability of this method for making HA/Polyactive™ 30/70 composite, since Polyactive™ 30/70 contains less PEG segments and a higher concentration of rigid PBT segments. The mobility of the PEG segments is largely affected by the existence of such rigid and high concentration of PBT segments. Our experimental results show that this method is indeed suitable for making HA/Polyactive™ 30/70 composites. The hydrogen bond/dipole interaction formation ability of PEG segment is not affected by the existence of relatively large amount of PBT segment. By using these coupling agents, the mechanical properties of composite can be significantly improved both in dry and wet state. The fractographical study of fracture surfaces revealed that the surface modified HA particles maintain better contact at fracture. It also showed that larger HA particles may initiate cracks and that such particles may be responsible for decrease in tensile strength of composites.

Introduction

Several kinds of polymer-hydroxyapatite (HA) composites have been developed as bone substitute materials [1,2,3]. The purposes of using HA as a filler in composites are to reinforce the polymer, especially to increase the stiffness, and to improve the materials' bone bonding properties which is essential for achieving early bone ingrowth and fixation of the implants by bone tissue. It has been found that adding a certain amount of hydroxyapatite (HA) to the polymer matrix may turn a non-bioactive polymer into a bone-bonding composite [1,2,3]. However, the lack of interfacial bonding between HA and polymer matrix hinders the synthesis of composites with satisfactory mechanical properties [3,4].

Polyactive™ is a copolymer from polyethylene glycol (PEG) and poly(butylene terephthalate)(PBT) with good biocompatibility. By changing the weight ratio of PEG/PBT, a series of copolymers with different structure and properties can be obtained. It has been found that when the weight ratio of PEG/PBT is higher than 55/45, Polyactive™ has bone bonding abilities and is biodegradable [5,6,7,8]. However, when the ratio of PEG is higher then the polymer has poor mechanical properties and can not be used as a load bearing biomaterial. When the weight ratio of PEG is lower, e.g. 30%, the material has much higher stiffness and strength but has no bone-bonding ability. A logical way to get a stronger material with bone bonding ability is thus to use HA as filler to reinforce Polyactive™ with 30 % PEG and 70 % PBT (Polyactive™ 30/70).

In earlier studies we have developed a new and specific method to introduce interfacial bonding between HA and Polyactive™ 70/30 by using polyelectrolytes such as polyacrylic acid (PAA) and poly(ethylene-co-maleic acid) (EMa) [9]. The principle is that both PAA and EMa can be firmly adsorbed onto the surface of HA [10,11,12,13], and also can form hydrogen bond or dipole complexes with PEG [14]. In this way the HA particles will adhere better to the polymer matrix, so that the resulting composite material will possess sufficient strength [9].

Our first studies on Polyactive™ 70/30 showed promising results, and more research had to be performed on the application of this method in PEG/PBT polymers with a proportion of 30/70. It is known that the molecular weight of both PEG and PAA plays an important role in forming intermolecular complexes [15]. Polyactive™ 30/70 has less PEG segments and a higher PBT content in its structure The varation in PEG/PBT ratio will affect the domain size and the aggregation structure of the coploymer. Therefore, the hydrogen bond formation ability of PEG probably will be affected by the existence of more and longer rigid PBT segments [16], making it necessary to study the suitability of this surface modification method of HA for

HA/Polyactive^{TM} 30/70 composites.

Materials and Methods

Polyacrylic acid (Mw = 5000, 50% water solution) and poly(ethylene-co-maleic anhydride) were obtained from Aldrich. Poly(ethylene-co-maleic acid) was prepared by dissolving poly(ethylene-co-maleic anhydride) in distilled water. Hydroxyapatite was synthesized and sintered in our laboratory. It was milled and sieved to powder with particle size range from 1-45 μ . Polyactive^{TM} 70/30 was obtained from HC Implants bv, the Netherlands. The molecular weight is about $1x10^5$ Dalton.

Coating of HA Particles

Aqueous PAA and EMa solutions were prepared and used for coating the HA powder. The pH of the PAA and EMa solutions were adjusted to pH 7 by using 10% NaOH solution, and the final concentrations of PAA and EMa were 2.5% and 1.5% respectively. HA was sintered, ground and sieved to a fraction with particle size 1-45 μ (figure 1).

HA particles (200 gram) were put into 200 ml PAA or EMa solution. The suspensions were stirred for 20 hours at room temperature and then the particles were separated from the solution by centrifuging. After the particles were re-suspended in distilled water and washed three times, the particles were first dried at 110 ^{0}C overnight and then in a vacuum oven at 80 ^{o}C for at least 72 hours. Control HA particles underwent the same procedure but with NaCl solution instead of EMA or PAA solution.

Characterization of HA particles

HA particles were characterized by specific surface measurement (BET) method, scanning electron microscopy (SEM) and Coulter Particle Counter before and after surface modification by PAA and EMa, as described elsewhere [9].

The amount of surface adsorbed EMa and PAA was quantitatively measured by using a Total Organic Carbon analyser (TOC, D.C-190, T.O.C. Analyser). An amount of 0.54 grams of coated HA particles was first dissolved in 100 ml hydrochloride acid solution of pH 1, then 10 ml of such solution was used for analysing the carbon content in TOC.

In order to measure the effect of PAA or EMa modification on the surface property changes of HA particles, a semi-quantitative sedimentation method was used : 0.5 g particles were transfered to a test tube of a diameter 1.4 cm containing 10 ml distilled water. After shaking, the time needed for the supernant to become clear was recorded.

Preparation of Composites

Surface modified and control HA particles were premixed with Polyactive^{TM} 30/70 granules at 25% and 50% weight percentage and then blended twice at 200 ^{o}C using a single screw extruder (Codlin, 20x25) . The obtained granulated materials were hot pressed into 2 mm thick and 50 X 50 cm^2 sheets at a temperature of 230 ^{o}C and 20 ton pressure . Standard dumb-bell specimens were cut from the sheet by using an ISO R37 type 1 die, and were then used for mechanical and other testing. All the specimens were kept at room temperature for 4 days before mechanical testing was performed.

Mechanical Testing

In order to evaluate the effectiveness of the surface treatment, we determined the tensile strength, elongation at break and the elastic modulus of composites in both dry and wet state (after swelling in distilled water). The wet state testing was carried out after the specimens had been immersed in distilled water for 48 hours. Then the specimens were taken out of the distilled water and kept wet during the testing process. Tensile tests were performed in a Hounsfield HN200 testing machine. The crosshead speed was 50mm/min., and the gauge length was 25mm. In order to determine the elastic modulus, an strain gauge extensometer (Instron) was used to measure the specimen extension. Ten specimens were used for each test. The area under the stress-strain curve (AUC) was also calculated to estimate and compare the fracture fracture energy of the 50% filler composites.

Swelling Degree of the Composites

Rectangular specimens with size 2 x 10 x 20 mm^3 were used for swelling tests in distilled water at room temperature. For each composition, the swelling test was performed with two specimens.

At certain time intervals, the specimens were taken out from the distilled water, and the water at the surface was quickly removed with tissue paper. The swelling degree at different time intervals was calculated according to following equation

Error!

where Sw stands for swelling degree and W_t is the weight of the sample at time t, W_0 is the weight of sample in dry state at the beginning of testing.

Fracture surface study

The fracture surfaces of mechanical testing specimens were observed by light microscopy and Scanning Electron Microscopy (Philips 525). All the samples were sputter coated with gold.

Results

Characteristics of Particles

Table 1 summarizes the characteristics of HA particles before and after surface modification by PAA and EMa.

Table 1. The characteristics of particles

	Size (microns) by SEM	surface area (m^2/g)	sedimentation time	amount of coating (mgC/g)
HA	1-50	1.75	50 minutes	as control
EMa-HA	1-50	n.d	3 hours	0.57
PAA-HA	1-50	n.d	20 hours	2.29

Note: n.d = not determined

Figure 1. SEM pictures of HA particles used in this study. Note the porous structure of the large HA particles. The sizes of the HA particles were measured to be 1 -50 microns.

Figure 1 gives a SEM micrograph of the hydroxyapatite particles used in this study . The SEM study showed that the large HA particles had a porous structure, and the HA particle sizes were in between 1 and 50 microns. There was almost no change in the particle size and the surface morphology after surface modification by EMa and PAA as reported previously (see chapter 2).

Swelling degree of the composites

Figure 2 gives the swelling degree vs. time curves of the composites. Swelling gradually reached equilibrium after 48 hours of immersion in distilled water for all the composites. The swelling degrees of 25% EMa and 25% PAA-composites are somewhat lower than that of HA composites with the same filler content but the three types of 50% composites showed nearly the same swelling degrees.

Figure 2. Swelling degree of composites as a function of time. Note the swelling degrees of all the composites nearly reached equilibram after 48 hours immersion in distilled water.

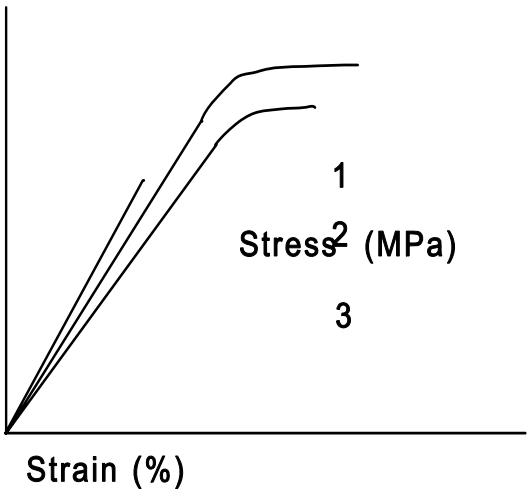


Figure 3. An illustration of the typical stress-strain curve of 50% filler composites. 1. 50%HA composites in dry state; 2. 50% PAA-HA composites and 3. EMa-HA composites.

Mechanical Properties

Figure 3 is an illustration of a typical stress - strain curve of 50% w/w composites in dry state. The differences among the mechanical properties of the composites can be clearly seen from the figure.

Elastic moduli

Increase of filler content increases the elastic modulus of the composites in dry state. Water causes a distinct decrease in elastic modulus (Figure 4). However, the EMa-HA and PAA-HA composites maintained higher elastic moduli after being immersed in distilled water. There was no significant difference between the elastic moduli of EMa and PAA-HA composites both in dry and in wet state.

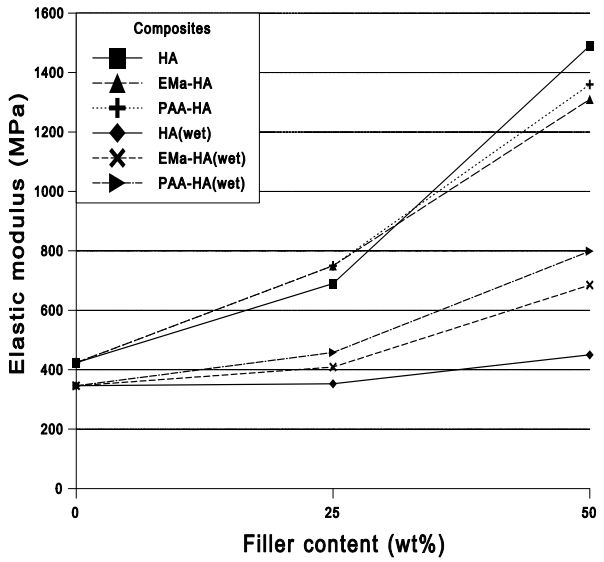


Figure 4. Elastic moduli of composites both in dry and wet state (distilled water). The effect of the coating can be clearly see from the E-moduli of the composites in wet state.

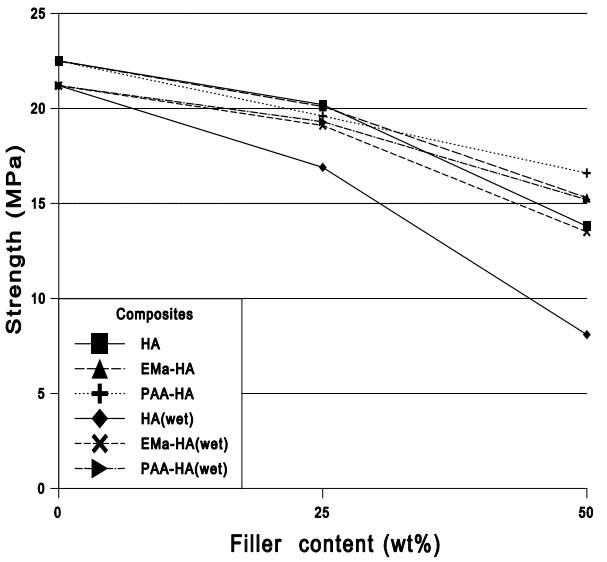


Figure 5. Tensile strength of composites both in dry and wet state (in distilled water). The Tensile strength of HA composites was significantly decreased by immersion in water, while the composites with coating maintained higher strength.

Tensile Strength

The tensile strengths of composites are shown in figure 5. It can be seen that the

strengths of all the composites in dry state are slightly decreased when adding 25% filler. A larger filler amount causes a further decrease in strength. Water also has a negative effect on the strength of the composites. However , the wet strengths of composites with surface modified HA particles remained significantly higher than those with unmodified filler.

Elongation at break

It can be seen that the elongation at break of composites decreases with an increase of filler amount (figure 6). Water causes an increase of elongation at break. Both composites with surface modified HA particles have higher elongation at break than unmodified HA composite. PAA modified composites gave the highest value.

Relative fracture energy of the composites

The difference in AUC of the composites is obvious (Figure 7). The composites with PAA surface modified HA particles have a much higher fracture energy than composites with unmodified HA particles both in dry and in wet state.

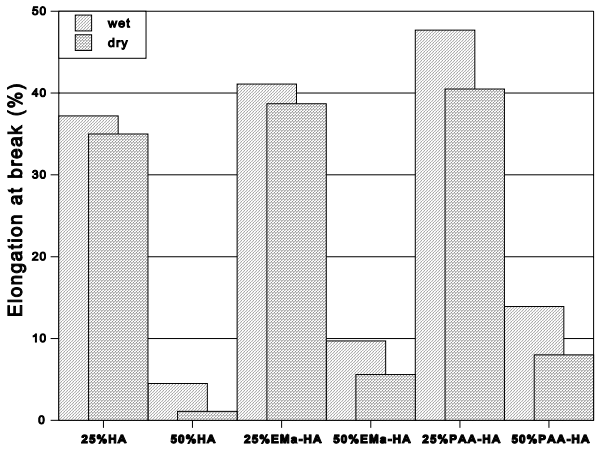


Figure 6. Elongation at break of the composite both in dry and wet state (distilled water). The elongation at break for unfilled Polyactive^{TM} 30/70 are 390 ± 85 (dry) and 187 ± 91(wet) respectively.

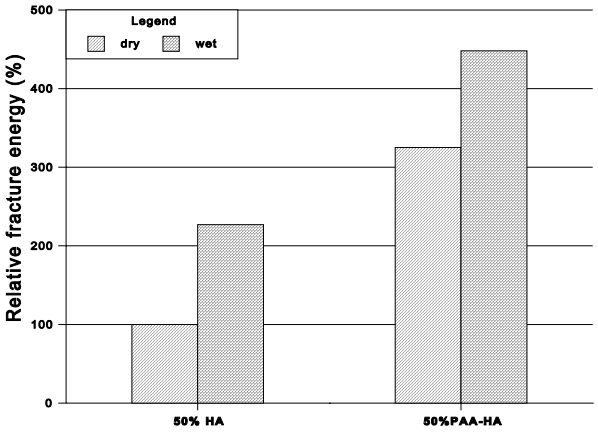
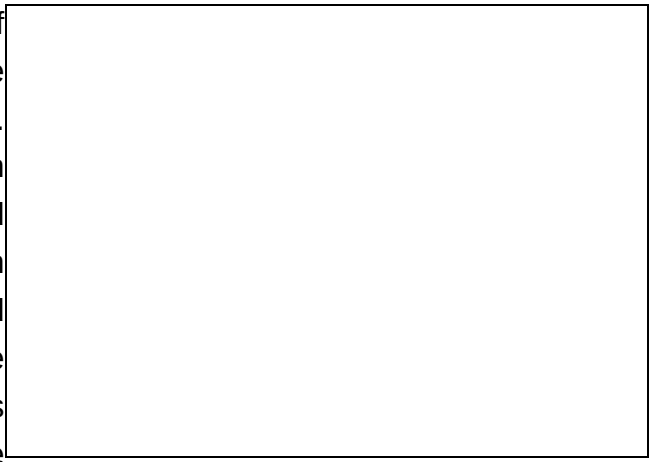


Figure 7. The relative fracture energy of the 50% filler composites both in dry and in wet state. The fracture energy of the 50% HA composites in dry state was considered as 100%.

Fractography study by SEM



The fracture surface study of composites shows that the fracture surface can be roughly classified into two zones. They are referred to as rough zone (with high deformation) (figure 8 a, b, c) and smooth zone (figure 8 d, e, f) . Based on the observation that the air bubbles could always be found in the rough zone of the fracture surface in the unperfect specimens (Figure 9), it was concluded that the fracture was initiated in the rough zone and that the crack propagated through the smooth zone. Within the high deformation zone, polymer threads are observed. The HA particles which can be seen in this zone all have larger sizes. Within the smooth zone, there are no polymer threads present and all sizes of HA particles can be found. The composites with surface modified HA particles and with unmodified HA particles show differences in the appearance of the high deformation zone. Larger areas of these rough zones can be observed in the fracture surfaces of EMa-HA and PAA-HA composites. In the smooth lower deformation zone, the surface modified HA particles show a more intimate contact with the polymer matrix than in the case of unmodified filler particles (figure 8 d, e, f).

Figure 8. SEM pictures of the fracture surface of composites. (a) and (d) are the rough zone and smooth zone of 50% HA composite. (b) and (e) are the rough zone and smooth zone of 50% EMa-HA composite. (c) and (f) are the rough zone and smooth zone of PAA-HA composite. Note the obvious differences in smooth areas. Loosely embedded HA particles can be observed in (d), while in (e) and (f), particles still maintain more intimate contact with polymer matrix. In rough zones, relatively long polymer threads can be observed in (b) and (c).

Discussion

Using a filler can be an effective way to improve stiffness of the composites. In this study, it was shown that the elastic moduli of composites were increased both in dry and in wet state. The composites with unmodified HA particles, however, became brittle when the filler amount was 50%, and the strength of the composite decreased drastically at this high amount of filler. This may be attributed to the lack of interaction between the HA particles and Polyactive™ matrix, indicating the necessity of introducing some kind of interaction between the inorganic filler and the organic matrix.

Polycarboxylic acids have been reported to be readily absorbed to HA surfaces [10,12,13,14]. PAA and EMa can form a hydrogen bond complex with PEG not only in aqueous mixtures, but in heat blended systems as well [15]. We have shown that PAA and EMa most probably form complexes with PEG segments in Polyactive™ 70/30 [9]. For Polyactive™ 70/30, the concentration of PEG segments is high and therefore PEG segments have more chance and mobility to form complexes with PAA or EMa. However, when increasing the amount of PBT and keeping a constant PEG segment length, the glass transition temperature of PEG segment will increase and the melting enthalpy of PEG will decrease until no PEG crystals can be detected as has been reported by Fakirov et al [16]. It can be explained by the influence of a higher concentration and longer hard segments of PBT [16]. The increase of T_g and decreased crystalinity of PEG phase means that the mobility of PEG segments is restricted by the high concentration of longer PBT blocks .

In spite of the the mobility of PEG segments being restricted , we can still observe the effect of surface modification of HA particles. For 25% filler composites, such an effect is evident from the higher elongation at break of EMa-HA and PAA-HA composites both in dry and wet state (figure 3, 6). For 50% filler composites, the effect of surface modification can be deduced from the higher tensile strengths, elongation at break, elastic moduli and relative fracture energy as compared to unmodified HA composites both in dry state and in wet state. These results suggest that the surface modification of HA with PAA and EMa improves the interface of HA with Polyactive™ 30/70 in spite of the low amount of PEG present. The interfacial improvement leads to a better load transfer throughout the material, and result in a better mechanical properties and higher fracture energy.

Swelling in water caused a significant decrease in the mechanical properties of the composites. Two factors are considered to cause: the absorption of water by polymer matrix and by HA filler. As can be seen from figure 10, the equilibrium swelling degree of the composites was higher than the expected values if it is considered that the filler did not absorb water. It is quite clear that the filler does contribute to the water

absorption of the composites. The absorbed water by filler was most likely present on the surface of the filler or in the pores of the filler particles. The presence of water on the surface of filler will certainly affect the adhesion of filler to the polymer matrix.

From the fracture surface study of composites we conclude that the crack initiates at rough zones and propagates through the smooth zones (figure 8, 9). In the rough zone , only the larger

Figure 10. The equilibrium swelling degree of the composites. Note the difference between the measured values and the expected values based on the assumption that the filler did not take up water.

particles can be seen. This suggests that the larger particles may have initiated cracks at their interface with polymer matrix, probably because the larger particles had a porous structure. Air trapped within the pores resulted in incomplete filling of the pores of the particles by polymer and in formation of less intact interface. In the smooth zone where the crack propagates through, the surface modified HA particles maintain a better contact with the polymer matrix (Figure 8, e and f) proving the existence of bonding between them. The occurrence of voids between unmodified HA and the polymer matrix after the sample had been broken shows the lack of interaction between the particles and polymer matrix.

The fractographical study also reveals that the weak point of the composite is still the interface of HA and the polymer matrix. The observation of relatively large amounts of HA particles on the fracture surface of the composite indicates that the fracture frequently occurred at the interface of HA with polymer. Further optimizing the surface modification process, such as to modify the surface of filler to render it more hydrophobic or to realize chemical linkage between filler and polymer matrix is thus necessary.

Conclusions

Surface modification of HA particles by using polyacrylic acid and poly(ethylene-co-maleic acid) was demonstrated to be an effective way to improve the interface of HA with Polyactive™ 30/70 as it was the case with Polyactive™ 70/30. It has been shown that the hydrogen bond formation ability of PEG is not affected by the existence of relatively larger amount of PBT segment. Composites with surface modified HA particles maintain better mechanical properties when they are in aqueous environment and at high filler content (50%). Our results also suggest that smaller HA particles with relatively smooth surface will be a better filler for the synthesis of HA/Polyactive™ composites.

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Chapter 4

Intermolecular Complexation Between PEG/PBT Block copolymer and Polyelectrolytes Polyacrylic Acid and Maleic Acid Copolymer

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ABSTRACT

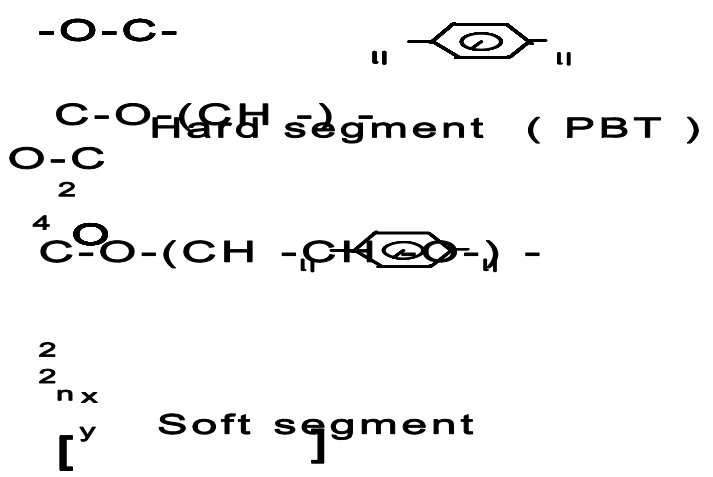
The intermolecular complexation (IPC) between PEG/PBT copolymer and polyelectrolytes such as polyacrylic acid (PAA) and ethylene-co-maleic acid (EMa) copolymer was studied by means of DSC, TGA and IR. The study results indicated that the PEG segment which had a molecular weight of 1000 D still had the ability to form IPC with PAA or EMa at lower pH due to the presence of PBT segment. The presence of PBT segment stabilized the IPC through the hydrophobic or dipole interaction between PBT and PAA or EMa. At slightly basic pH, interaction between PEG and PAA or EMa was also observed, but the interaction was likely attributed to dipole interaction. These findings indicated that it is possible to modify the bulk properties of PEG based block copolymers by forming IPC with polyacid.

Introduction

The study of the interactions between macromolecules is important both for understanding the structures and functions of biological systems and for developing new materials. Due to the difficulties in studying the complexation formation of natural macromolecules, synthetic polymers are often used as model system as well for the study.

In synthetic polymers, it is well known that polyethylene glycol (PEG) is capable of forming complexes with various compounds, with macromolecules such as polyacrylic acid and maleic acid copolymers as well as with low molecules such as urea [1,2].

The formation of inter polymer complexes (IPC) between PEG and polyacid is based on hydrogen bonding [1,2]. A large number of investigations dealing with IPCs formed in aqueous solution and usually containing 1:1 ratios of interacting units have been reported [3,4,5,6]. It has been observed that for the formation of an IPC between PEG and PAA, the molecular weight of the PEG has to exceed a value of 6000 [7]. It was also reported that hydrophobic interactions between the two polymers play an important role in the stability of the complexes. Thus , complexes with poly(methacrylic acid) (PMAA) were formed with PEG of molecular weight exceeding 2000, as compared with 6000 for PAA [7]. Introducing hydrophobic groups in the PEG molecule has the similar effect: stable complexes of PAA or PMAA could be obtained with low molecular weight PEG if they were substituted by hydrophobic groups [6, 8, 9, 10, 11].



For the formation of IPC with PEG, one interesting questions is wether it is possible to decrease PEG molecular weight further by increase the chain length of hydrophobic group? If it is possible, then it will offer a possibility to modify the properties of many PEG block copolymers simply by forming IPC with polyacrylic acid, since it was shown that the bulk physical properties of PEG itself can be significantly improved by forming IPC with polyacrylic acid [12]. To answer the question, a PEG/PBT block copolymer was chosen both for its structural suitability (figure 1) and its possible applications.

This poly(ether ester) block copolymer is a representative of thermoplastic elastomers

(polyurethanes, polystyrene-block-polybutadienes and other block copolymers). It combines the good physical characteristics of chemically cured elastomers with the easy processing ability of thermoplastics. The first commercialized PEG/PBT block copolymer elastomer was introduced by DuPont under the trade name of Hytrel™. Other similar products are Arnitel™ from Akzo Plastics and Pelprene™ from Toyobo. Recently, it was introduced as a bone replacement material (trade name Polyactive™) because of the bone bonding ability it has [13,14]. In an effort to make a new bone bonding composite from Polyactive™ and hydroxyapatite (HA), we found that by surface treatment of HA with polyacrylic acid or ethylene maleic acid copolymer (EMa), the interface between the HA and polymer Polyactive™ matrix was significantly improved [15, 16]. Therefore we speculate that such improvement is due to the inter polymer complexes formation between the HA surface adsorbed PAA or EMa with PEG segments of Polyactive™. Since the molecular weight of PEG segment within Polyactive™ is only 1000, it was unknown wether such IPC can be formed and what kind of interaction exists between them. Therefore in this paper we studied the inter polymer complexation formation ability of PAA or EMa with PEG segments of Polyactive™.

Experimental Procedures

Materials

Polyactive™ 70/30 (the figures indicate the weight ratio of PEG/PBT) was obtained from HC Implant bv, The Netherlands. PEG segment has a molecular weight 1000, and the molecular weight of Polyactive™ is about 100,000. Polyacrylic acid (Mw =5000) 50% water solution and poly(ethylene-co-maleic anhydride) were purchased from Aldrich. Poly(ethylene-co-maleic acid) was obtained by dissolving poly(ethylene-co-maleic anhydride) in water. Both PAA and EMa were used in 2.5% (w/w) water solution.. The pH of PAA and EMa solution were adjusted by either using hydrochloride acid (1 M) or sodium hydroxide (5 % w/w).

Preparation of samples

10% Polyactive™ 70/30 chloroform solution was used to make solvent casting film. After fully evaporating chloroform in vacuum at room temperature, the films (0.1 mm in thickness) was put into either PAA solution or EMa solution. the pH of the solution used in the experiment were 3.6, 6.9 and 7.4. After one hour swelling in PAA or EMa solution , the films were taken out and shortly rinsed with distilled water and then dried in vacuum oven. Dried films were used for DSC, TGA and IR spectrophotometer measurement. A Polyactive™ sample which was swelled in distilled water and subsequently dried was used as control in all the experiments.

Instrumentation

DSC measurements were carried out in a Du Pont 910 series. About 10-15 mg sample was put into a sample pan and put into the sample chamber. After the sample was cooled down from room temperature to -90 ^{o}C by using liquid nitrogen, DSC curves were recorded from -80 to 350 ^{o}C in a temperature increase rate of 10 ^{o}C/min..

TGA was used to study the decomposition behaviour of the polymers. It was performed from room temperature to 350 ^{o}C at a increasing rate of 10 ^{o}C/min. 10-15 mg samples were used in each measurement.

A Perkin Elmer 783 IR spectrophotometer was used. Transmission was recorded from 4000 to 200 wavenumber.

Results and Discussion

DSC analysis

Figure 2 a is a typical DSC curve of PEG/PBT block copolymer. Two glass transition temperatures and two melting peaks can be seen from the curve. A glass transition temperature of PEG segments can be observed at -50 ^{o}C and followed immediately by a crystallization peak and a melting peak of PEG from -20 to 30 ^{o}C. The observed another glass transition temperature at 56 ^{o}C may be ascribed to the amorphous PBT phase in the polymer [17,18]. A crystallization peak of PBT was observed after T_g^{PBT} and followed by the melting peak of PBT, which can be observed around 155 ^{o}C. The shape of this broad endotherm is due to the crystallites which are different in dimensions and regularity depending on hard-segment length [17]. From the DSC curve it is known that at room temperature the polymer consists of 2 amorphous phases, i.e. one PEG, one PBT phases, and one crystalline phase of PBT . As for the length of PBT segments, x should be larger than 3, because only when the length of PBT exceeds a certain value, PBT can form crystals [16].

After the films were immersed in PAA solution and subsequently dried. It can be seen from the DSC curve that the glass transition temperature of the PEG segments was still at about -50 ^{o}C (figure 2 and table 1). The melting of crystalline PEG also took place in the range of -20 to 30 ^{o}C. Prominent changes were observed in the melting enthalpy of PEG crystals (table 1) and in T_g^{PBT} , which was shifted to 61-63 ^{o}C in all PAA treated samples despite the treatment at different pH. The changes in T_g^{PBT} indicate certain kinds of interaction took place between PAA and PBT segments. The change in the shape of the crystallization peak of PBT after the treatment indicates that the mobility of PBT segment was hindered by the presence of PAA due to the interaction, therefore that the melting peak of PBT was diminished in PAA treated samples. Another prominent change was that a broad endothermal peak from 40 - 140 ^{o}C only appeared in PAA treated samples at pH 3.6.

Figure 2. DSC curves of PAA treated samples at different pH. (a) control sample; (b) treated at pH 7.4; (c) treated at pH 6.9; (d) treated at pH 3.6.

Table 1. T_g and melting enthalpy ΔH^{PEG} of PAA treated samples at different pH

pH	T_g^{PEG} (^oC)	ΔH^{PEG} (J/g)	T_g^{PBT} (^oC)
3.6	-50	5.48	61
6.9	-50	19.13	63
7.4	-50	17.23	62
Control	-50	21.48	56

The decrease in the crystallinity of PEG (table 1) indicated that the mobility of some PEG segments were inhibited by the presence of PAA. It can also be seen that the decrease of the crystallinity of PEG strongly depends on the pH value at which the samples were treated. At lower pH, i.e., at pH 3.6, $\_H_m^{PEG}$ of PEG was 5.48 J/g, while at pH of 6.9, a higher crystallinity of PEG was observed ($\_H_m^{PEG}$ = 19.13 J/g). However, when the pH was further increased to 7.4, the crystallinity was slight decreased again ($\_H_m^{PEG}$ = 17.23 J/g). These results strongly suggest that the interaction of PAA with PEG depends on the pH. The strongest interaction observed at lower pH (pH3.6) is probably due to the hydrogen bond formation, since the hydrogen bond IPC can only be formed at pH lower than 4 [1,2].

The observed broad endotherm peak from 40 -140 ^oC in samples treated at pH 3.6 is probably due to the disassociation of PAA with PEG, since such hydrogen bond complexes tend to dissociate at elevated temperature [19]. Therefore, it can be concluded that there are interactions between PAA and PEG segments, and that the interaction strongly depends on the pH. The strongest interaction observed at pH 3.6 is most likely due to the hydrogen bond complexes formation between PAA and PEG segments. At elevated pH, hydrogen bond complexes can be easily break up due to the ionization of PAA. It has been shown that only a small percentage (< 10%) of ionization of the carboxylic groups of PAA was sufficient to break up the complex [20] However, the interaction between PAA and PEG still exists, but to a less extent. Such interaction might be ascribed to dipole or hydrophobic interaction between the PAA and PEG segments [2]. However, the observed T_g^{PEG} for all the PAA treated samples indicates that not all the PEG segments were involved in the interaction with PAA, perhaps this is due to the slow diffusion rate of PAA through the already formed PAA-PEG complexes at the out surface of the sample.

The observed PBT melting peak in all PAA treated samples indicated that the crystallinity of PBT was not affected by the treatment of PAA. That the observed T_g^{PBT} was shifted to a higher temperature indicated that interactions also exist between PBT segments and PAA. Since the shifting of T_g^{PBT} was independent of pH, thus the interaction between PAA and PBT is likely to be hydrophobic interaction.

Figure 4. DSC curves of EMa treated samples at different pH. (a) control sample; (b) treated at pH 7.4; (c) treated at pH 6.9; (d) treated at 3.6.

Table 2. Transition temperatures of EMa treated samples at different pH

pH	T_g^{PEG} (^oC)	$\_H_m^{PEG}$ (J/g)	T_g^{PBT} (^oC)
3.6	-51	14.72	59
6.9	-50	21.13	?
7.4	-50	17.76	58
Control	-50	21.48	56

For EMa treated samples, the same T_g^{PEG} and PEG crystallization peak appeared at about -50 ^oC and -30 to -20 ^oC respectively. The crystallinity of the PEG in EMa treated samples were also decreased by the treatment both at pH 3.6 and 7.4 but to a less extent as compared to PAA treated samples. There was almost no changes in the PEG crystallinity in the sample treated at pH 6.9. The dependence of the crystallinity of PEG on the pH of the treatment also suggested

hydrogen bond complex formation between EMa and PEG segments at lower pH.

In EMa treated samples, a slight increase in T_g^{PBT} was observed both at pH 3.6 and 7.4 (table 2 and figure 4), while at pH 6.9, due to the existence of a small endothermal peak, T_g^{PBT} was hardly detectable. The diminishing of crystallization peak of PBT in EMa treated samples means that the mobility of PBT segment was affected by the treatment. However, the interaction between EMA and PBT also depends on the pH. Increase of the pH gradually decreases both the crystallization peak and the melting peak of PBT until it is totally diminished at pH 7.4. This result suggests a different interaction mechanism between EMa and PBT segments other than a hydrophobic interaction. A dipole interaction between EMa and PBT segments probably took place in the process.

TGA analysis

The TGA analysis curves (figure 5, 6) clearly show the effect of treatment at different pH.

Figure 5. TGA curves of PAA treated samples at differnet pH.

Figure 6. TGA curves of EMa treated samples at different pH

Figure 8 gives the values of the changes in decomposition temperature relative to that of Polyactive^{TM} 70/30. It can be seen that at pH 3.6, PAA treated film has the highest decomposition temperature of 320 ^{o}C. At slightly basic pH, i.e. pH 7.4, EMa treated samples also have a relatively higher decomposition temperature of 260 ^{o}C, while at near neutral pH, the changes in decomposition temperature were very small. These data suggest that both at acidic and slightly basic pH, there are stronger interactions between Polyactive^{TM} and PAA or EMa. At nearly neutral pH, there is only little interaction between the polymer and PAA or EMa These TGA data suggested same interaction pattern as that suggested by DSC data.

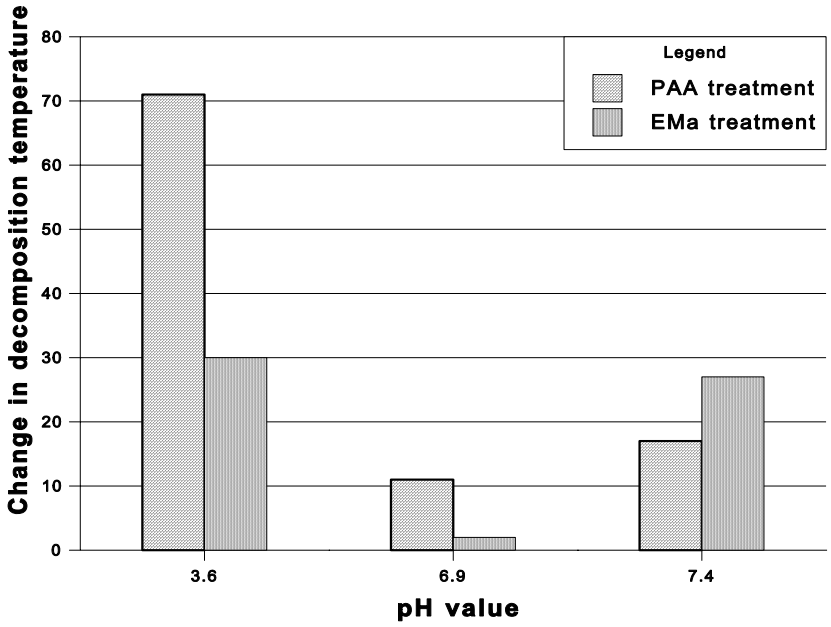


Figure 7. The changes in the decomposition temperature of Polyactive^{TM} when treated at different pH. The decomposition temperature of untreated Polyactive^{TM} is 249 ^{o}C**.**

IR spectra study

The IR spectra (figure 9, 10) indicated a strong and broad absorption band from 3750-2200 cm-1from the samples treated at acidic pH 3.6. Such a broad peak was due to the overlap of bands from hydrogen bonding and CH_2 vibration. The existence of hydrogen bond can be attributed to the hydrogen bond between PAA (or EMa) and PEG segment of Polyactive^{TM} 70/30. No such band could be observed in the other samples treated at near neutral or slightly

basic pH.

Above results clearly showed that IPC can formed between Polyactive™ 70/30 and PAA (or EMa) at lower pH. Table 3 summarized the type of interactions between Polyactive™ and polyacid.

Figure 8. IR spectra of PAA treated samples at different pH. (a) treated at pH 3.6; (b) treated at pH 6.9; (c) treated at pH 7.4; (d) control sample. Note a broad peak in spectrum (a), which indicates the existence of hydrogen bonding in the sample.

Figure 9. IR spectra of EMa treated samples at different pH. (a) treated at pH 3.6; (b) treated at pH 7.4; (c) control untreated sample. Note there is also a broad peak in spectrum a, which indicates hydrogen bonding in the sample treated at pH 3.6.

Table 3. The type and intensity of the interactions between Polyactive™ and polyacid

	pH 3.6		pH6.9		pH 7.4	
	PAA	EMa	PAA	EMa	PAA	EMa
PEG segments	H-bonding	H-bonding	weak dipole	weak dipole	dipole	dipole

PBT segments	hydrophobic	hydrophobic	hydrophobic	hydrophobic + dipole (?)	hydrophobic	hydrophobic + dipole (?)
Polyactive	++++	+++	+/-	+/-	+	++

Note. ++++ = very strong; +++ = stronger; ++ = strong; + = medium; +/- = weak

Conclusion

Combining the DSC, TGA and IR results, we conclude that at pH 3.6, the PAA can form hydrogen bond complexes with PEG 1000 segments in a PEG/PBT block copolymer due to the stabilization effect of hydrophobic interaction between PAA and PBT segments. A dipole interaction between PAA and PEG segments was also observed at elevated pH. EMa copolymer also can form IPC through hydrogen bonding with PEG segments in PEG/PBT block copolymer, but the interaction between EMa and PBT might attribute to dipole interaction rather than hydrophobic interaction. A further study is needed to elucidate the interaction mechanism .

The experimental results also showed the possibility to modify the properties of such block copolymers by forming IPC.

References

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Chapter 5

Nano-apatite/Polymer Composites: Mechanical and Physicochemical Characteristics

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Abstract

Hydrothermally synthesized acicular nano-apatite (Nap) was used as filler to make composites with a polyethylene glycol/poly(butylene terephthalate) (PEG/PBT) block copolymer (Polyactive™ 70/30). The nano-apatite had a particle diameter of 9-25 nm and a length of 80-200 nm. The mechanical properties , the physicochemical characteristics of the composites, such as Young's modulus, swelling degree in water, and the calcification behaviour, have been determined. It was found that nano-apatite had a strong ability to promote the calcification of composites when incorporated into Polyactive™ 70/30, while polyacrylic acid (PAA) coating of Nap had an adverse effect on the calcification of composites, presumably due to the formation of complexes between PAA and PEG segments. Nano-apatite had a prominent stiffening effect for Polyactive™ 70/30 in dry state but had a poor stiffening effect for composites in an aqueous environment due to the hygroscopic nature and/or the formation of aggregates. PAA coating on Nap almost has no additional effect on the mechanical properties of composites both in dry state or in an aqueous environment. To reinforce the polymer by Nap, achieving more homogeneous dispersion of Nap in the polymer matrix and surface modifications to render the powders less hygroscopic appears to be necessary.

Introduction

Composites, due to the possibility of combining the advantages of different materials, have attracted much attention from material scientists. Because of its biocompatibility and bone bonding ability [1-5], hydroxyapatite (HA) has been used as a bone substitute material as such, but also as a filler in composites with organic polymers. In these cases, synthetic HA is usually used in the form of polygonal sintered coarse particles with polycrystaline structure, which have little similarity to natural bone mineral as far as crystal size and shape are concerned. Some researchers have suggested that better osteoconductivity would be achieved if HA had more similarity to bone mineral in composition, crystal structure, crystallinity, crystal size and morphology [6, 7, 8]. Hydrothermally synthesized nano-apatite is a kind of carbonated apatite which has an acicular or needle-like shape [9]. It has much more similarity to natural bone mineral in the mentioned compositional and morphological aspects and therefore better osteoconductivity is expected. In addition to its similarity to bone mineral, the nano-apatite (Nap) may possess other special properties due to its submicron size and consequently huge specific surface area. Since nano particles showed quantum size effects in their electronic, optical and chemical properties, there has been conducted much research in this area of synthetic materials chemistry [10,11] and applications in composites with organic polymers [10-16]. When using such nano-particles to make composites with organic polymers, provided homogeneous dispersion of the nano-particles could be achieved at the microscopic level, the mechanical properties are expected to be further improved and / or new unexpected features might appear [10, 11].

Polyactive^{TM}, a block copolymer from Poly(butylene terephathalate)(PBT) and Poly (ethylene glycol)(PEG) , is the only bone bonding polymer known up until now [17]. The bone bonding properties of the polymer are considered to be derived from the ability of PEG segments to complex calcium ions. However, the same PEG segments render the material gel-like when submersed in water and therefore its mechanical properties are quite poor. In an attempt to develop a more bioactive and stronger material as bone substitute material, we used hydrothermally synthesized Nap as filler to make composites with Polyactive^{TM} 70/30. Polyacrylic acid (PAA) was used as coupling agent to improve the interface of Nap with PolyactiveTM, since PAA has been proved to be effective in improving the interface of sintered HA with Polyactive^{TM} [18,19].

Materials and Methods

Nano-apatite (Nap)

Nap was hydrothermally synthesized as described elsewhere[8]. To improve the interface of Nap with PEG/PBT polymer, polyacrylic acid was used as coating. The coating process: 80 gram hydrothermally synthesized Nap was transferred to 1800 ml 2 mM Polyacrylic acid sodium salt solution (pH adjusted to 6 using 1 M HCl) and stirred for 24 hours. Then the pH of the suspension was brought down to 5 and washed with ethanol to remove unabsorbed PAA. Finally the Nap was thoroughly washed with acetone. The non-coated Nap underwent the same procedure, omitting PAA from the solution.

Characterization of Nap

The size and the shape of Nap and PAA coated Nap was characterized by transmission electron microscopy (TEM, Philips 410). The presence of PAA coating on the surface of Nap was determined by Infra-red spectrophotometer (IR, Perkin Elmer 783) using KBr tablets. The amount of PAA coating was determined by thermal gravimetrical analysis (TGA, Du Pont 990) using a temperature increase rate of 10 ^{o}C/min..

Composites

PEG/PBT copolymer (Polyactive^{TM} 70/30, HC Implants bv, the Netherlands) has a PEG/PBT ratio of 70/30, the molecular weight of PEG being 1000 Dalton. Certain amounts of PAA coated and non-coated Nap were mixed into a 15% (w/w) Polyactive^{TM} 70/30 chloroform solution. After being intensively stirred, the suspension was dropped into a large amount of diethyl ether. The precipitate was dried first in air and then in a vacuum oven at 50 ^{0}C. Composite mixtures with 10%, 25% and 50% weight percentage Nap were obtained. After full removal of the ether, the precipitate was chopped into small pieces and used for hot press moulding at 195 ^{0}C and 20 ton of pressure.

Swelling degree of the composites

Samples for swelling tests were cut from the hot press sheets with a size of 1 x 1 x 0.2 cm. The swelling test was carried out in distilled water at room temperature . The swelling degree of the composites was calculated according to the following equation:

Error!

where Sw stands for swelling degree at certain time interval, Wt for the weight of the tested specimens after immersion in water at time t, and Wo for the weight of the tested specimens at the beginning of testing.

Mechanical testing

Rectangular sheets of 2 mm thickness were made and dumbbell shaped specimens for mechanical testing were cut from the sheet with a cutting die (ISO R37 type 1 die). The E-modulus, tensile strength, elongation at break were determined in a Houndsfield testing machine at a testing speed 50 mm/minute at room temperature. The mechanical properties were determined in the dry state and after immersion in PBS solution. In order to accurately measure the E-modulus, a strain gauge extension meter (Instron) was used.

In vitro calcification of the composites

It is generally believed that the in vitro calcification ability of biomaterials has a correlation with the bone-bonding ability in vivo. Therefore we performed an in vitro test in 1.5 times Simulated Body Fluid (1.5 SBF) which has a ionic concentration 1.5 times of the standard concentration of SBF [25]. Samples with a size about 1.5 x 1.5 cm^2 were used for the in vitro calcification of the nano-composite. Each sample of certain composition was put into a polystyrene beaker with 30 ml 1.5 SBF and kept at 37 °C in a shaking water bath. At day 3, 6, and 9, samples were taken out and carefully washed by distilled water. After drying and sputter coating with carbon, the samples were subjected to scanning electron microscopy observation and EDX determination.

RESULTS

Characterization of Nap

The as synthesized nano-apatite powder particles (Nap) had an acicular shape with a width of 9- 25 nm and a length of 80-200 nm (figure 1). It had a BET specific surface of 60-80 m^2/g. The size and the shape had not been changed by the PAA coating process .

The IR spectra of PAA coated powder clearly show the existence of PAA on the surface of the particles (figure 2). The band at 2880 cm^{-1} indicates the existence of CH_2 vibration. The peak at 1720 cm^{-1} indicates hydrogen bonding between the -C=O and the H-O-C- of the PAA.. The peak at 1568 cm^{-1} comes from the stretch vibration of C=O groups. The band at 1410 cm^{-1} is from the vibration of -C-O-H.

Figure 1. PAA coated Nap used in this study. The size and the shape of Nap was not changed by the treatment with PAA.

Figure 2. IR spectra of Nap (A) and PAA Nap (B) used in this study. Note the peaks in spectrum B at 2880, 1568 and 1410 cm^{-1} indicating the existence of PAA on the Nap.

The amount of PAA coating on the surface of Nap powder as determined by TGA is 5.7 % by weight (figure 3) .

Figure 3. The TGA curve of PAA Nap which indicated that there was about 5.7% polyacrylic acid on the surface of Nap.

Swelling degree of Nap/polymer composites

Incorporating Nap into the polymer decreased the uptake of water for the composite although the uptake was more than would be expected on basis of proportionality. Swelling gradually reached equilibrium after the samples were soaked in distilled water for 24 hours. The PAA coated Nap composites have a slightly lower swelling degree as compared to the corresponding composites with non-coated filler (figure 4).

Figure 4. The swelling degree of the composites. Note the swelling degree nearly reached equilibrium after 24 hours

immersion in water.

Mechanical Properties of the composites

The tensile tests showed that the tensile strength and elongation at break had decreased by the incorporation of both non-coated and coated filler. Swelling in water caused a decrease in mechanical properties for all the composites. Although the elastic modulus of 25% PAA-coated Nap/polymer composites in the wet state was higher than that of non-coated Nap/polymer composites, generally speaking , the effect of PAA coating can be barely seen from the mechanical properties. An increase of the filler amount decreases the tensile strength and elongation at break. Composites with 50% filler had very poor mechanical properties. Composites with 50% PAA coated filler, probably due to the formation of hydrogen bond complexation between the PEG segment and PAA molecules, were difficult to process into satisfactory samples for mechanical testing.

Table 1. Mechanical properties of the nano-composites in dry state

Filler content (%)	E-Modulus (MPa) Nap PAA-Nap	tensile strength (MPa) Nap PAA-Nap	elongation (%) Nap PAA-Nap
0	30.5 ± 2.1	7.0 ± 0.2	375 ±100
10	49.1 ±1.7 56.0± 6.3	6.8 ±0.5 6.5± 0.3	343± 73 354 ±29
25	82.1± 6.3 79.2 ±3.3	5.8 ±0.2 6.0 ±0.3	270 ±16 137 ±60
50	242± 27.9 n.d.*	4.8± 0.9 n.d.	8.7 ±3 n.d

* n.d = not determined

Table 2. Mechanical properties of the nano-composites after being immersed in PBS for 24 hours

Filler content (%)	E-Modulus (MPa) Nap PAA-Nap	tensile strength (MPa) Nap PAA-Nap	elongation (%) Nap PAA-Nap
0	7.1± 0.4	4.4± 0.3	87.2 ± 9.1
10	17.7 ± 1.7 16.7± 1.9	3.9± 0.2 3.8 ±0.2	91 ± 16 80± 11
25	15.5± 0.6 18.5± 0.7	2.9± 0.3 2.8± 0.2	51± 9 51± 8
50	11.4 ±1.0. n.d	0.6± 0.1 n.d	4.8 ±0.5 n.d.

* n.d = not determined

The calcification behaviour of the composites

The calcification experiment showed that the incorporation of non-coated Nap into the polymer matrix significantly promoted calcification of the composites in 1.5 SBF (figure 6, 7). Composites with untreated Nap filler showed much more calcification in 1.5 SBF as compared to unfilled Polyactive™ 70/30 in which no calcification was found. Composites with 10% non-coated Nap filler induced significant amounts calciumphosphate precipitation on its surface (figure 7-b). The thickness of the calcification layer increased with increase of the soaking time in 1.5 SBF. The more Nap present in the composites, the

more calcification layer would be obtained in 1.5 SBF (figure 7-d). In contrast, Unfilled Polyactive^{TM} 70/30 failed to induce calcification even after 9 days immersion in 1.5 SBF (figure 7-a).

Composites with PAA coated Nap showed a different calcification behaviour as compared to that of non-coated Nap/polymer composites. While 10% PAA-Nap composites still showed mineral precipitation from 1.5SBF after 6 days immersion, the 25% PAA-Nap/polymer composites could not induce precipitation after 6 days immersion in the same medium.

Figure 7. (a) Polyactive™ 70/30 samples incubated in 1.5 SBF for 3 days. No calcium and phosphate can be detected on the surface of the sample; (b) Composites with 10% Nap after 3 days immersion in 1.5 SBF. The sample was covered by a calcium phosphate layer; (c) Calcium phosphate layer on the 25% Nap composites after 3 days immersion in 1.5 SBF; (d) A thick calcium phosphate layer was found on top of 50% Nap composites after 6 days immersion (cross section); (e) After 6 days immersion in 1.5 SBF, 10% PAA coated Nap composites could also induce calcium phosphate precipitation on its surface.

Discussion

Generally speaking, using a filler is an effective means to increase the stiffness of a polymer. This is also the case when we use Nap in combination with PEG/PBT copolymer. When the Nap/polymer composites were tested in dry state, it seems that the Nap (with or without PAA coating) had a prominent effect on the elastic modulus of the composites. When the Nap filler content was as high as 50% by weight, the elastic modulus of the composites could be as about eight times higher as that of unfilled polymer. However, the decrease in strength indicate that the Nap as filler has no reinforcing effect in terms of tensile strength. Although we have shown that by using PAA as coating [18], the interface of sintered large HA particles with Polyactive™ 70/30 could be distinctly improved, it seems to have less effect on the mechanical properties of the composites in the case of nano-apatite.

Incorporating Nap decreased the swelling degree of the composites (figure 4 and figure 8), although more water was taken up than would be expected on basis of the assumption that the filler particles do not absorb. In figure 8 this expected swelling behaviour is plotted together with the found values. It is obvious that the filler does contribute to the water uptake. Extrapolation of the found swelling degree values to 100

Wt% filler shows an excess of about 25% by weight absorbed water. The hygroscopic nature of the nano-apatite powder was already noticed in the laboratory - extremely dry storage condition being necessary to prevent the free flowing powder from aggregation and humidification - and is apparently still present in the composites. It is unclear wether the water uptake by the powder takes place through adsorption at the surface of the particles (60 -80 m^2) or through absorbtion in capillaries of clusters of the acicular material. The combined water uptake of polymer and filler has a fatal effect on the mechanical properties of composites, especially for the high filler content composites. Swelling in PBS caused 50% filler containing composites to lose nearly all of the tensile strength and at the same time a drastic decrease in elastic modulus ocurred. Composites with 10% filler content can maintain relatively reasonable strength and elastic modulus when compared to that of unfilled polymer. PAA coating seems to have no effect on the mechanical properties of composites although the coating has a slight effect on the swelling degree of composites which can be explained as an indication of complex formation between PAA and PEG segments of the polymer [18,19](figure 8).

One other important factor that determines the mechanical properties of the Nap/polymer composites is the dispersion of the particles in polymer matrix. It has been indicated that only when the dispersion of the nano-particles achieves the microscopic level, a significant improve in mechanical properties can be expected [10,11]. Unfortunately, such microscopic level dispersion is very difficulty to achieve under the present conditions. In this experiment, agglomeration of the nano-particles is, besides water absorption, responsible for the observed decrease in tensile strength. It is also a reason why the effect of the PAA coating could not be found back in the mechanical properties.

Previous studies have shown that postoperative calcified Polyactive^{TM} contained needle shape carbonated apatite crystals when implanted in vivo. This post-operative calcification probably played an important role for Polyactive^{TM} in achieving bone-bonding [20, 21]. Pre-operatively added Nap to the polymer may promote early bone bonding by accelerating the calcification rate. In fact we found increased calcification rates in this in vitro experiments.

In this experiment Polyactive^{TM} 70/30, for which calcification has been reported both in vitro and in vivo [17, 20-22], failed to induce precipitation from 1.5 SBF even after 9 days immersion. Incorporating of Nap into Polyactive^{TM}, however, significantly promoted the calcification of the composites in 1.5 SBF. All the composites showed a calcification layer on their surfaces after 3 days immersion in 1.5 SBF. Therefore, Nap probably also has the ability to improve bone bonding rates of the composites when implanted in vivo. The strong calcification inducing capacity of Nap is probably due to the larger specific surface area of the particles and the resulting high Ca^{++} and HPO_4^{2-} concentrations due to the dissolution of Nap.

PAA coated Nap also has the capacity to promote the calcification of the composites. This can be seen from the calcification induced on the surface of 10% PAA-Nap after 6 days

immersion in 1.5 SBF. However, the calcification inducing ability of PAA-Nap seems to be lower than that of Nap, because 10% PAA-Nap composites only showed calcification after 6 days immersion, while no calcification on 25% PAA-Nap composites could be observed after 6 days immersion. Polyacrylic acid may affect the dissolution behaviour of the Nap, but it is also a possibility that the calcification rate of PAA-Nap composites was decreased by the formation of dipole complexes between PEG segments of Polyactive^{TM} and PAA molecules [23]. Where PEG segments have the capacity to chelate calcium ions from the solution by forming a helix structure in aqueous solution [24], the formation of the complexes between the PEG and PAA might have decreased this capacity by interfering with the helix conformation of PEG, and thus with the calcification of the composites.

Conclusion

Nano-apatite has a prominent stiffening effect for Polyactive^{TM} 70/30 in dry state. It has a poor stiffening effect for composites in an aqueous environment. Due to the hygroscopic nature and/or formation of aggregates the wet strength was impaired by the filler in all the composites. PAA coating on Nap almost has no additional effect on the mechanical properties of composites both in dry state or and in an aqueous environment. On the other hand, while Nap has the ability to promote the calcification of composites when incorporated into Polyactive^{TM} 70/30. PAA coating of Nap had an adverse effect on the calcification of composites presumably due to the formation of complexes between PAA and PEG segments. To reinforce the polymer by Nap, achieving more homogeneous dispersion of Nap in the polymer matrix and surface modifications to render the powders less hygroscopic appears to be necessary.

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Chapter 6

A Novel in Vitro Model to Study the Calcification of Biomaterials

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Abstract

A novel in vitro model based on a solution mainly composed of sodium, calcium, chloride and phosphate ions, was developed to study the calcification of biomaterials at near physiological conditions. This model, due to its ability to quickly calcify the tested materials, is called Accelerated Calcification Solution (ACS). Polyactive™ 30/70, Polyactive™ 70/30 and its composites with nanoapatite were used as testing materials because of their known calcification behaviour. The experimental results showed that Polyactive™ 70/30 and its composites could induce calcium phosphate precipitation from ACS in a relatively short period with different rates, while the polymer without filler failed to induce calcium phosphate precipitation in more conventional Simulated Body Fluide (1.5 SBF) in 9 days immersion in this study. Nanoapatite has the ability to promote calcification because nano-apatite/Polyactive™ 70/30 composites could quickly induce calcium phosphate precipitation in ACS as well as in 1.5 SBF. Polyactive™ 30/70 did not induce calcium phosphate precipitation in ACS. As these results agreed very well with the known *in vitro* and *in vivo* calcification behaviour of Polyactive™, we conclude that the ACS solution is a suitable model to study the calcification behaviour of biomaterials.

Introduction

Calcification or formation of a calcium phosphate phase in biomaterials is of great importance in biomedical science. Studies have demonstrated that calcification plays an important role in the bone bonding process of bone prosthetic materials such as Polyactive™ (a block copolymer of polyethylene glycol and Poly(butylene terephthalate)) [1,2], hydroxyapatite [4,21], Bioglass [16], glass ceramics [18], and silica gel [22,23]. On the other hand, calcification is not a desirable process in other applications of biomaterials, such as in bioprosthetic heart valves (bovine pericardium and porcine aortic heart valves)[11,29] polyurethane heart valves and blood pump blades etc [8, 32]. Calcification is a complicated biological process which involves many factors like calcium binding proteins, and cellular response of the host to the implants [30] , and there is no doubt that intrinsic factors of the material play an important role in the process of calcification. Examples of intrinsic factors are calcium ion complexing ability of the material [2, 31], the dissolution rate of calcium phosphate from the implanted material [4, 21], the hydroxyl groups formed on the surface of the material [22,23] and the negative charges due to pretreatment [26].

Methods to promote or to prevent the calcification process largely depend on the understanding of the mechanism of the calcification process. Also, in developing new materials for which the possibility to become calcified is of great significance, it is important to know how to control the calcification process by engineering the structure or the surface of the material. For both purposes a simple and rapid in vitro method is needed to examine the calcification ability of the material before performing cost and time consuming animal experiments. There have been developed several in vitro tests such as the use of Simulated Body Fluid (SBF) [17] for studying the materials' ability to induce calcium apatite formation [22, 23, 24, 25] and several other in vitro models for studying bioprosthetic heart valves and implantable polyurethane [14, 15, 33,]. In practice, we found that those models are not sensitive enough or take a long time to reveal differences between the ability of materials to induce calcification, or are unstable as a solution at room temperature. In this study, a new in vitro model using "Accelerated Calcification Solution (ACS)" has been developed. A series of materials with differing rates of calcification was used to evaluate the effectiveness of ACS .

Polyactive™ (HC Implants bv, the Netherlands), a block copolymer from polyethylene glycol and poly(butylene terephthalate), was chosen as testing material because its ability to calcify has been well characterized [2, 3, 27, 28]. By changing the weight ratio of PEG/PBT, a series of copolymers with different ability to be calcified can be obtained. In this study, we chose Polyactive™ 30/70 (which will not calcify *in vivo*), Polyactive™ 70/30 (which can be calcified *in vivo* and has bone-bonding ability) and a series of Polyactive™ 70/30 apatite composites with 10%, 25%, and 50% weight percentage of a nanoapatite mineral powder. These composites have shown strong calcification in SBF.

Materials and Methods

Accelerated Calcification Solution (ACS)

ACS was prepared by dissolving analytical grade NaCl, $CaCl_2$ and K_2HPO_4 to the following ion concentrations: $[Na^+]$=136.8 mM, $[Cl^-]$=144.5 mM, $[K^+]$=4.64 mM, $[Ca^{++}]$=3.87 mM, and $[HPO_4^{3-}]$=2.32 mM. The solution was buffered with 50 mM Tris buffer at pH 7.4 at room temperature.

1.5 SBF, which has a ionic concentration 1.5 times of normal SBF, was also used to compare with ACS.

Table 1 gives the ionic concentrations of SBF, 1.5 SBF and ACS.

Table 1. Ionic concentrations of SBF, 1.5 SBF and ACS

	Concentration /mM							
	Na^+	K^+	Ca^{++}	Mg^{++}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
SBF	142	5	2.5	1.5	27	103	1	0.5
1.5 SBF	213	7.5	3.8	2.3	6.3	223	1.5	0.75
ACS	136.8	4.64	3.87	/	/	144.5	2.32	/

Materials for ACS testing

Polyactive™ 30/70 and 70/30 (the figures represent the PEG/PBT ratio) were obtained from HC Implants bv, the Netherlands. The molecular weight of PEG segments is 1000 Dalton. Nano-sized hydroxyapatite (nanoapatite) was synthesized by a hydrothermal process described elsewhere [34].

A certain amount of nanoapatite was transferred to a Polyactive™ 70/30 chloroform solution. After mixing by vigorously stirring, the mixture was precipitated in a large amount of ether. After drying, the precipitates were used for hot press moulding. Composites with 10%, 25%, 50% weight percentage nanoapatite were made. Polyactive™ 30/70 and 70/30 granulates were directly used for hot press moulding. The pressed plates were 2 mm thick . The samples with size 10 x 10 x 2 mm for ACS immersion testing were cut from the hot press plates.

ACS and 1.5SBF immersion experiment

Each sample was put into either 30 ml of ACS or 30 ml 1.5 SBF in a polystyrene

disposable beaker, sealed and put in a shaking water bath at 37 ^{0}C. At day 3, all the medium was refreshed. At 3 and 6 days, the samples were taken out. After careful rinsing in distilled water and subsequently drying, samples were carbon coated for SEM and EDX analysis. Some precipitate on top of the samples from ACS solution was scratched off for infra-red analysis and powder X-ray diffraction analysis (XRD).

Results

SEM and EDX results

A. ACS immersion

At day 3, all the samples were covered by a layer of mineral except Polyactive^{TM} 30/70. This layer was composed of calcium and phosphate as confirmed by EDX (figure 1), and crystal plates were found to have grown from the surface of the tested materials (fig.2). For different materials , the coverage of the mineral was different. Polyactive^{TM} 70/30 was incompletely covered by the Ca/P crystals (figure 2 a and d). Composites with nanoapatite filler were all completely covered by the crystal plates (figure 2, b and e) but with different mineral thickness. The thickness of covered mineral layer was increasing with the filler content At day 6, except for Polyactive^{TM} 30/70, all the materials were completely covered by a thick calcium phosphate layer. In some occasions, some segregated larger globule Ca/P mineral spots were found on top of Polyactive^{TM} 30/70 with sizes more than 100 μm (figure 3). No precipitation was found on the walls of the polystyrene disposable beaker throughout the experiment.

Figure 1. The EDX analyse results showed that the precipitation on the material was mainly composed of calcium and phosphate.

Figure 2. SEM pictures showing the calcification developed on the materials. (a) The incomplete coverage of Polyactive^{TM} 70/30 by mineral layer after 3 day immersion in ACS. (b) The complete coverage of 10% nanoapatite-polymer composites by efflorescence mineral crystals after 3 day immersion in ACS. (c) The cross

section of 10% nanoapatite composites showing calcification developed from top (right) of the material towards the bottom (left) within the matrix. (d) Crystals on top of Polyactive™ 70/30 after 6 days immersion in ACS. (e) Very dense crystal plates on 50% nanoapatite-polymer composites after 6 day immersion in ACS. (f) Morphological changes of the mineral layer. The bottom part of the mineral is dense while the crystal plates still exist on top of the dense mineral layer. This suggests that the crystal plates gradually transformed to a dense mineral layer, possibly accompanied by phase structure transformation.

Calcification of the subsurface material was also found . Such calcification had developed from the top of the material towards the bottom of the sample as seen from the cross section of the material (figure 2, c).

B. 1.5 SBF immersion

Polyactive™ 70/30 and Polyactive™ 30/70 both failed to induce calcium phosphate precipitation after 6 days immersion, not even after increase of the immersion time to 9 days. However, the composites of nanoapatite/Polyactive 70/30 had induced calcium phosphate precipitation when immersed for 3 days (figure 4)

Infra-red and XRD spectra of the Ca/P mineral layer induced in ACS

The IR spectra of the mineral layer were basically similar regardless of the composition of the materials used in this study (figure 5). The broad bands at 3800 - 3000 and 1615 cm^{-1} are H_2O absorptions. The peaks at 1080 , 1030, 964 cm^{-1} are absorptions from PO_4^{-3} group. HPO_4^{-2} bands can be seen at 923, 868 and 530 cm^{-1}, and those are typical for octacalcium phosphate (OCP, $Ca_8H_2(PO_3)_6.5H_2O$). However, some of the typical HPO_4^{2-} bands of OCP are shoulders and can be barely seen (1280, 1190, 530 cm^{-1}). A small peak from OH group is found at 630 cm^{-1}. The XRD Spectra and the computer data analysis suggested that the calcium phosphate layer formed on the materials has the octacalcium phosphate structure (figure 6).

Figure 5. The Infra-red spectra of minerals induced from ACS on different materials: 1. On Polyactive™ 70/30. 2. On 50% nanoapatite composites.

Figure 6. The XRD spectrum of the mineral induced from ACS after 6 days immersion (A). B is an apatite spectrum.

Discussion

An ideal in vitro model to evaluate the calcification ability of biomaterials should be able to produce a mineral layer on the surface of the material within a short period of time, and the results should be predictive for the in vivo results. Polyactive^{TM} 70/30, as has been demonstrated, can be calcified both in vitro and in vivo while Polyactive^{TM} 30/70 does not has the ability to become calcified [1,2]. In our study, the results of the ACS model agreed very well with these in vivo tests, because Polyactive^{TM} 70/30 became calcified, in contrast to Polyactive^{TM} 30/70. On the other hand, 1.5 SBF, although capable of inducing a Ca/P layer on nano-composites, failed to produce a Ca/P layer on Polyactive^{TM} 70/30 not even while 9 days immersion. In these respects, ACS is a preferable solution for in vitro testing, because of its ability to produce Ca/P layer on the biomaterals within short periods, and the results correlating very well with in vivo experiment results.

The ACS in this study is a highly supersaturated calcium and phosphate solution similar to the solution used by Golomb et al [15]. The $[Ca^{2+}]$ $[HPO_4^{2-}]$ = 9 mM^2, and [Ca]/[P] was kept at 1.67, which is the same ratio as in hydroxyapatite. The existence of relatively large amounts of Na^+ and Cl^-, from NaCl, actually decreases the ion activity product (IAP) of Ca^{++} and HPO_4^{2-}, resulting in IAP < $[Ca^{2+}][HPO_4^{2-}]$ = 9 mM^2. So ACS solution is quite a stable solution as compared to the solution used by Golomb and Wagner [15].

The first step of calcium phosphate mineral formation is, as in any crystal precipitation, nucleation from the supersaturated solution. However, supersaturation alone doesn't mean that the nucleation will occur. In order for nucleation to occur, a certain amount of energy is needed for the system to overcome the activation energy barrier. The value of the activation energy is the critical factor in determining the rate of nucleation in a supersaturated system. Generally speaking, there are two ways to lower the activation energy, 1). increase the supersaturation degree, or 2) at a given supersaturation, decrease the interfacial energy [10]; and the latter can

be done by introducing some kind of solid surface, where the solid/ion cluster interfacial energy can be lower than the surface/solution and ion cluster/solution interface energies. Under those conditions nucleation can occur at the solid surface followed by crystal growth.

It has been demonstrated that Polyactive^{TM} 70/30 has the ability to absorb Ca^{++} from the calcium containing media probably through a Ca^{++} complexing mechanism of PEG segments [1,2,31]. So, when Polyactive^{TM} 70/30 samples are put in ACS solution, due to the high content of PEG segments, Ca^{2+} and HPO_4^{2-} will diffuse into the polymer matrix. Because of the calcium ion complexing ability of PEG segment, the Ca^{2+} concentration within the polymer matrix and near the surface can reach high values locally. It is likely that the Ca^{2+} rich surface will favour the nucleation by either or both the above mentioned mechanism. The nucleation of the mineral occurs at the surface of polymer first and is followed by the mineral crystal growth on the surface nucleation sites. Subsurface calcification can develop towards the centre of the sample due to the relatively high concentrations of the Ca^{2+} and HPO_4^{2-} in the permeable polymer matrix. On the other hand, the availability of space for crystal growth will favour the Ca/P precipitation on the surface of the already formed Ca/P layer.

According to the above discussion, in order to decrease the activation energy for nucleation, the supersaturation of the solution should be as high as possible, but should not exceed the critical supersaturation above which homogenous nucleation will occur and which renders the solution unstable.

The Ca/P layer induced from ACS is mainly composed of OCP according to IR and XRD Spectra. The IR spectrum of the mineral is quite similar to that of OCP, except for some of the shoulders (1280, 1190, 530 cm^{-1}) and the OH band at 630 cm^{-1}. XRD results also showed it has a OCP structure. SEM observation showed that the original plate-like crystals (typical OCP crystal morphology) were gradually transformed to a dense crystal layer with the plate like crystal on top. Therefore we do not exclude the possibility that the calcium phosphate layer was composed of OCP and calcium apatite transformed from OCP. The transformation of OCP to apatite is possible. The transformation mechanisms have been described by other authors [6, 7, 9, 10, 19, 20].

Nanoapatite was suggested to have better osteoconductivity than pure hydroxyapatite, due to its similarity to bone mineral in morphology, crystal structure, composition and crystallinity [34]. However, no direct evidence to prove this has been given. In the present and previous experiments, it has been found that addition of nano-apatite to Polyactive^{TM} indeed improves the polymer's ability to calcify both in SBF and ACS. The possible mechanisms of the improvement are: 1) the nano-apatite dispersed in Polyactive^{TM} may act as a nucleation site for the OCP-like phase. 2) incorporating of nano-apatite to the polymer may increase the Ca^{++} and HPO_4^{2-} concentrations within the polymer. Both mechanisms will decrease the interfacial energy of the solid surface. Thus, a Ca/P mineral layer can be formed on the surface of polymer

within a shorter period of time. It has been also found that addition of calcium phosphate to Polyactive™ also promoted the calification both *in vitro* and *in vivo*[12,13].

Our experimental results suggested that the supersaturation of ACS is beneficial for the speed of testing the calcification ability of materials. 1.5 SBF failed to give a layer of mineral on Polyactive™ 70/30 within 9 days immersion period. Two factors may attribute to this. The first factor is that the IAP, product of $[Ca^{++}]$ $[HPO_4^{2-}]$, in 1.5 SBF is less than IAP in ACS, which means that the interfacial energy of Polyactive™ 70/30 is higher in 1.5 SBF than in ACS. The second factor is the presence of Mg^{2+} and other ions in SBF. It is known that Mg^{2+} can inhibit the nucleation of apatite , as has been shown for the induction period of apatite nucleation on silica gel [5, 24]. Hence, as compared to the ACS, the induction time of the nucleation in 1.5 SBF may be quite long, and therefore no Ca/P precipitation occurs within a short period of time, unless the material has a very strong ability to calcify.

In Polyactive™ 30/70 materials, some segregated, quite large Ca/P globular spots could be observed on the surface (figure 3). Those globules have a very different morphology from the Ca/P layer on Polyactive™ 70/30 (figure 2), and do not necessarily indicate an ability of the material to calcify. In our experience, a confluent mineral layer formation is the indication of such ability.

From our results, we conclude that ACS is a suitable model solution for the examination of the calcification ability of materials in vitro. Although SBF has proved to be capable of inducing precipitation of a carbonated calcium phosphate layer on certain materials, even concentrated solutions failed to induce precipitation on Polyactive™ 70/30 after 9 days immersion in our former experiment. Where this polymer has been shown to become rapidly calcified in vivo, SBF does not seem to be a proper model for fast scrutinizing differences in material's calcification ability. ACS, however, forms a thick layer of calcium phosphate on the 70/30 polymer and its composites. Therefore, ACS may be used for more rapid screening of materials on their ability to calcify in vivo.

Conclusion

A novel and simple in vitro model for the study of the calcification of biomaterials has been developed in this study. In combination with the known calcification behaviour of Polyactive™, this model solution has proved to be fast and effective for comparing the calcification rate of biomaterials. This study also showed that by incorporating nano-apatite to Polyactive™, the calcification rate of the resulted materials can be significantly enhanced.

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Chapter 7

Surface Modification of Nano-apatite by Grafting Organic Polymer

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Abstract

Since surface properties of hydroxyapatite (HA) play an important role in its performance, surface modification of HA has gained much attention from researchers. Silane coupling agents have been the focus of the research. In this paper, however, an effective surface modification method was developed by using hexamethylene diisocyanate as coupling agent. Polyethylene glycol (Mw = 1500) was successfully coupled to the surface of nano sized apatite particles (nano-apatite). Various methods were used to characterize the surface modified nano-apatite. Infra-red spectra confirmed the existence of a layer of polymer with both urethane and ether linkage on the surface of nano-apatite. The amount of the polymer as determined by Total Organic Carbon Analysis (TOC) and Thermal Gravimetric analysis (TGA) was about 20% in weight. The direct evidence that the polymer was chemically bound to the surface of nano-apatite through the reaction with hydroxyl groups of nano-apatite came from ^{1}H MAS NMR spectra. The proton MAS NMR spectra indicated that the amount of hydroxyl groups of nano-apatite was decreased after the grafting reaction. It is concluded that this grafting method provides an effective way to graft various organic molecules on to the surface of hydroxyapatite.

Introduction

Hydroxyapatite (HA), due to its structural similarity to bone mineral, enamel and dentin, has gained much attention from biologists and biomaterial scientists [1,2,3]. Numerous investigations have dealt with the structure-property relationship and the application of hydroxyapatite as a biomaterial [1-11]. Nowadays, HA has been successfully used as: bone void filler [8,9], coating of dental and orthopedic implants [7,10,11], filler of inorganic/polymer composites [12-15], substrate for the column chromatography of protein [16] and cell culture carrier [17].

In most of the cases, the surface properties of the HA play an important role in its application, as its surface is in direct contact with the environment (body fluids, etc.) when in use. Therefore, to control or to manipulate the surface properties of HA is of great importance [15,18-25].

Surface modification of HA by organic molecules or polymers will provide an effective means to manipulate the surface properties of HA. In our point of view, there are two ways to modify the surface of HA by organic molecules. The first method is through surface adsorption. It is known that many polymers and proteins can be firmly adsorbed onto the surface of HA [26-30]. The second approach is to graft organic molecules through covalent bonding to the hydroxyl groups which are available on the crystal surface of HA. The direct application of these surface modification methods will be in the field of HA/polymer composites, column chromatography of protein, cell culture carrier and carrier of catalysts in chemical engineering.

The hydroxyl group present on the surface of HA seems to be a reactive group of which use can be made to graft organic molecules. Silane coupling agents have been the focus of the research. Nishizawa et al modified the calcium phosphate ceramics by using organic silane coupling agents, and claimed that the silane coupling agents were covalently bonded to the surface of the ceramics through the reaction with surface hydroxyl groups of the ceramics [18]. Labella et al, also speculated that silane could be coupled to the surface of HA through the reaction with the surface hydroxyl group of HA [15]. However, there had been no direct evidence that the surface hydroxyl group was involved in these reactions. Since HA has the ability to absorb certain organic molecules through hydrogen bonding, an important issue of the present study is to find wether the hydroxyl groups indeed have the ability to react with organic functional groups.

The nature of NMR renders it an effective technique for the structural investigation of calcium phosphates. Previous studies have utilized the favourable NMR properties of ^{31}P and ^{19}F nuclei to obtain quantitative structural information from high-resolution solid state NMR spectra of calcium phosphates, including mineralized tissue[31-34]. High resolution ^{1}H NMR techniques have also been used to study the hydroxyl group, structural water, surface adsorbed

water, and surface adsorbed proteins in synthetic hydroxyapatite [35-38].

The larger range of proton NMR chemical shifts (ca. 20 ppm) observed for oxygen-bound hydrogen in solid and the sensitivity to hydrogen-bonding effects makes it a very useful technique for studying biologically relevant calcium phosphates. The orientation at the magic angle of 54.7^0 with respect to the magnetic field (MAS NMR) and high sample spinning speed (can be as high as 8 kHz) can result in highly resolved ^{1}H-MAS NMR spectra [35]. All these advantages of the ^{1}H MAS NMR make it an ideal technique to study the reactivity of hydroxyl groups of HA towards organic functional groups.

In our previous study, we have shown the necessity of surface modification of HA particles in making HA/Polymer composites [24]. In an attempt to develop an effective and versatile method to modify the surface of HA, we tried to use bifunctional-group organic chemicals as coupling agents to introduce polymers onto the surface of HA. In this study, we looked at the feasibility of using diisocyanate as coupling agent to introduce polyethylene glycol (PEG) to the surface of HA particles. We aimed at improving the interface of HA with a PEG/PBT block copolymer, which has bone-bonding property and can be used as a bone substitute material [39, 40]. ^{1}H MAS NMR was specially used to study whether the hydroxyl groups of HA can react with isocyanate functional group.

Materials and Methods

Nano-apatite was hydrothermally synthesized as described elsewhere [41] and was freeze dried. The obtained powder consists of whisker shaped particles of 300 nm in length and 10-30 nm in width. The specific surface area is 70 m^2/g (N_2, BET). Polyethylene glycol 1500 was purchased from Aldrich. Hexamethylene diisocyanate (HMDI) was also obtained from Aldrich, and it was distilled and dried before using. Dimethyl formamide (DMF) (Aldrich) was distilled before use.

Surface grafting reaction

After thoroughly being dried at 120^0C for 48 hours, 4 grams of nano-apatite was charged to an erlenmeyer flask together with 75 ml dry DMF, 3 ml dry Hexamethylene diisocyanate and 0.06 ml dibutyltindilaurate. The suspension was stirred with a magnetic stirrer and bubbled with nitrogen. The temperature was increased to 60 ^{o}C and kept for 4 hours under the protection of nitrogen. Then 20 grams of PEG (Mw=1500) was added to the suspension together with 20 ml DMF and stirred overnight. The powders were separated by centrifuging and further washed by DMF and ethanol 3 times. Then the powder was precipitated in ether. After decanting the ether, the powders were first dried at room temperature and then at 50 ^{o}C to fully remove ether.

Characterization of surface grafted nano-apatite

Transmission electron microscopy (TEM Philips 450) was used to observe size or shape changes of the particles before and after the nano-apatite was surface modified by grafting PEG.

Infrared (IR) spectrophotometry (Perkin Elmer 783) was used to characterize the surface grafted polymer and nano-apatite. The spectra were recorded in 4000-200 cm $^{-1}$ region. The grafted nano-apatite was used directly for IR measurement in KBr tablets. In order to characterize the surface grafted polymer a certain amount of grafted nano-apatite was dissolved in 1M HCl to remove the mineral. The insoluble polymer was separated by filtration and washed 3 times by distilled water. Then the polymer was dried in 60 ^{0}C for 24 hours and characterized by IR using KBr tablets.

Total organic carbon analysis (DC-190 TOC analyzer) and thermal gravimetric analysis (TGA, Du pont 910) were used to determine the amount of surface grafted polymer. For TOC measurement, 0.54 gram surface grafted nano-apatite was put in 100 ml HCl solution (pH=1). 10 ml of the suspension was used for TOC measurement. The amount of polymer can be calculated from the carbon content of the grafted nano-apatite. TGA measurements were performed at 5 ^{0}C/min from room temperature up to 700 ^{0}C. Both grafted and non-grafted nano-apatite were used for these measurements.

Solid NMR was performed in a Brucker solid NMR equipment with magic-angle spinning techniques. Sample spinning speed was 7500 Hz. Tetra methyl silane (TMS) was used as reference substance.

Results

TEM observation

TEM observation indicated that the size and shape of the nano-apatite remained unchanged after the surface grafting with PEG but the nano-apatite had more tendency to aggregate (figure 1).

IR spectra study

Figure 2 gives the IR spectra of nano-apatite, surface grafted nano-apatite and the grafted polymer. The polymer on the surface of nano-apatite was identified by its urethane linkage, CH_2 vibration and C-O-C vibration. Some of the main absorption peaks were identified as follows: the peak at 3335 cm^{-1} belongs to the N-H hydrogen bond. The twin peaks at 2935 and 2861 cm^{-1} belong to the CH_2 stretch vibration. The peak at 1734 cm^{-1} can be assigned

to -C=O. An amide II band (N-H and C=O stretching vibration) can be observed at 1577 cm^{-1}. The shoulder at 1100cm^{-1} is the -C-O-C vibration of PEG. The IR spectra confirms that there is a layer of HMDI coupled PEG on nano-apatite.

Figure 1. TEM photograph shows that the nano-apatite still retains the original size and shape after surface modification by PEG grafting, although it has a strong tendency to aggregate due to the surface grafted polymer. Note the needles at the edge of the cluster. (bar = 200 nm).

Figure 2. IR spectra of nano-apatite before surface modification (1), after surface modification (2) and the polymer on the surface of nano-apatite (3). Note the appearance of $-CH_2-$ vibration at 2835 and 2861 cm^{-1}, urethane linkage at 1577 cm^{-1}, and -C-O-C- vibration at 1100 cm^{-1} in spectrum (3). The huge PO_4^{3-} peak can be seen at 1040 cm^{-1} from spectrum (1) and (2).

The amount of grafted PEG

The amount of this layer was measured by both Total Organic Carbon Analysis and Thermal Gravimetric Analysis. Both methods gave similar results. The TOC measurement gave 20.6 wt% polymer on grafted nano-apatite. The TGA result (Figure 3) showed that there was 21.8 wt% organic matter on the surface of grafted nano-apatite.

Solid ^{1}H-NMR MAS study

Figure 4 gives the ^{1}H NMR MAS spectra of PEG surface grafted nano-apatite and control nano-apatite. Nano-apatite shows several peaks in the spectrum. According to the literature [35, 36], the highest upfield peak usually belongs to the structural hydroxyl group. Therefore we assigned the peak at -0.059 ppm to the structural hydroxyl group of apatite. The downfield chemical shifts at 6.77 and 5.5 (shoulder) are probably from the two types of surface absorbed water i.e. loosely bound and firmly bound water. The down field peak at 13 ppm is from the hydrogen bonding protons in the structure of nano-apatite.

After surface grafting, the line height of structural OH decreased drastically which indicated that the amount of the -OH groups was decreased due to the reaction with the isocyanate group. Surface absorbed water on nano-apatite was removed by the reaction with isocyanate. The assignment of new peaks at 3.58 and 1.39 ppm is still unclear, they are most probably from the surface absorbed polymers through hydrogen bonding.

Discussion

The nano-apatite used in our study was a nonstoichiometric apatite with a general formula $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-X}$ $(0<X<2)$ [41]. Although it has less hydroxyl groups than stoichiometric hydroxyapatite, due to the nano size of the apatite and the large specific surface area (70 m^2/gram), there are relatively large amounts of atoms on the surface and hence a large fraction of surface hydroxyl groups can be expected. Also Yesinovski and Eckert [35] have indicated in their study that there existed a significant fraction of hydroxyl groups at the surface of hydroxyapatite particles which had a specific surface area of 37 m^2/gram. The large specific surface area of nano-apatite makes it possible to study the reactivity of the surface hydroxyl groups.

IR spectra confirmed that the polymer on the surface of nano-apatite had the urethane

linkage and polyether structure. TGA and TOC results showed the relatively large amount of polymer present on the surface of nano-apatite. The existence of such a large amount of polymer can not be simply explained by adsorption, since the samples were extensively washed by DMF. Solid proton NMR MAS spectra of nano-apatite showed the peak of structural hydroxyl group and the surface adsorbed water with positions that are very well in agreement with the literature [35,36]. After the reaction

Figure 3. The TGA graph shows that the organic polymer on the surface of nano-apatite was about 20% by weight. The temperature increase rate was 5 ^{o}C/min.

Figure 4. ^{1}H MAS NMR spectra of nano-apatite before (1) and after (2) surface modification. A huge OH peak can be seen at - 0.059 ppm on nano-apatite. This peak was decreased after the surface modification.

the peak height of the structural hydroxyl group at -0.059 ppm was significantly decreased. This clearly indicated that the hydroxyl group was involved in the reaction. The decrease of the OH peak in the NMR MAS spectrum also confirmed that there existed large amount of OH groups at the surface of nano-apatite.

The successful bonding of polymer to nano-apatite will provide the possibility to make HA-polymer composites in which HA particles are chemically bound to a polymer matrix , which in turn will result in improvement of mechanical properties of composites.

Grafting of PEG on to nano-apatite did not alter the size and shape of nano-apatite as indicated by TEM. Therefore the grafting reaction was purely a surface reaction.

Grafting organic substances or polymers to the surface of nano-apatite will offer an effective way to modify the surface properties of apatite-like materials which we believe will be very useful in expanding the field of application of these compounds.

Conclusion

By using hexamethylene diisocyanate, we successfully grafted PEG 1500 molecules to the surface of nano-apatite. It was proved that - OH groups at the surface of nano-apatite have reactivity towards organic functional groups, and also that high percentages of the OH groups are situated at the surface of nano-apatite particles. Our results also indicate it is possible by surface grafting to modify the surface properties of nano-apatite and hence to manipulate the surface properties of apatite.

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Chapter 8

A Study on the Grafting Reaction of Isocyanates with Hydoxyapatite Particles

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Abstract

The surface grafting reactions of a series of isocyanates with hydroxyapatite particles at different temperatures were studied by Infra-red spectrophotometry (IR) and thermal gravimetric analysis (TGA). The study results show that both hexamethylene diisocyanate (HMDI) and isocyanateoethyl methacrylate (ICEM) react readily with HA, while ethyl isocyanato acetate (EIA) and butyl isocyanate (BIC) have lower reactivity towards HA particles. It has also been found that the reaction of ICEM with HA follows the second order reaction mechanism, while the reaction of HMDI with HA does not follow the second order mechanism due to the complexity of the reaction. Based on the study, it is concluded that ICEM and HMDI are suitable coupling agents for the coupling of polymers due to their reactivity towards HA.

Introduction

Over the last decade, many efforts have been made toward the development of new bone substitute materials. Among them hydroxyapatite/polymer composites have attracted much attention since such composites may have osteoconductivity due to the presence of hydroxyapatite (HA) [1,2,3,4,5]. In making HA/polymer composites, the interfacial strength between filler and polymer offers a matter of concern, as lack of adhesion between the two phases will result in an early failure at the interface and thus in a decrease of mechanical properties, especially in terms of tensile strength. Several methods have been developed aimed at improving the adhesion between HA and polymer matrix. These methods include the use of coupling agents, such as silane [6,7,8], zirconyl salts [9], polyacid [10,11], and the introduction of a chemical linkage to octacalcium phosphate by co-precipitation [12,13]. In an effort to realize chemical linkage between HA and organic polymer, we have found that organic compounds with isocyanate groups can react readily with surface hydroxyl groups of hydroxyapatite [14], thus allowing us to chemically graft organic polymers to the surface of HA [14, 15].

The reaction kinetics between isocyanates and hydroxyl groups in solvent has been studied extensively [16,17]. However, there are no kinetic data on the reaction occurring at liquid/solid interfaces, as in the isocyanate-HA system. The reaction between a liquid and a solid is quite different from a reaction in solution, as many physical-chemical processes such as diffusion and adsorption of liquid reactant to the surface of solid are involved in addition to the chemical reaction. In order to optimize the grafting process with isocyanate, we performed this study on several isocyanates, namely: isocyanateoethyl methacrylate (ICEM), hexamethylene diisocyanate (HMDI), ethyl isocyanate acetate (EIA) and butyl isocyanate (BIC).

Materials and methods

Isocyanateoethyl methacrylate (ICEM) was purchased from Polyscience with hydroquinone as stabilizer, and it was used without further purification. Hexamethylene diisocyanate (HMDI), butyl isocyanate (BIC) and ethyl isocyanatoacetate (EIA) and dibutyl tindilaurate were purchased from Aldrich and used without further purification. Nonsintered Hydroxyapatite (HA) powder was from Merck. It has been proved by IR and x-ray diffraction (XRD) spectroscopy to be a poorly crystallized carbonated hydroxyapatite. The powder has a BET specific surface area of 66 m^2/g. HA was dried at 125 ^oC for at least 48 hours before being used. DMF was dried over molecular sieves.

The grafting Procedure

Table 1. The concentrations of isocyanates and the reaction temperatures used in the study

Isocyanate	Concentration(s) (M)	Reaction temperature (^{o}C)	Comments
ICEM	0.13	20, 50, 70	
HMDI	0.15, 0.04	20, 50, 75 20, 65	samples were quenched with CH_3OH
EIA	0.30	50	
BIC	0.30	70	

The grafting was performed at different reaction temperatures and by using an isocyanate solution in DMF of specific molarity as indicated in table 1. A typical procedure is as follows: 10 gram dried HA, 98 ml DMF and 0.013 mol ICEM (2 ml) were put into a 250 ml flask. 0.1 ml dibutyltin dilaurate was used as catalyst of the reaction. Hydroquinone was used as inhibitor of the polymerization (150 ppm). The reaction was kept at certain temperature under the protection of N_2. At certain time intervals, a 1.5 ml sample was taken from the reaction vessel, the powder separated by centrifuging. The powder was washed with DMF 3 times and further washed by $CHCl_3$ for 2 times to remove DMF. Samples were dried at 60 ^{o}C. In order to study the effect of the inhibitor, a control reaction without inhibitor was also carried out at 50 ^{o}C.

$OCN\text{-}CH_2CH_2\text{-}OC(O)\text{-}C(CH_3)=CH_2$	ICEM	Mw = 156
$OCN\text{-}CH_2CH_2CH_2CH_2CH_2CH_2\text{-}NCO$	HMDI	Mw = 168
$OCN\text{-}CH_2C(O)O\text{-}CH_2CH_3$	EIA	Mw = 129
$OCN\text{-}CH_2CH_2CH_2\text{-}CH_3$	BIC	Mw = 99

Figure 1. The isocyanates used in the study.

TGA and IR spectroscopy

Thermal gravimetric analysis (TGA, Du Pont 910) was performed from room temperature to 700 ^{o}C at a rate of 10 ^{o}C/min and using 20-30 mg samples. The weight loss

during the heating process was determined. The amount of grafted isocyanate was supposed to be equal to the weight loss and expressed as a molar percentage of the powder's total weight.

Infra-red spectra (Perkin Elmer 783) were recorded from 4000 - 200 cm^{-1} by using KBr tablets.

Reaction Order of the Grafting Reaction

The raction order was estimated by fitting the reaction data obtained from TGA measurements to the classical first order or second order kinetic equation:

For a first order reaction : $t = C - D \log(a-x)$

For a second order reaction : $t = C + D \log[(a-x)/(b-x)]$

where C and D are constants, t is the reaction time, a and b are the starting concentrations of the reactants (the initial concentration of hydroxyl groups on the surface of HA was taken as 0.1 M as an estimation and considered to be homoggenously distributed through the reaction medium). x is the concentration of disappeared reactant at time t which equals the molar concentration of grafted material at that time.

Results

ICEM grafting

Figure 2 gives a typical IR spectrum of ICEM grafted HA powder. The presence of amide peaks at 1660 and 1570 cm^{-1}, and the presence of ester carbonyl band at 1730 cm^{-1} indicate the existence of bound ICEM on HA (figure 2 and 3). The peak at 1270 cm^{-1} is the ester absorption band

Figure 2. IR spectrum of a typical ICEM grafted HA powder. The huge peak at 1030 cm^{-1} and the peaks at 605, 560

cm^{-1} are the P-O absorption bands of hydroxyapatite. A broad peak at 3420 cm^{-1} is from the absorption band of H_2O which is present in the KBr tablet. The presence of ester carbonyl band of ICEM at 1730 cm^{-1} (★), amide bands at 1660 and 1570 cm^{-1}(✧), and a ester absorption band from the urethane linkage at 1270 cm^{-1} (↑) indicates that ICEM was chemically bound to the surface of HA.

of -C(=O)-O- which resulted from the coupling reaction of isocyanate group with surface hydroxyl groups of HA.. The changes of the absorption intensity of these peaks indicate that the amount of grafted ICEM was increased by increase of the reaction time (figure 3). The intensity of the ester carbonyl absorption band at 1730 cm^{-1} is lower than that of the amide carbonyl band at 1660 cm^{-1} (figure 3) after 24 hours reaction at 50 ^oC in the presence of hydroquinone inhibitor while at 70 ^oC, the intensity of ester carbonyl band is slightly higher than that of the amide carbonyl band at 1660 cm^{-1} (figure 4). Deviating IR spectra were obtained from the control reaction products without using inhibitor (figure 5). It can be observed that with the increase of the reaction time, the intensity of the ester carbonyl absorption band at 1730 cm^{-1} was increased significantly and was much higher than the amide carbonyl band at 1660 cm^{-1} (figure 5).

Figure 3. IR spectra of ICEM grafted HA powder particles at 50 ^oC. The intensity of the ester carbonyl absorption band (★, 1730 cm^{-1}) as well as the amide bands (✧, 1660 cm^{-1} for amide carbonyl and 1570 cm^{-1} for amide -N-H)

was increased with the increase of reaction time. Also note the relative intensity of ester carbonyl band (1730 cm^{-1}) to amide carbonyl band (1660 cm^{-1}). (a) 2 hours reaction; (b) 9.5 hours reaction; (c) 22 hours reaction.

Figure 4. IR spectra of ICEM grafted HA powder at 70 ^{o}C. Note the relative intensity of ester carbonyl band at 1730 cm^{-1} (★) to that of amide band at 1660 cm^{-1}(✧). (a) 12 hours reaction; (b) 36 hours reaction.

Figure 5. IR spectra of ICEM grafted HA powder at 50 ^{o}C without using hydroquinone as polymerization inhibitor. Note the relative intensity of ester carbonyl band at 1730 cm^{-1} (★) to that of amide carbonyl band at 1660 cm^{-1}(✧). (a) 1 hour reaction; (b) 7.5 hours reaction; (c) 24 hours reaction.

Figure 6. Grafting yields of ICEM at different reaction temperature and reaction time. Note the increase of the yield when no polymerization inhibitor is present.

Figure 6 gives the amount of grafted ICEM at different temperatures and different time intervals as determined by TGA. As can be seen from figure 6, generally speaking, the reaction strongly depends on the reaction temperature, the reaction at 70 ^{o}C could reach "completion" within 12 hours. At 50 ^{o}C, the reaction reached nearly the same final level after 22 hours reaction. At 20 ^{o}C, almost no grafting reaction occurred.

Figure 7. The kinetic data fitting of ICEM grafting shows a linear relation of raction time with log[(a-x)/(b-x)], indicating that the reaction is a second order reaction. (a) Reaction at 50 ^{o}C; (b) Reaction at 70 ^{o}C.

The fitting of the kinetic data obtained by the TGA measurement revealed that the reaction of ICEM with HA is a second order reaction (figure 7).

HMDI grafting

The IR spectra (figure 8) indicate the existence of HMDI on the surface of HA by the presence of strong CH_2 vibration bands at 2940 and 2850 cm^{-1}, amide bands at 1620 cm^{-1} and 1575 cm^{-1}. It is also noted that the intensity of CH_2 vibration bands and the amide carbonyl band increases with the increase of reaction time (figure 8). Since the reaction was quenced by CH_3OH, both the urethane linkage which resulted from the coupling reaction of HMDI and HA and the urethane linkage from the quenching reaction may contribute to these newly emerged amide absorption bands. In caculating the grafting yield, the molecular weight of the adduct of HMDI and methanol was used (200 instead of 168).

Increase of the temperature also increases the reaction rate and the amount of grafted HMDI (figure 9). The grafting reaction at 75 ^{o}C had a saturation yield of 1.5 mmol/gHA. At 20 ^{o}C, the reaction had a yield of 0.3 mmol/gHA after 5 hours reaction. It has been found that the reaction proceeds very fast during the first 5 hours.

Figure 8. IR spectra of HMDI grafted HA powder particles at room temperature. The intensity of the CH_2 bands (Δ, 2960 and 2850 cm^{-1}) as well as that of amide bands (✧, 1620, 1575 cm^{-1}) was increased with the increase of reaction time. (a) 1 hour reaction; (b) 3 hours reaction; (c) 23 hours reaction.

Figure 9. The grafting yields of HMDI at different reaction temperature and different reaction time.

EIA grafting

The weight losses of EIA treated powder were in the order of 2% wt (0.16 mmol/gHA) throughout the whole reaction period (1- 24 hours) as determined by TGA. The IR spectrum showed that there were small peaks at 2940 and 2850 cm^{-1}, which correspond to the -CH_2 vibration band. A small amide absorption band at 1570 cm^{-1} could also be observed (figure 10). The broad peak at 1640 cm^{-1} is largely due to the H_2O absorption from the KBr tablet.No ester carbonyl absorption (1730 cm^{-1}) could be detected in the spectra.

BIC grafting

The amount of organic mater on the surface of HA was 1.7% wt (0.23 mmol/gHA) both at 70 ^oC and 20 ^oC after 24 hours reaction. IR spectra also show small peaks of CH_2 vibration at 2940 and 2850 cm^{-1}, and a small amide peak at 1570 cm^{-1} (figure 10). The broad peak at 1640 cm^{-1} is due to the H_2O absorption from the KBr tablet.

Figure 10. IR spectra of BIC (a) and EIA (b) grafted powders after 24 hours reaction. (a) BIC grafted at 70 ^{o}C; (b) EIA grafted at 50 ^{o}C. Small amide absorption bands can be observed at 1570 cm^{-1}(✧).

Discussion

A reaction between liquid and solid is very complex due to the fact that both chemical and physical processes are involved in a heterogeneous reaction. A typical heterogeneous reaction which occurs at the interface of solid/liquid may often consist of the following steps:
(a). Diffusion of the reactants from the bulk of the liquid phase to the interface of solid/liquid. If an additional layer of solid product and inert materials (e.g. the products from the reaction of liquid and solid reactants) is present at the interface, the reactants would have to overcome the resistance of this layer before they could reach the surface of the solid phase.
(b). Chemical reaction between the liquid reactants and the solid reactants.
Therefore, factors such as the diffusion characteristics of the liquid phase, the interfacial energy of the liquid reactants/solid, and the chemical kinetic (activation energy, concentration of the reactants, temperature, etc.) may affect the reaction.

A homogeneous reaction between hydroxyl group and isocyanate with alkyl tin as catalyst is a second order reaction [16]. Since the alkyl-tin catalyst is a specific catalyst for the reaction of hydroxyl with isocyanate, it is believed that an intermediate is formed prior to the condensation. In a homogeneous system, the reaction rate can be written as:

$$-d[NCO]/dt = K_{obs}\,[NCO][OH]$$

ICEM grafting

Generally speaking the reaction of isocyanate groups with hydroxyl groups will result in a urethane or carbamate (-O-C(=O)-NH) linkage, which is characterized by a secondary amide absorption band -C(=O)-NH between 1695 - 1615 cm^{-1}, In the case of ICEM grafting, an ester linkage from the methacrylate will be introduced at 1730 cm^{-1}. The presence of these functional groups provide the possibility to monitor the reaction by IR spectrophotometer.

As can be seen from from spectra of figure 3 and 4, the appearance of amide peaks at 1660 cm^{-1}, 1570 cm^{-1} and an ester absorption C(=O)-O- at 1270 cm^{-1} strongly indicates that a urethane linkage was created. The ester absorption band at 1730 cm^{-1} which stands for the presence of the methacrylate part of ICEM can also be seen. The combination of newly appeared absorption bands and the totally dissappearence of isocyante absorption band at 2200 cm^{-1} leads to the conclusion that ICEM was grafted to HA. We also found in a previous solid ^{1}H NMR study that the amount of apatite hydroxyl groups had been decreased after HMDI and polyethylene glycol grafting [14]. The NMR study together with the IR spectrophotometry study led to the conclusion that it is the structural hydroxyl group of HA instead of phosphate proton which reacted with isocyanate. The present study confirms our previous finding that the organic isocyanate groups can react with the surface hydroxyl groups of HA..

For this heterogenous reaction of HA with ICEM, the kinetic data can be nicely fitted into the second order reaction equation as stated previously (figure 7), indicating that the reaction between HA and ICEM is still a second order reaction.

Increase of the reaction temperature favours the grafting rate. The grafting amount was significantly increased with increase of the reaction temperature, either in the presence or without the presence of hydroquinone inhibitor. Without inhibitor, the yield was higher than that in the presence of inhibitor. The IR spectra clearly show that in the presence of inhibitor, the amide carbonyl absorption band (1660 cm^{-1}) is higher than or comparable to the ester carbonyl band (1730 cm^{-1}) after 24 hours reaction (figure 3, 4). Without inhibitor, the ester carbonyl absorption band is much higher than the amide carbonyl band which clearly indicates that polymerization took place. Two possible mechanisms may be involved in the polymerization: (1). polymerization of ICEM, along the methacrylate groups, prior to the grafting. (2). grafting of ICEM to HA followed by polymerization with uncoupled ICEM.

Both mechanisms will contribute to the higher yield of grafting of ICEM.

In the presence of hydroquinone inhibitor, the IR spectra show that the intensity of amide carbonyl absorption band is higher than that of ester carbonyl band. The TGA determination shows that the weight percentage of grafted ICEM is lower than when the inhibitor is absent. Also the kinetic data which show a nice fit to second order reaction kinetics suggest that the presence of inhibitor apparently prevented the polymerization of ICEM. For the purpose of

using ICEM as a coupling agent between HA and a polymer matrix (i.e. PMMA, poly(HEMA)), it is, therefore, necessary to use inhibitor to prevent the polymerization in the grafting stage.

The results also show that both the concentration and the reaction temperature will affect the grafting reaction. At low concentration (0.13 M) and room temperature, almost no grafting took place.

Estimating the surface hydroxyl groups of HA from the saturation yield of the reaction gives about 0.7 mmol OH/g HA. If we assume that the Merck powder used in this study is a stoichiometric hydroxyapatite, then the total amount of hydroxyl groups of HA should be 2 mmol/gHA, thus the result means that at least about 35% of the hydroxyl groups of HA is present on the surface.

Briefly, for the purpose of effectively introducing a double bond to the surface of HA particles, moderate reaction temperatures (i.e. 50 ^{o}C) and lower concentrations of ICEM as well as the use of inhibitor is necessary,

HMDI grafting

The presence of the carbamate peaks at 1620 cm^{-1} , 1575 cm^{-1}, 1260 cm^{-1} and CH_2 bands at 2940, 2850 cm^{-1} confirms that grafting reaction took place at the surface of HA even at room temperature and a low concentration of HMDI (0.042 M) (figure 8). The changes in the intensity of the carbamate bands as well as the CH_2 bands at 2960 and 2850 cm^{-1} indicate that the grafting yield increases with increase of the reaction time. The study by TGA also indicates that the reaction proceeds easily even at room temperature (figure 9) and a low concentration (0.042 M). The reaction achieves saturation within about 7 hours. Increase of the reaction temperature and the concentration of HMDI significantly increases the reaction rate as well as the saturation yield of grafted material.

Increase of the concentration of isocyanate and of the reaction temperature certainly can be expected to increase the reaction rate and the grafted amount within a certain period of the reaction according to the rate equation of the isocyanate-alcohol system. Kinetic analysis becomes more complecated in this case because the reaction of one isocyanate group imobilizes the other at the same molecule. Moreover, two side reactions, namely allophanate formation and oligomer formation, have to be considered (figure 11). Although both reactions can be largely avoided by using low concentrations of isocyanate and low reaction temperatures (<120 ^{o}C), the allophanate formation can still take place at 40 ^{o}C in solution. Actually, this reaction has been used to modify the surface of polyurethane at 40 ^{o}C with the same alkyl tin catalyst [18].

Both of the side reactions will lead to a higher grafting yields. The fact that the apparant saturation yields are higher at higher temperature (figure 9) raises the suspicious that indeed such side reactions played a role in determing the results.

Figure 11. Two possible side reactions of HMDI grafting.

EIA grafting

The fact that the TGA data show that there was 2% wt organic matter on the surface of HA, and the IR data do not show the expected intensity of amide absorption bands as in the case of ICEM and HMDI (figure 10), indicates that the reaction between EIA and HA does not proceed as easily as ICEM and HMDI.

Although we used the same concentration of isocyanate groups in EIA grafting as in the case of HMDI (0.30 N), the higher reaction temperature (50 ^{o}C) apparently did not promote the reaction of EIA with HA. It seems that the isocyanate groups of EIA have lower reaction activity

towards HA.

BIC grafting

TGA data and IR spectra also indicated the reaction between BIC and HA does not take place as easily as in the case of ICEM and HMDI, in spite of a higher reaction temperature (70 ^{o}C) and the same isocyanate group concentration as in the case of HMDI (0.30 N). It indicates that also BIC has a lower reactivity towards HA.

It is well know that the reactivity of isocyanate groups is affected by the structure of the isocyanate, the reaction solvent and the concentration of catalyst. Also the physical process, like the diffusion and the absorption of the isocyanate to the surface of HA, will play an important role in determining eventual grafting yield. It may be assumed that the isocyanate should first be absorbed to the surface of HA before the real reaction can take place. The absorption site of the isocyanate and the configuration of the absorbed isocyanate may also quite important for the chemical reaction, as the absorption site as well as the configuration will determine the availability of the surface hydroxyl groups of HA. Although we believe that the reaction takes place between the surface hydroxyl groups of HA and the isocyanate groups, the exact reaction mechanism is still unkown. Further study is needed in order to elucidate the mechanism.

In view of the purpose of using isocyanates as coupling agents, HMDI and ICEM are the suitable coupling agents for following polymer grafting.

Conclusion

The reaction of isocyanates with HA is affected by the reaction temperature and the concentration of the isocyanates. The reactivity of isocyanates towards the surface hydroxyl groups of HA was strongly affected by the structure of the isocyanates. ICEM and HMDI can readily react with HA, while EIA and BIC hardly react with HA. Under comparable conditions, ICEM and HMDI are the more suitable coupling agents for the grafting of polymers.

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Chapter 9

Covalent Bonding of PMMA, PBMA and Poly(HEMA) to Hydroxyapatite Particles

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Abstract

In our earlier study, we have shown that the surface hydroxyl groups of hydroxyapatite have the ability to react with organic isocyanate groups. In this study, we studied the feasibility of grafting poly(methyl methacrylate) (PMMA), poly(n-butyl methacrylate) (PBMA) and Poly(hydroxylethyl methacrylate) (poly(HEMA) by making use of the reaction of isocyanate groups with the hydroxyl groups on the surface of HA. Double bonds were first introduced to the surface of HA via the coupling reaction of isocyanateoethylmethacrylate (ICEM) with HA, or through hexamethylene diisocyanate (HMDI) with hydroxylethyl methacrylate (HEMA) and HA, then followed by radical polymerization in MMA, BMA or HEMA. Infra-red spectra indicated the existence of polymers on the surface of HA. Thermogravimetric analysis also confirmed the presence of grafted polymer on the surface of HA powder particles (20-26% wt). The polymers gave typical PMMA, PBMA or poly(HEMA) infra-red spectra, with the exception of amide bands, which resulted from the coupling reaction of ICEM or HMDI with hydroxy groups of HA or HEMA. Therefore, it is concluded that the polymers were chemically bonded to the surface of HA through the isocyanate groups of ICEM or HMDI.

Introduction

Hydroxyapatite (HA) has found many applications in orthopaedic and maxillo-facial surgery [1-5]. HA particles also have been used as filler to make HA/polymer composites [6-10]. Such composites have some unique advantages over their conventional constituting components. First, HA/polymer composites combine the oseteoconductivity of HA with the easy processing ability of polymer. Second, in combination with the wide variety of mechanical properties of polymers, the HA/polymer composites can be made either for load bearing or for none-load bearing purposes. Finally, completely degradable HA/polymer composites can be made by using a biodegradable polymer matrix. Such biodegradable composites will have the ability to induce new bone growth and to gradually degrade, thus enabling the load to gradually transfer from the material to the newly grown bone.

In making HA/polymer composites, the interfacial strength between HA and polymer is one of the major factors which will determine the ultimate mechanical properties of the composites. Lack of adhesion between HA and polymer matrix usually results in a early failure at the interface of HA/polymer. Therefore, in view of the mechanical properties of the composites, it is necessary to improve the interface of HA/polymer composites.

Various methods have been developed to improve the interface of HA with polymer matrix: silane coupling agents [10-13], zirconyl salts [14], polyacids [15,16] and chemically coupled hydroxyethyl methacrylate (HEMA) to octacalcium phosphate by a co-precipitation method [17,18]. We have shown that it is also feasible to chemically graft polyethylene glycol (PEG) to the surface of nano-apatite by using hexamethylene diisocyanate (HMDI) [19] as a binding moiety to the mineral surface. Solid ^{1}H MAS NMR proved that the isocyanate-PEG adduct was covalently bound to the surface of apatite [19]. Based on this finding, we designed experiments to couple polymethyl methacrylate (PMMA) to HA particles through isocyanateoethyl methacrylate [20].

PMMA is the most commonly used polymer in bone cement for the fixation of total hip prosthesis [10, 13, 17, 18, 21-24]. PBMA also has been used in bone cements because of its advantages in aspects such as lower exotherm, higher fracture toughness and superior fatigue life, as well as lower toxicity to soft tissue and dental pulp [13, 25]. Poly(HEMA), due to its satisfactory biocompatability, is also used to make composites with HA [26, 27] or as additives in dental resins [28, 29]. HA has been used in these cases as filler to improve both the mechanical properties and the osteoconductivity of the materials. If a chemical linkage between HA and PMMA or poly(HEMA) can be realized, a stronger composite can be expected.

Materials and Methods

Non-sintered HA powder was purchased from Merck. It has a specific surface area of 66

m^2/g. IR and XRD studies of the HA indicated that it was a poorly crystallized, carbonated hydroxyapatite (figure 1a, 1b). The powder was dried at 120 ^oC for at least 48 hours before use. Hexamethylene diisocyanate (HMDI), hydroxyethyl methacrylate (HEMA), benzoyl peroxide (BPO), and dibutyl tindilaurate were purchased from Aldrich and used without further purification. Methyl methacrylate (MMA), n-butyl methacrylate (BMA), N,N - dimethyl - p - toluidine (DMPT) and dimethyl formamide (DMF) were from Fluka and used as received; Only DMF was dried over molecular sieves. Isocyanateoethylmethacrylate (ICEM) was from Polyscience, Eppelheim, Germany and used without further purification. The solvents used in this study were all of p. a. quality.

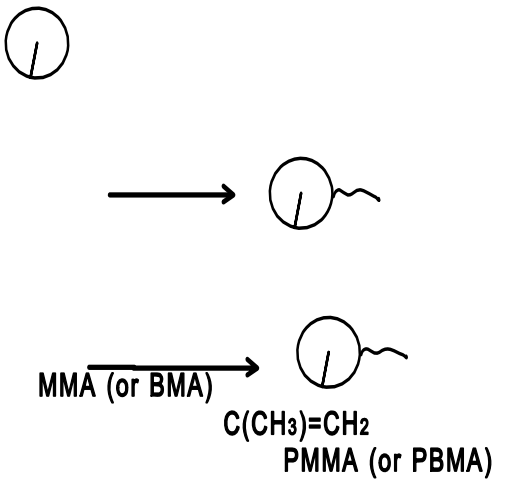
Figure 2 gives the schemes of the PMMA and poly(HEMA) grafting procedure.

Figure 1. The IR (a) and XRD (b) spectra indicate that the HA used in this study was a poorly crystallized carbonated hydroxyapatite. The vibration bands in (a) at 1450 and 1420 cm^{-1} are from carbonate groups (Δ). The

XRD spectrum indicates that the HA has a low crystallinity.

-OH + OCN-$CH_2CH_2OC(O)C(CH_3)=CH_2$ (ICEM)

Scheme 1. PMMA or PBMA grafting by using ICEM as coupling agent



Scheme 2. Grafting PMMA or poly(HEMA) by using HMDI and HEMA as coupling agent

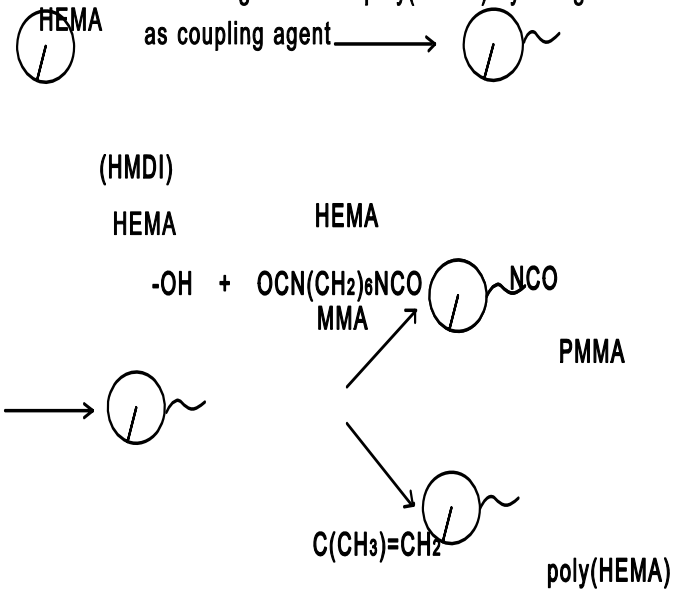


Figure 2. Two schemes for grafting PMMA, PBMA or poly(HEMA) to the surface of HA by either using ICEM or using HMDI - HEMA.

ICEM coupling

20 g of dried HA powder was suspended in 200 ml of dry DMF under N_2 protection. 4 ml of ICEM and 0.3 ml dibutyl-tindilaurate were added and the reaction mixture was kept at

50°C for 24 hours (hydroquinone was used as inibitor (150 ppm)). The powder was then separated by centrifuging and extensively washed with DMF (3 times), $CHCl_3$ (2 times) respectively, then dried at room temperature. After drying, the powder was characterized by TGA and IR. ICEM grafted powder was used for PMMA, PBMA or poly(HEMA) grafting.

HEMA coupling

20 g of dried HA was suspended in 300 ml DMF. 10 ml HMDI and 0.3 ml catalyst (dibutyl- tindilaurate) were added. The mixture was kept for 8 hours at 50 °C under the protection of N_2. Then 8 ml of HEMA was added and the mixture was kept for 5 more hours in the same reaction conditions. The reaction was stopped by adding a larger quantity of methanol. The HEMA-coupled powder was separated by centrifuging and washed by ethanol for 3 times and dried at room temperature. The obtained powder was characterized by IR and TGA.

PMMA grafting

To graft PMMA to the ICEM or HEMA grafted powder, 3 g grafted powder was mixed with 3.5 g MMA and 3.5 g PMMA beads of a cold curing acrylic resin system, 1% BPO and 2% DMPT (relative to powder and liquid respectively) were used as initiator and accelerator. After curing, the resin blocks were dissolved in chloroform and the powder was separated by centrifuging and extensively washed by large amounts of solvent. Finally it was dried in a oven at 60 °C.

Grafted polymer was isolated by dissolving the HA in a methanol-HCl (1M) solution, and washing the insoluble polymer with distilled water.

PBMA grafting

12 g ICEM grafted powder was mixed with 15 g BMA monomer and 15 g PMMA beads. Also, 1% BPO and 2% DMPT were used as initiator and accelerator. After curing, the resin blocks were dissolved in chloroform and the powder was separated by centrifuging and extensively washed by large amounts of solvent. Finally it was dried in a oven at 60 °C.

Poly(HEMA) grafting

1 gram of HEMA coupled HA powder was mixed with 1 ml HEMA and 2 ml DMF. 0.01 g AIBN was used as initiator. The mixture was bubbled with N_2 and kept in a sealed glass vial at 70 °C under magnetically stirring for about 4 hours when the stirring became difficult due to the increased viscosity of the reaction system. The stirring was stopped and the reaction vial was kept at 70 °C for 12 hours. After the reaction, the mixture was suspended in excess methanol. The grafted particles was separated by centrifuging, and further washed for 3 times by methanol. The poly(HEMA) grafted particles were dried at 70 °C. Grafted polymer was isolated by dissolving the HA in HCl (1M). The insoluble polymer was washed by distilled water and

dried at 60 ^{o}C .

Infra-red spectra (IR) and thermal gravimetric analysis (TGA) of the powders

IR (Perkin Elmer 783) spectra were obtained by using KBr tablets. The spectra were recorded from 4000 - 200 cm^{-1}. TGA (Du pont 990) was performed from room temperature to 700 ^{o}C at a rate of 10 ^{o}C/min.

Grafting Efficiency of Polymers

Grafting efficiency of polymers was determined according to following equation:

$$G = W / N$$

where G stands for the grafting efficiency of polymer, W is the weight of grafted polymer on 1 gram of HA. N is the molar number of coupled ICEM or HEMA on 1 gram of HA.

Results

ICEM grafting (HA-ICEM)

Fig. 3 gives the IR spectra of ICEM grafted HA. The -CH_2 stretch peak and the ester carbonyl peak can clearly be seen at 2960, 1740 cm^{-1} resp. The spectrum indicates the existence of ICEM on HA. The weight percentage as determined by TGA was 7.1%. (table 1).

Table 1. The grafting efficency of polymers via different procedures.

	Weight %	mmol/gHA	G (g/mmol)
ICEM	7.1	0.49	
ICEM-PMMA	28	/	0.59
ICEM-PBMA	20	/	0.32
HMDI	4	0.24	/
HMDI-HEMA	6.1	0.16	
HMDI-HEMA-PMMA	26	/	1.68
HMDI-HEMA-P(HEMA)	23	/	1.37

HEMA coupling (HA-HMDI-HEMA)

Fig. 4 gives the IR spectrum a of HEMA- coupled HA. The -CH_2 band, at 2940 and 2860 cm^{-1}, the ester -C=O band at 1730 cm^{-1}, an amide II band (-CO-NH) at 1570 cm^{-1} and a -C=O peak from amide at 1660 cm^{-1}. Therefore, it was proved that HEMA was coupled to HA through HMDI. The amount of HMDI-HEMA was 6 % wt as determined by TGA (table 1).

Figure 3. IR spectra from HA-ICEM (a) and ICEM (b). The -CH_2 band at 2960 cm^{-1} (*), the ester carbonyl band (★) at 1740 cm^{-1} and the amide absorption bands (✧) at 1660 cm^{-1} and 1570 cm^{-1} are all visible in the HA-ICEM spectrum. The isocyanate peak at 2200 cm^{-1} completely disappeared after grafting.

Figure 4. IR spectrum from HA-HMDI-HEMA. The -CH_2 bands at 2940 and 2860 cm^{-1} (*); the ester carbonyl band at 1725 cm^{-1} (★); the amide bands at 1660 and 1570 cm^{-1}(✧).

PMMA grafting

PMMA was grafted to HA via either ICEM (refer to as HA-ICEM-PMMA, table 1) or HMDI-HEMA (refer to as HA-HMDI-HEMA, table 1).

a. HA-ICEM-PMMA

Fig. 5 shows the IR spectrum of PMMA grafted HA powder. The band at 3000 cm^{-1} can be assigned to -CH_3 stretch vibration. 2960, 2850 cm^{-1} can be assigned to CH_2 stretch vibration. A very strong band at 1735 cm^{-1} belongs to the carbonyl group. 1275, 1245 cm^{-1} are the bands from -C-O-C-. The amide bands at 1660, 1570 cm^{-1} are still visible. In the IR spectrum, therefore, a PMMA spectrum is clearly recognizable. The amount of grafted PMMA was about 28% wt according to TGA determination.

Figure 5. IR spectra of, (a), HA-ICEM; (b), HA-ICEM-PMMA; (c), ICEM-PMMA. Note the ester carbonyl band at 1735 cm^{-1} (★). Spectrum c is quite similar to a PMMA spectrum except for the amide band at 1550 cm^{-1} (✧).

b. HA-HMDI-HEMA-PMMA

Fig. 6 presents the spectrum from HA-HMDI-HEMA-PMMA (figure 6-a) as well as the spectrum from the grafted polymer (figure 6-b).

It can be seen that both in figure 6-a and 6-b, a hydrogen bonded -N-H band at 3320 cm^{-1}, a -CH_3 band at 3000 cm^{-1}, and -CH_2 bands at 2960, 2850 cm^{-1} occur. A very strong carbonyl band appears at 1735 cm^{-1}, as well as amide I and amide II bands at 1630 and 1580

cm^{-1} respectively. The -C-O-C peaks occurs at 1275, 1250, 1200 and 1155 cm^{-1}. The spectra provide a strong indication that PMMA was grafted to HA through HMDI-HEMA.

The amount of grafted polymer was 26% wt by TGA measurement.

Figure 6. IR spectra of, (a), HA-HMDI-HEMA-PMMA; (b), HMDI-HEMA-PMMA. Spectrum b indicates the existence of a ester carbonyl band at 1735 cm^{-1} (★), amide bands at 1630 and 1570 cm^{-1} (✧) and other bands typical for PMMA, proving that the PMMA was coupled to HA via HMDI-HEMA.

PBMA grafting

Figure 7 is the IR spectra of PBMA grafted HA (7-a) as well as grafted PBMA (7-b). CH_2 bands at 2960, 2860 cm^{-1}, a carbonyl band at 1735 cm^{-1}, amide bands at 1655, 1555 cm^{-1} are all visible in both spectra. Spectrum b is quite similar to a PBMA spectrum except for the amide bands.

The TGA measurement gives 28% wt. organic matter on HA powder.

Poly(HEMA) grafting (HA-HMDI-poly(HEMA))

The IR spectra obtained from poly(HEMA) grafted HA particles are given in Fig. 8. A -CH_2 band at 2960 cm^{-1}; a very strong ester carbonyl band at 1730 cm^{-1}, a -C-H band at 1460 cm^{-1}, -C-O-C- bands at 1275, 1250 cm^{-1} and amide I and II bands at 1630, 1560 cm^{-1} indicate that the poly(HEMA) grafted was coupled to HA through HMDI. No residual free isocyanate groups could be detected in the spectrum at 2270 cm^{-1}.

The amount of poly(HEMA) grafted to the HA was 23% wt as measured by TGA.

Grafting Efficiency of Polymers

Table 1 gives the grafting efficiency of polymers by different coupling methods. Using HMDI-HEMA as coupling spacer resulted in a higher grafting efficiency than in the case of ICEM.

Figure 7. IR spectra of , (a), HA-ICEM-PBMA and (b), ICEM-PBMA. Spectrum b shows the presence of an ester carbonyl band at 1730 cm^{-1}(★) and amide bands at 1655, 1555 cm^{-1}(✧). The latter indicate that the PBMA was coupled to HA via chemical bonding**.**

Figure 8. IR spectra from (a), HA-HMDI-poly(HEMA) and the (b), grafted polymer HMDI-poly(HEMA) after dissolving of HA. The existence of amide bands (✧) results from the reaction of isocyanates with hydroxyl groups

of HA and HEMA.

Discussion

We have shown that the surface hydroxyl groups of hydroxyapatite have the ability to react with organic isocyanate groups [19,20]. Therefore the hydroxyl groups on the surface of HA provide reactive sites for chemically coupling of organic polymers.

The experimental results clearly show that PMMA, PBMA and poly(HEMA), three commonly used biomaterials, can be chemically bonded to hydroxyapatite surfaces by using one of the mentioned coupling methods.

All grafted (PMMA, PBMA or HEMA) HA particles showed the existence of polymers on the surface of HA. By dissolving the grafted HA particles in acid, the insoluble polymer gave typical PMMA, PBMA or poly(HEMA) infra-red spectra, with the exception of the amide bands, resulting from the coupling reaction of ICEM or HMDI with hydroxygroups of HA or HEMA. Therefore, it is concluded that PMMA or poly(HEMA) was chemically bonded to the surface of HA through the isocyanate groups of ICEM or HMDI. The weight increase data (24-26% wt%) of polymer also indicated that such large amount of polymers on the surface of HA are unlikely to be caused by adsorption.

The first step to realize the coupling was to couple ICEM or HMDI to HA through the reaction of -NCO with surface hydroxyl group. Under the used reaction conditions , the amount of ICEM coupled to HA was 7.1 %wt which is 0.49 mmol ICEM/gHA. In the case of the diisocyanate, HMDI, it was 4 % wt. or 0.24 mmol/gHA. After HEMA had been coupled to HA-HMDI, the weight percentage increased to 6.1%, that means 0.16 mmol HEMA/gHA. Where the reaction between isocyanate and HEMA hydroxyl groups is on molecular basis, it must be concluded that 8 mmol (some 30%) of the isocyanate groups were inaccessible or inactivated by certain terminants like H_2O.

After polymerizing either HA-HMDI-HEMA or HA-ICEM particles in MMA, BMA or HEMA monomers, the weight percentage of organic matter on HA was increased. Considering the grafting efficiency, it is apparent that HA-HMDI-HEMA has a higher efficiency than HA-ICEM, although ICEM had a higher percentage on HA (0.49 mmol/gHA). There are two possible reasons for the lower grafting efficiency of HA-ICEM. The first one is that some of the ICEM coupled to HA had already been polymerized during the coupling process. This would lead to a higher ICEM weight percentage on HA but, thereafter, to a lower grafting efficiency for PMMA. The second possibility is that the chain length of molecules anchored on the HA surface is of importance. ICEM is shorter (C6) than HEMA-HMDI (C10) which may result in a difference in their probability to be polymerized. The effect of the spacer on the grafting efficiency still needs to be studied. The small difference between the grafting efficiency of HA-HMDI-HEMA-PMMA and HA-HMDI-HEMA-poly(HEMA) may result from the way of polymerization. HA-HMDI-HEMA-PMMA was prepared by bulk polymerization while HA-HMDI-HEMA-poly(HEMA) was prepared by solution polymerization in DMF.

Conclusion

PMMA, PBMA and poly(HEMA) were successfully coupled to the HA particles via covalent bonding of isocyanate groups. The possibility to realize chemical bonding between HA and a polymer matrix provides a wide range of possibilities to intergrate HA in composites.

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Chapter 10

Composite Biomaterials with Chemical Bonding Between Hydroxyapatite Filler Particles and PEG/ PBT Copolymer Matrix

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Abstract

In an effort to make composites from hydroxyapatite and a PEG/PBT copolymer (Polyactive™ 70/30), chemical linkages were introduced between the filler particles and polymer matrix by using hexamethylene diisocyanate as coupling agent. Infra-red spectra (IR) and thermal gravimetric analysis (TGA) confirm the presence of Polyactive™ 70/30 on the surface of HA filler particles. The amount of chemically bound polymer was 4.7 wt. % as determined by TGA. The mechanical properties of the composites, i.e. tensile strength and Young's modulus, were significantly improved by the introduction of chemical linkage between the filler particles and the polymer matrix as compared to the control composites. This method provides a effective way to introduce chemical linkage between HA filler particles and polymer matrix. By optimizing the grafting process, a further improvement of the mechanical properties of the composites can be expected.

Introduction

A commercialized biomedical polymer, polyethylene glycol and poly(butylene terephthalate) (PEG/PBT) block copolymer (Trade name: Polyactive^{TM}) with a PEG content higher than 55 wt %, has bone bonding properties when implanted in vivo [1, 2]. This special characteristic makes the copolymer very attractive as a bone replacement material. Increasing the PEG amount will lead to a faster bone bonding rate, but to relatively poor mechanical properties [1]. Applying a calcium phosphate layer on the copolymer has been shown to enhance bone-bonding rates [3] and therefore the use of HA filler particles to form composites may improve upon both bone-bonding rate and mechanical properties.

Hydroxyapatite (HA) particles have been used as a filler to make composites with polymers[4-11]. The incorporation of HA filler in a polymer matrix may combine the osteoconductivity of HA with the ease of processing of polymers. Also, the wide range of mechanical and biological properties of polymers offer the possibility to make composites with various desired properties, e.g. biodegradation.

In making HA/polymer composites, the interfacial problem between HA and the polymer matrix is one of the major factors which will determine the ultimate mechanical properties of the composites. Lack of adhesion between the two phases usually results in a early failure at the interface of filler/polymer matrix.

Various methods have been developed to improve the interface of HA with polymer matrix. Silane coupling agents [11-14], zirconyl salts [15] and polyacids [16,17] have been used to this purpose and structural modification of the polymer matrix, e.g. introducing acrylic acid groups to polyethylene, also proved to be an effective method [13].

In our previous work, we have found that organic isocyanate groups can react readily with the surface hydroxyl groups of HA [18]. Based on this finding, various polymers, including PEG, PMMA and poly(HEMA) have been coupled to the surface of HA via isocyanates [19,20]. This finding also made the achievement of direct chemical linkage between HA filler particles and polymer matrix possible.

In this paper, we tried to use commercial HA powder as a filler to make composites with Polyactive 70/30. Direct chemical bonding of the polymer to the particle surface by diisocyanate had the purpose to improve the interface between the mineral and the polymer matrix.

Materials and Methods

HA powder

The HA powder used in this study was purchased from Merck. It is a poorly crystallized carbonated apatite as determined by Infra-red and powder x-ray diffraction spectroscopy (see

Chapter 9, figure 1). It has a specific surface area of 66 m^2 as determined by BET method (N_2 was used as adsorption gas). The HA powder was kept at 120 ^oC for at least 48 hours before use.

Chemicals

Hexamethylene diisocyanate (HMDI) and dibutyl tindilaurate were purchased from Aldrich and used as received without further purification. Polyactive^{TM} 70/30 with a PEG molecular weight of 1000 Dalton and an overall Mw of 90,000 was obtained from HC Implants bv, the Netherlands. Chloroform (HPLC grade, Biosolve LTD, the Netherlands) was dried over calcium hydride. Other solvents were all of p.a. quality.

HA/Polymer composites

Surface grafting of Polyactive 70/30 to HA particles

Surface grafting of Polyactive 70/30 to HA particles was achieved by using HMDI as coupling agent. A typical procedure is as follows:

80 gram HA particles and 800 ml dried chloroform were put in a 1500 ml erlenmeyer flask under the protection of N_2, and stirred for 30 min., 5.04 ml HMDI (0.03 mol) and 0.1 ml dibutyl tindilaurate were added to the suspension. The suspension was stirred at room temperature for 5 hours before 62 g predried (60 ^oC in vacuum) Polyactive^{TM} 70/30 granulate was added and dissolved in the chloroform. The suspension was stirred for another 48 hours. The grafted HA particles were then separated by centrifuging at 4000 rpm, resuspended in $CHCl_3$ and washed 3 times.

HA/Polyactive^{TM} composites

The surface grafted HA was transferred to a 10 wt. % Polyactive^{TM} 70/30 chloroform solution in ratios of 10 - 30 % in volume. The mixture was stirred while the chloroform evaporated untill a high viscous mixture was obtained. The mixture was then precipitated in excess of ether. After drying the precipitates were chopped into small pieces.

Control HA/Polyactive^{TM} composites were also made by directly mixing dried but untreated HA in 10 wt. % Polyactive^{TM} 70/30 chloroform solution for 12 hours, and by the same precipitation procedure as mentioned above.

The chopped HA/Polyactive precipitates were used to make samples for mechanical and other tests by hot press moulding. Composite plates of 15 x 15 x 0.2 cm^3 were made by hot pressing at 195^oC and 20 ton pressure. Dumbbell shaped samples for mechanical testing were cut from the hot press sheet with an ISO R37 type 1 die.

Swelling degree of the composites

Rectangular samples with size 0.8 x 2 x 0.2 cm^3 were used for the test. Samples were immersed in distilled water at 20 ^oC in polystyrene beakers. At certain time intervals, the samples were taken out, and the surface water was quickly removed by using tissue paper. The weight of the sample was measured and the swelling degree of the composites was caculated by:

Error!

where S_w stands for the swelling degree, W_t is the sample weight after immersion in water at time t. W_0 is the sample weight before immersion in water. Three samples were used for each composition, and the mean value was caculated.

Mechanical Testing

Mechanical testing, especially tensile testing has been shown to be a sensitive test to study the interface of filler and polymer. Tensile tests were performed by using a Hounsfield mechanical testing machine at a crosshead speed of 50 mm/min. E-modulus, tensile strength and elongation at break of the composites were determined at room temperature both in dry and in wet state (after immersion in pH 7.2 Tris buffer for 24 hours). In order to accurately measure the E-modulus, an Instron extension meter was used. For each composition, 6 samples were tested.

Characterization of the surface grafted HA particles

The HA particles were characterized by thermal gravimetrical anylisis (TGA, Du Pont 990) and Infra-red spectroscopy (Perkin Elmer 763) before and after surface grafting.

A sample of grafted HA powder (PA-HA) was taken from the reaction vessel and quenched by adding methanol, and then thoroughly washed by $CHCl_3$ for 3 times. After drying at 60 ^oC, the grafted powder was used for IR and TGA measurement. IR spectra were recorded from 4000-200 cm^{-1} by using KBr tablets. TGA was performed on 20-30 mg samples from room temperature to 700 ^oC at a temperature increase rate of 10 ^oC/min.

Results

Characterization of grafted HA powder:

The IR spectrum of HA powder indicates that the powder is carbonated apatite due to the existence of 1455, 1415 cm^{-1} absorption (Chapter 9, figure 1).

After grafting of HMDI, some new bands emerged as the result of HMDI coupling (figure 1): -CH_2- absorption at 2940 cm^{-1} and 2860 $cm^{-1,}$ amide bands at 1630 cm^{-1} and 1580 cm^{-1} due to the reaction of isocyanate groups either with surface -OH groups of HA or with the quenching methanol.

When HA had been grafted with Polyactive^{TM} 70/30 via HMDI, there appeared more bands which indicated that Polyactive^{TM} 70/30 was coupled to the surface of HA via HMDI (figure 1). The band at 1725 cm^{-1} is the >C=O stretch vibration from coupled Polyactive. Not surprisingly, another band of -CO-O- can be observed at 1280 cm^{-1}. The band at 735 cm^{-1} is from the C-H vibration of benzene rings. Therefore, the IR spectra clearly showed the existence of Polyactive^{TM} 70/30 on the surface of HA.

Figure 1. IR spectra of surface grafted HA with HMDI (a) and Polyactive 70/30 (b). Note the increase of the absorption intensity of CH_2 bands (✧,2940 and 2860 cm^{-1}) and the amide bands (★, 1630 and 1580 cm^{-1}). Also note the ester (➔, 1280 cm^{-1}) and benzene(→, 735 cm^{-1}) absorption bands in spectrum (b), which indicate the existence of Polyactive 70/30.

The amount of surface grafted HMDI and Polyactive were determined by TGA. As seen from figure 2, the amount of grafted HMDI was found to be 2.6% and after Polyactive^{TM} 70/30 grafting, the weight of the organic part increased to 7.3 %. Therefore, the amount of grafted Polyactive^{TM} 70/30 was 4.7 wt % .

Figure 2. TGA curves of surface grafted HA powder. The weight of grafted Polyactive can be caculated from the two curves. (a) is the curve from HMDI grafted HA, and (b) is TGA curve from Polyactive grafted HA.

Figure 3. Swelling degree versus time curves of the composites. Note the swelling degree reached equilibrium after 24 hours immersion in distilled water at room temperature.

Swelling degree of composites

The swelling degree of the composites was decreased by the addition of HA filler (figure 3, 4). The swelling degree of all the composites reached equilibrium after 24 hour immersion in water

Figure 4. Equilibrium swelling degree of the composites. The dashed line represents the expected swelling behaviour of the composites if it is assumed that the HA filler does not absorb water.

Figure 5. Typical load-displacement curves of the composites. When increase the filler content, both the elastic moduli and the strengths of the composites are increased, but the surface grafted HA composites have higher values as compared to the control HA composites.

(figure 3). There was not much difference in the equilibrium swelling degrees between PA-HA

composites and control HA composites (figure 4).

Mechanical properties of the composites

Figure 5 gives typical load-displacement curves of composites in dry state. Generally speaking, the elastic modulus and the strength of the composites were increased when the filler amount was increased from 10% to 30%. The effect of Polyactive grafting can be observed from the graph. Composites with PA-HA filler have better mechanical properties when compared to HA filled composites. When composites were immersed in Tris buffer (pH7.2), the mechanical properties of the composites drastically decreased, due to swelling of the polymer matrix and/or to deterioration of the filler/matrix interface.

Figure 6. E-modulus of the composites in dry state (a), and in wet (b) state.

A. Elastic modulus

Incorporating filler to polymer matrix had a prominent effect on the elastic modulus of the composites (figure 6a), especially when the filler content was as high as 30% by volume. PA-HA filled composites had higher elastic moduli than HA filled composites.

After immersion in Tris buffer for 24 hours, the elastic modulus had significantly decreased. When the filler amount is less than 20%, the filler almost had no effect on the elastic modulus of the composites in wet state. Increase of the filler amount to 30%, caused further increase of the modulus in case of PA-HA filler, where the mechanical properties of HA filled composites became so poor that the elastic modulus could not be determined (figure 6b).

Figure 7. Strength of the composites in dry state (a), and in wet (b) state.

B. Tensile strength

The strength of Polyactive™ 70/30 initially dropped by the incorporatinn of 10% filler (figure 7a), but increased again with further increase of the filler volume. PA-HA composites had higher tensile strengths than HA composites.

After immersion in Tris buffer for 24 hours, the strength (including that of unfilled Polyactive™) had decreased. The filler, however, caused a greater loss in strength as compared to unfilled Polyactive™ 70/30. It can be seen from figure 8b, that after incorporating 20% filler to the polymer, nearly half of its strength, from 5.7 MPa to about 2.2 MPa was lost. With further increase of the HA filler to 30%, ungrafted HA composites lost almost all of their strength, in contrast to the PA-HA grafted HA composites which retained about the same strength.

Figure 8. Elongation at break of the composites in dry state (a), and in wet (b) state.

C. Elongation at break

Incorporating the filler caused a drop of the elongation at break, no matter wether the filler was surface modified or not (figure 8a).

Immersion in Tris buffer caused a drastic loss in elongation at break even when the filler amount was only 10%. Composites with 30% HA filler were extremely brittle with small elongations while composites with 30% PA-HA filler still maintained a pronounced elongation at break of 29%.

Discussion

Covalent bonding of HA with polymer matrix has the potential to significantly improve the interface of HA and polymer matrix, therefore leading to a significant improvement of mechanical properties of composites.

Due to the presence of hydroxyl end groups and carboxylic end groups in Polyactive^{TM} 70/30, covalent bonding of HA with polymer can be achieved by using HMDI as coupling agent. The presence of Polyactive^{TM} 70/30 on the surface of HA has been confirmed by IR spectroscopy and the amount of grafted Polyactive^{TM} 70/30 could be determined by TGA measurement.

Covalent bonding Polyactive^{TM} to HA has a distinct effect on the mechanical properties of the composites, especially at higher filler content. The elastic modulus, strength and elongation at break were all higher for PA-HA composites as compared to HA filled composites(figure 5). The most pronounced effect was found between surface grafted and ungrafted HA composites at higher filler content, especially in the case of the elastic modulus. This agreed well with our previous results [16,17,23] and the results of Jones et al [12], in which it was found that only at filler amounts higher than 6%, significant differences could be found for silane treated versus non-silane treated materials. Apparently the interfacial interactions become more important when the filler amount is higher.

Immersion in water will cause a decrease in mechanical properties for both PA-HA composites and HA composites. Swelling will decrease the mechanical properties of Polyactive^{TM}, and cause a loose embedding of HA particles in polymer matrix. Thus HA composites at 30% by volume of HA almost completely lost their strength in contrast to the surface grafted HA composites. Apparently the grafted HA particles could maintain a better contact because of the bonding between HA and polymer matrix.

An interesting finding is that 30% grafted filler composites had a higher tensile strength than 10% grafted filler composites, especially in the wet state, allthough both were less strong than unfilled Polyactive^{TM}. This is different from results with

nano-apatite fillers in our previous work [23] where an increase of the filler volume was found to only further decrease the strength. In this light the results of the swelling test are helpfull in explaining the difference. If the filler per se is assumed to have zero water uptake, then the the equilibrium swelling degrees of the composites should follow the dashed line of Fig. 4. The actual swelling degree values were found to be lower with the current Merck-powder fillers, but in the case of the nano-apatite fillers, the swelling degree values were found to be higher than the theoretically expected values. In this last case the filler apparently took up water by itself destroying any interaction between it and the matrix. With the current fillers showing lower than expected swelling degrees it appears that the quality of the interactions between filler and matrix is much higher and even prevents part of the matrix material from swelling in water. Where ungrafted or grafted fillers do not differ in this respect, adsorption of matrix molecules to the filler surface is apparently as effective as covalent bonding. When mechanically loaded, however, the difference becomes clearer and do grafted filler composites show better mechanical properties than control HA composites, both in dry and in wet state.

Several theoretical models have been proposed to predict the Young's modulus of a composite system from the mechanical properties of the matrix and the filler. Among the models, the Voigt model and the Reuss model are believed to provide the upper limit and lower limit of the composites respectively. In the Voigt model, a homogenous and elastically isotropic composition with continuity of strain across the interface is assumed. The equation of the Voigt model can be written as a simple rule of mixture:

$$E_c = V_f E_f + V_m E_m$$

where E is the Young's modulus, V is the volume fraction, c, f, and m denoting the composites system, filler phase and matrix phase respectively.

Reuss model, which assumes that the stress will be identical in each phase, can be written

Error!

as follow:

or

Error!

If we take the Young's modulus of filler and matrix as 70 GPa and 37 MPa respectively, according to the Voigt model, the composites with 10%, 20% and 30% volume of filler should

have Young's moduli 7 GPa, 14 GPa, and 21 GPa. These theoretical values are much higher than the actually found values. The Reuss model gives lower Young's moduli as compared to the found values of the composite system. Figure 9 gives the theoretical value of Voigt and Reuss models in comparison with the realistic found value of the composites. Therefore, the current composite system behaves somewhere between the model of Reuss and Voigt model, partially as a constant strain system in which the composite modulus is higher than the values predicted by Reuss model, and well below the upper limit predicted by Voigt model. The fact that introducing interfacial bonding of the filler/matrix resulted in higher Young's modulus raises the question whether if more matrix molecules could be chemically bound to the filler surface, moduli much closer to the theoretical values of Voigt model could be achieved. In this experiment, only 4 wt% Polyactive^{TM} 70/30 was bound to HA filler, which is much lower than the theoretical value. Considering the bound HMDI is 2.6 wt% , which is 0.13 mmol/gHA, in theory, 12 grams Polyactive^{TM} (Mw = 90,000 Dalton) can be bound to 1 gram HA. By optimizing the grafting procedure, the mechanical properties of the composites might very probably be further improved.

Figure 9. The experimental Young's modulus in comparision with the theoretical values predicted by Voigt and Reuss models.

Conclusion

A copolyether-ester (Polyactive™ 70/30) was successfully bound to HA filler particles via HMDI, establishing a covalent bonding of HA filler particles to polymer matrix. The mechanical testing results showed that the chemical bonding of HA filler to polymer matrix had a distinct effect on the mechanical properties of the composites. The Young's modulus, tensile strength and elongation at break were significantly improved by grafting, especially at higher filler fractions. A further improvement of the mechanical properties can be expected if higher grafting percentages of Polyactive™ to HA can be achieved.

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Chapter 11

General Discussion

Hydroxyapatite (HA)/polymer composites have been studied extensively in recent years [1-10]. It has been found that the use of HA as filler may turn an initially non-bioactive materials into a bone-bonding composite. Besides the bone-bonding properties of the composites, the possibility to improve the mechanical properties of composites as well as the possibility to make the HA/polymer composite systems biodegradable by using biodegradable polymer matrices make composite systems very attractive [4-6, 9,10].

When a composite is intended to use in a load bearing situation, the mechanical properties are of primary concern. The interface between HA and polymer matrix will play an critical role in determining the mechanical properties of the composites. Lack of adhesion between the two phases usually will result in a early failure at the interface of HA and polymer matrix.

Introduction of interactions such as hydrogen bonds, dipole interaction, hydrophobic interaction to the interface is expected to improve the interface of HA with polymer matrix. For example, bone, a natural biocomposite, is mainly composed of bone mineral (a hydroxyapatite-like mineral), organic matrix of type I collagen and non-collagenous proteins [11]. The bone minerals are bound to collagen by non-collagenous proteins via ionic bonds, hydrogen bonds and hydrophobic interactions [11,12]. These interactions distribute the load throughout the matrix and bone mineral by transferring the load across the interface, thus giving bone its unique composite behaviour. Therefore, we expect that the mechanical properties of the composites can be improved if certain kinds of interactions can be introduced between hydroxyapatite and polymer matrix.

1. Polyacids as bonding agents in HA/Polyactive^{TM} composites

To improve the interface of HA with Polyactive^{TM}, polyacrylic acid (PAA) and ethylene-maleic acid copolymer (EMa) were used to introduce interactions between HA and Polyactive^{TM}.

It is well documented that PAA and maleic acid copolymer can be firmly adsorbed on the surface of HA [13,14], Also, PAA and maleic acid copolymer can form hydrogen bond complexes with polyethylene glycol (PEG) at acidic pH [15]. At higher pH, the complexation can still occur, but to a less extent, due to the dipole interaction [16]. Based on these facts, PAA and EMa were chosen to precoat the sintered HA particles (1 - 45 μm in diameter) in aqueous solution. These procoated HA particles proved to be effective in improving the mechanical

properties of the composites when used as filler in both Polyactive™ 70/30 and Polyactive™ 30/70 composites (chapter 2, 3). The effectiveness of the coatings on HA/Polyactive™ 30/70 composites was not expected, because there are less PEG segments and more PBT segments in Polyactive™ 30/70. A further study found that hydrophobic interactions are present between PAA (or EMA) and PBT segments, independent of pH. This is in contrast to the hydrogen bonds or dipole interactions between PAA (or EMa) and PEG segments (chapter 4) which depend on the pH. Therfore we conclude that the effectiveness of PAA (EMa) in improving the interface of HA/Polyactive™ is due to both the interactions between PAA (EMa) and PEG segments, and the interactions between PAA(EMa) and PBT segment.

2. Mechanical properties and in vitro calcification of nano-apatite/Polyactive™ Composites

Nano-apatite was used as filler to improve the bioactivity of composites. Since nano-apatite is more similar to natural bone mineral in aspects such as the size, crystallinity, crystal structure and composition [17] more bioactivity was expected from this type of filler. It was found that incorporation of a small amount of nano-apatite (10% wt) to Polyactive™ 70/30 greatly improved the calcification rate of composites in simulated body fluids (chapter 5) and another supersaturated calcium phosphate solution (ACS) (chapter 6). Since it is generally believed that calcification of biomaterial is the prerequisite for achieving bone-bonding, the nano-apatite composites may offer the advantages of inducing early bone-bonding in vivo[18, 19, 20, 21].

Although the incorporation of nano-apatite into polymer matrix improves the calcification rate of the composites, it decreased the tensile strength of Polyactive™ 70/30. The use of PAA as coating on nano-apatite had no effect on the mechanical properties of the composites. On the contrary, the use of PAA had an adverse effect on the calcification rate of the composites, presumably due to the formation of complexes with PEG segments of Polyactive™ and therefore interfering with the calcium ion complexation ability of PEG segments (chapter 5).

The dispersion of the nano particles in the polymer matrix may play an important role in determining the mechanical properties of the composites. It can be seen from the mechanical testing results that although there was complexation between the PAA coating and Polyactive™, no improvement in mechanical properties was achieved. Therefore, we speculate that the aggregation of the nano-apatite in the polymer matrix is responsible for the decrease in mechanical strength. Aggregation of the very fine particles is very difficult to avoid and thus special techniques are required for preparing nano-apatite composites. The observed increased water uptake due to the presence of nano-apatite may also be responsible for the decrease in mechanical strength.

3. Grafting of polymers to HA particles

Grafting of polymers onto the surface of HA particles may offer possibilities to modify the surface characteristics of HA and to introduce covalent bonds between HA and the polymer matrix.

The grafting of polymers to the surface of HA was realized by using isocyanate containing reagents. It has been found by solid ^{1}H MAS NMR and IR analysis that the organic isocyanate group can react with the surface hydroxyl groups of HA (chapter 7). The reaction seems to follow a second order reaction mechanism in the presence of alkyl-tin catalyst despite the heterogenous nature of the reaction system (chapter 8). With isocyanates such as hexamethylene diisocyanate (HMDI) and isocyanateoethylmethacrylates (ICEM) as coupling reagents various polymers can be grafted to the surface of hydroxyapatite particles. The grafted polymers may be hydrophobic such as polymethyl methacrylate (PMMA), polybutyl methacrylate (PBMA) or hydrophilic such as PEG, Polyactive^{TM} 70/30 and polyhydroxylethyl methacrylate (polyHEMA) (chapter 8, 9, 10). Thus the grafting of polymer will offer an effective way to modify the surface characteristics of HA and the realization of chemical bonding between HA and polymer matrix greatly improves the mechanical properties of the composites (chapter 10).

The use of isocyanate as coupling agents offers an alternative for silane coupling agents, since the effectiveness and the water resistance of silane coupling agents in HA is still a matter of controversy [22-26].

General conclusions

Based on the experimental results, we can draw some general conclusions:

1. The use of PAA and EMa as bonding agents is effective in improving the interface of sintered larger HA particles with Polyactive^{TM} due to the interactions of PAA (EMa) with both the soft segments (PEG) and hard segments (PBT).

2. Nano-apatite has a prominent effect in accelerating the calcification rate of the composites but has a poor effect in reinforcing the composites, possibly due to the difficulty of achieving a homogeneous dispersion of nano-apatite particles in the polymer matrix. The use of PAA as bonding agent has no effect on the mechanical properties and has a negative effect on the calcification rate of the composites due to the interactions between PAA and Polyactive^{TM}.

3. The isocyanate group reacts with surface hydroxyl groups of HA and forms a covalent bond with the mineral.

4. Isocyanate group containing coupling agents offer a simple and effective way to graft various polymers to the surface of hydroxyapatite. It is an alternative for silane coupling agents. It will be very useful in surface modification of hydroxyapatite particles for different purposes.

5. The realization of chemical bonding between HA particles and polymer matrix by the

use of isocyanate coupling agents greatly improves the mechanical properties of the composites.

Future directions

Some of the findings in this thesis provide a basis for future studies.

1. The experiments concerning the use of PAA and EMa as bonding agents (Chapter 2, 3, 4) indicate that the PAA and EMa have strong interactions with both PEG and PBT segments of Polyactive^{TM}, suggesting a possible method to modify the mechanical and other properties (except for bone bonding properties) of PEG or PBT containing block copolymers in other applications.

2. The use of isocyanate coupling agents to graft biodegradable polymers such as polylactide and polyglycolide to HA would be very useful in making biodegradable bioactive composites for bone replacement. It also could offer a way to make novel HA/collagen composites as bone substitute material which would be much more similar to natural bone in composition.

3. HA particles grafted with polymers may be used directly to make highly loaded HA/polymer composites by sintering at relatively low temperatures (< 300 ^{o}C) or by other methods. The brittleness of high temperature sintered HA may be improved by the introduction of polymers.

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Summary

To improve the mechanical properties and the bioactivity of Polyacitve™, hydroxyapatite particles were chosen as filler to reinforce the polymer. In making composites, the interface between hydroxyapatite particles and polymer plays an important role in determining the ultimate mechanical properties of the composites. Therefore, much effort has been devoted to the development of methods to improve the interface of HA and polymer in this study. Although silane coupling agents have been widely used in making various mineral/polymer composites, such as in dental restorative resin and bone replacement materials, their effect on the HA/polymer composites is still doubtfull. Therefore, two new methods to improve the interface between HA and Polyactive™ were developed in this thesis.

In **chapter 2**, polyacrylic acid (PAA) and ethylene-maleic acid (EMa) copolymer were used as coating of HA particles to introduce interaction between sintered large HA particles (1- 45 μ) and PEG segments of Polyactive™ to improve the interface between HA and Polyactive™ 70/30. The effectiveness of using PAA and EMa as bonding agent was confirmed by mechanical testing and SEM observations. In **chapter 3**, PAA and EMa were also found to be effective in improving the interface of HA with Polyactive™ 30/70 which has much less PEG segments than Polyactive™ 70/30. A further study reported in **chapter 4** indicates that besides the pH dependent interactions such as hydrogen bonds and dipole interactions between PAA (or EMA) and the PEG soft segments of Polyactive™ there also exists a pH independent hydrophobic interaction between PAA (or EMa) and the PBT hard segments of Polyactive™.

Hydrothermally synthesized nano-apatite acicular particles are reported to have more similarity to natural bone minerals in many aspects and thus more bioactivity is expected from nano-apatite/Polyactive™ composites. It has been found in **chapter 5** that addition of small a amount of nano-apatite (10% wt.) to Polyactive™ 70/30 matrix greatly accelerates the calcification rate of composites in 1.5 times SBF solution which suggests that nano-apatite is very likely to be more bioactive than sintered coarse HA particles. However, the use of nano-apatite as filler decreased the tensile strength. The use of PAA as coating to improve the interface of nano-apatite with Polyactive™ had no effect on the mechanical properties of the composites and had a negative effect on the calcification rate of the composites, which in turn suggested that another method or coupling agent should be considered. The fact that with these nano-apatite particles no homogeneous dispersion within polymer matrix could be achieved might be the reason for the decreased mechanical properties.

Since it is very difficult to induce calcification in Polyactive™ 70/30 in SBF, an *in vitro* model called accelerated calcification solution (ACS) was developed to evaluate the calcification behaviour of Polyactive™ and its composites (**chapter 6)**. Calcification can be induced in Polyactive™ 70/30 and its composites within a short period in ACS at 37 ^{o}C. The calcification process of a material in ACS mimics, at least to some extent, the in *vivo* biological mineralization process since the process starts with the nucleation of octacalcium phosphate (OCP) and transformation of OCP to apatite during the crystal growth. This in vitro calcification model has been proved to be useful in evaluating the calcification behaviour of biomaterials.

As an attempt to find another way to improve the interface of HA with Polyactive™ we studied the feasibility of using hexamethylene diisocyanate (HMDI) as coupling agent to covalently bond polymers such as polyethylene glycol to the surface of nano-apatite (**chapter 7**). Solid ^{1}H MAS NMR and IR spectra indicated that organic isocyanate groups of HMDI have the ability to react with surface hydroxyl groups of nano-apatite. Relatively large amounts of PEG 1500 (20 % wt.) can be bound to the surface of nano-apatite.

The reaction of organic isocyanate groups with surface hydroxyl groups of HA in the presence of alkyl-tin catalyst were further studied in **chapter 8**. It was found that the structure of the isocyanate strongly affects the reaction rate. The reaction of isocyanate seems to follow a second order reaction mechanism despite the heterogenous nature of the reaction. It is also indicated that isocyanateoethyl methacrylate (ICEM) and HMDI are

suitable coupling agents for polymer grafting reactions.

As a model system, a cold curing acrylate system was used to graft polyacrylates due to its easy handling and potential use in bone cements. In **chapter 9** it is reported now several polyacrylates, such as polymethyl methacrylate (PMMA), polyhydroxylethyl methacrylate (poly(HEMA)) and polybutyl methacrylate (PBMA) were successfully grafted to the surface of HA by using either ICEM or HMDI, or HMDI together with HEMA as coupling agents.

Finally, also Polyactive^{TM} 70/30 was successfully grafted to the surface of HA via HMDI (**chapter 10**), thus realizating a covalent bonding between HA and the Polyactive^{TM} 70/30 matrix. Mechanical testing showed that the mechanical properties of the composites were greatly improved by the introduction of a chemical bond between HA and the polymer matrix.

Based on the experimental results we conclude that introduction of covalent bonds between HA filler particles and polymer matrices with isocyanate coupling agents is an effective way to improve the mechanical properties of the composites. It also offers a method for the preparation of composites with unusual high filler/polymer ratio's.

Samenvatting

Teneinde de mechanische eigenschappen en de bioactiviteit van de kunststof Polyactive^{TM} te verbeteren werden hydroxyapatiet (HA) poederdeeltjes als vulstof toegepast. Bij de vervaardiging van composieten heeft het grensvlak tussen vulstofdeeltjes en het polymeer een belangrijke invloed op de uiteindelijk te bereiken mechanische eigenschappen. Er is daarom veel aandacht gewijd aan het ontwikkelen van methoden om de kwaliteit van het grensvlak tussen HA en het polymeer te verbeteren. Koppelstoffen op basis van silanen worden veelvuldig toegepast voor dit doel, zoals bijvoorbeeld in tandheelkundige restauratiematerialen en sommige botvervangingsmaterialen, maar de effectiviteit ervan in het geval van HA/polymeer composieten is twijfelachtig. Er werden daarom twee nieuwe methoden voor dit doel ontwikkeld en beschreven in dit proefschrift.

In **Hoofdstuk 2** wordt bechreven hoe polyacrylzuur (PAA) en een ethyleen-maleinezuuranhydride coplymeer (EMa) werden gebruikt als coating van relatief grote, gesinterde HA deeltjes (1 - 45 μm) teneinde de interactie tussen deze deeltjes en de polyethyleenglycol (PEG) segmenten van Polyactive^{TM} 70/30 te bevorderen. De effectiviteit van PAA en EMa in deze kon worden bevestigd aan de hand van mechanische testen en SEM waarnemeningen. In **Hoofdstuk 3** wordt beschreven dat PAA en EMa eveneens effectief zijn in het geval van HA en Polyactive^{TM} 30/70 dat veel minder PEG segmenten bevat dan het 70/30-materiaal. Een nadere studie in **Hoofdstuk 4** wees uit dat er naast pH afhankelijke interacties tussen PAA of EMa en de PEG segmenten - zoals waterstofbindingen en dipoolinteracties - ook pH onafhankelijke, hydrophobe interacties voorkomen tussen de coatings en de harde polybutyleentereftalaat (PBT) segmenten van het polymeer.

Van hydrothermisch gesynthetiseerde, naaldvormige nano-HAdeeltjes is eerder een betere overeenkomst met natuurlijk voorkomend botmineraal gerapporteerd. Hierom kan verwacht worden dat composieten van Polyactive^{TM} met deze nano-apatiet deeltjes ook een hogere bioactiviteit zullen vertonen. In **Hoofdstuk 5** wordt beschreven hoe toevoeging van een geringe hoeveelheid (10 gew%) van deze deeltjes aan een Polyactive^{TM} 70/30 matrix de verkalkingssnelheid van het composiet in 1.5 SBF-oplossing (Simulated Body Fluid) aanzienlijk verhoogt. Deze bevinding bevestigt het vermoeden dat nano-apatiet een hogere bioactiviteit bezit dan gesinterde HA-deeltjes. Het gebruik van nao-apatiet als vulstof had echter een nadelige invloed op de treksterkte van de composieten. Het coaten van de deeltjes met PAA bracht hier geen verbetering in en had daarentegen weer een daling van de verkalkingssnelheid tot gevolg. Er zal dus voor deze vulstof een andere koppelstof gevonden moeten worden. De reden voor de nadelige invloed van deze nano-apatiet vulstof op de mechanische eigenschappen van de composieten

wordt gezocht in het feit dat het bijzonder moeilijk bleek te zijn de deeltjes zonder agglomeraatvorming homogeen in de polymeermatrix te verdelen.

Het optreden van verkalking in Polyactive^{TM} 70/30 verloopt bijzonder traag en daarom werd een *in vitro* model, genaamd "accelerated calcification solution" (ACS), ontwikkeld om het calcificatiegedrag van Polyactive^{TM} en de composieten ervan te evalueren (**Hoofdstuk 6).** Met behulp van ACS kan verkalking van Polyactive^{TM} 70/30 en composieten bij 37 °C binnen een korte tijd worden bewerkstelligd. Het verkalkingsproces van een materiaal in ACS vertoont, althans in zekere mate, gelijkenis met *in vivo* verkalking omdat het proces begint met vorming van octacalciumfosfaat kiemen die gedurende de kristalvorming transformeren tot apatiet. Dit *in vitro* calcificatiemodel is zeer bruikbaar bevonden om het verkalkingsgedrag van materialen te bestuderen.

Als een aanzet tot het vinden van een andere methode om het grensvlak tussen HA en Polyactive^{TM} te verbeteren werd onderzoek gedaan naar de mogelijkheid om hexamethyleendiisocyanaat (HMDI) te gebruiken als koppelstof waarmee polymeren als polyethyleenglycol covalent aan het oppervlak van nano-apatiet deeltjes gebonden kunnen worden (**Hoofdstuk 7**). Vaste stof ^{1}H MAS NMR en IR spectra wezen uit dat de isocyanaatgroep in staat is tot reactie met de hydroxylgroepen aan het oppervlak van nano-apatiet. Relatief grote hoeveelheden PEG 1500 (20%) bleken zo aan het nano-apatiet oppervlak gebonden te kunnen worden.

De reactie van de organische isocyanaatgroep met oppervlakte hydroxylgroepen van HA in de aanwezigheid van aan alkyl-Tin kataysator wordt nader bestudeerd in **Hoofdstuk 8**. Gevonden werd dat de struktuur van het isocyanaat de reactiesnelheid sterk beinvloed. Het reactiemechanisme lijkt een tweede orde mechanisme te volgen ondanks het heterogene reactiemengsel. Het onderzoek wees eveneens uit dat isocyanoethylmethacrylaat (ICEM) en HMDI bijzonbder geschikte koppelstoffen zijn voor het enten van polymeren aan HA.

Een koudhardend acrylaat cement, gemakkelijk hanteerbaar en gebruikt als botcement, werd gebruikt als model systeem om polyacrylaten aan HA te enten. In **Hoofdstuk 9** wordt beschreven hoe acrylaten als polymethylmethacrylaat (PMMA), polyhydroxyethylmethacrylaat (polyHEMA), en polybutylmethacrylaat (PBMA) met succes aan het oppervlak van HA poederdeeltjes kunnen worden geënt door gebruikmaking van ICEM of HMDI of HMDI samen met HEMA als koppelagentia.

Tenslotte werd ook Polyactive^{TM} 70/30 met succes aan het oppervlak van HA deeltjes geënt door gebruikmaking van HMDI als koppelstof (**Hoofdstuk 10**). Hiermee werd dus een covalente binding verkregen tussen HA en de polymere matrix. Mechanische testen toonden aan dat de mechanische eigenschappen van aldus samengestelde composieten sterk verbeterd waren.

Gebaseerd op deze experimentele resultaten wordt geconcludeerd dat het introduceren van covalente bindingen tussen HA vulstofdeeltjes en de polymere matrix met behulp van isocyanaatgroepen bevattende koppelstoffen een effectieve manier is om de mechanische eigenschappen van de composieten te verbeteren. Ook de vervaardiging van composieten met ongebruikelijk hoge vulstof/polymeer verhoudingen kan langs deze weg worden gerealiseerd.

Samenvatting

Teneinde de mechanische eigenschappen en de bioactiviteit van de kunststof Polyactive™ te verbeteren werden hydroxyapatiet (HA) poederdeeltjes als vulstof toegepast. Bij de vervaardiging van composieten heeft het grensvlak tussen vulstofdeeltjes en het polymeer een belangrijke invloed op de uiteindelijk te bereiken mechanische eigenschappen. Er is daarom veel aandacht gewijd aan het ontwikkelen van methoden om de kwaliteit van het grensvlak tussen HA en het polymeer te verbeteren. Koppelstoffen op basis van silanen worden veelvuldig toegepast voor dit doel, zoals bijvoorbeeld in tandheelkundige restauratiematerialen en sommige botvervangingsmaterialen, maar de effectiviteit ervan in het geval van HA/polymeer composieten is twijfelachtig. Er werden daarom twee nieuwe methoden voor dit doel ontwikkeld en beschreven in dit proefschrift.

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Curriculum Vitae

Qing LIU was born on June 22, 1962 in Changzhi city, Shanxi Province, P. R. China, where he completed both his primary school and middle school (The First Middle School of Changzhi City). He went to Tsinghua University (Beijing) in 1979 after his graduation from middle school. In 1984, after spending 5 years of study at the Department of Chemical Engineering, he got his Bachelor of Engineering degree in the field of polymer chemistry and engineering. After spending three more years in studying biomaterials (supervised by Prof. Dr. Yue Yilun) at the Department of Polymer Materials and Science, Chengdu University of Science and Technology, he was awarded the Master of Engineering degree in 1987. He then worked as a Research Assistant at the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, P.R. China. In 1993, he went to Institute of Macromolecules Chemistry, Czech Academy of Science, Prague, Czech Republic, where he worked as a Visiting Scholar to study controlled drug release system (Prof. Dr. K. Ulbrich). He started his Ph.D research at Biomaterials Research Group, Leiden University in August, 1994. After the completion of his Ph.D studies, he will work as a Post-doctoral Research Fellow at the Department of Chemical Engineering (Prof. Dr. A. G. Mikos), Rice University, Houston, USA.