HA Coated Magnetic Nanoparticles for the Treatment of Osteoporosis

Ganesan Balasundaram[1] and Thomas J. Webster[1,2]
[1]Weldon School of Biomedical Engineering
[2]School of Material Engineering
Purdue University, West Lafayette, Indiana, 47907, USA

1. INTRODUCTION

There are several major barriers that exist for the use of any pharmaceutical agents to stimulate new bone formation. First, the agents can cause non-specific bone formation in areas not desirable. This is because these agents are often delivered in non-specific ways (such as through the mouth, directly into the blood stream, etc.). Second, if delivered locally to the tissue around the area of low bone density, they rapidly diffuse to adjacent tissues which limit their potential to promote prolonged bone formation in targeted areas of weak osteoporotic bone. In this experiment, we use nanotechnology (or the design of materials with 10^{-9} m dimensions) to develop novel drug-carrying systems that will specifically attach to osteoporotic (not healthy) bone. Moreover, some of these novel drug carrying systems will then distribute pharmaceutical agents locally to quickly increase bone mass. Magnetic nanoparticles are also of interest. Specifically, the main interest for the use of magnetic nanoparticles in biomedical applications is that an inhomogeneous external magnetic field exerts a force on them, and thus they can be manipulated or transported to a specific diseased tissue by a magnetic field gradient [1]. They also have controllable sizes, so that their dimensions can match either that of a virus (20–500 nm), of a protein (5–50 nm) or of a gene (2 nm wide and 10–100 nm long). In addition, magnetic particles are of interest because they do not retain any magnetism after removal of the magnetic field.

Specifically in this study, efforts will focus on the prolonged release of bioactive agents to efficiently regenerate enough bone for a patient to return to a normal active lifestyle. Specifically, inorganic biodegradable nanoparticles (including ceramics like hydroxyapatite or HA) will be functionalized with bioactive compounds that bond to bone of low mass. Such bioactive groups will be placed on the outer surface of the magnetic nanoparticle systems using various techniques (such as covalent chemical attachment). After bonding specifically to osteoporotic bone and not healthy bone, magnetic nanoparticle systems will deliver bioactive compounds to locally increase bone mass. Lastly, the outer coating of the embedded nanoparticle systems will be created to have different biodegradation rates for the release of bone-building agents over various time spans; this will allow for not only quick bone formation but also long-term sustained bone regeneration. One potential advantage of formulating HA magnetic nanoparticles are that as the magnetic particles accumulate, e.g., in bone tissue, they can play an important role in detection through MRI to locate, monitor and control drug activities.

II. EXPERIMENTAL

HA is chemically similar to the mineral component of bones and hard tissues in mammals. It is one of few materials that are classified as bioactive, meaning that it will support bone ingrowth and osseointegration when used in orthopaedic, dental and maxillofacial applications. This is because bone itself is composed of hydroxyapatite and other calcium phosphates. When loaded with bioactive compounds, such systems can also release the drug at therapeutic concentrations directly to the needed area. The implant of this type is capable of releasing the bioactive agents over the entire period of

resorption. To achieve a fast resorption rate, amorphous calcium phosphate and nanocrystalline HA drug delivery carriers are excellent candidates.

A. Chemical Synthesis / Material Characterization of Hydroxyapatite (HA) Coated Magnetic Nanoparticles

HA powders can be synthesized via numerous production routes, using a range of different reactants. Some processing techniques include: wet chemical methods (precipitation), hydrothermal techniques, sol-gel, and hydrolysis of other calcium phosphates. We synthesized HA nanoparticles by a wet chemical process followed by hydrothermal treatment. The wet chemical routes to synthesize magnetite nanoparticles are simpler, more tractable and more efficient with appreciable control over size, composition and sometimes even the shape of the nanoparticles. For the HA coated magnetic nanoparticles, the pH of magnetite (Fe_3O_4) nanoparticle dispersions was adjusted to 10 by adding NH_4OH followed by the addition of diammonium hydrogen phosphate and calcium nitrate. This precipitated HA is then processed hydrothermally at 70°C and 200°C for 20 h. High crystallization is achieved at relatively low temperatures but under a higher pressure than atmospheric. As a result, nano-sized amorphous calcium phosphate and nano-crystalline HA can be obtained.

Material properties of the synthesized products were characterized by X-ray diffraction, inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to measure Ca/P ratio, particle size analyzer to measure mean agglomerated particle size, BET to measure grain/particle size and TEM to analyze HA particle morphology. Standard techniques were followed for each characterization method.

B. Chemical Functionalization of Nanoparticles

For the attachment of bioactive compounds, we will use silane chemistry [2]. Alkoxysilanes were chosen in this study because of their ability to graft to hydroxyl terminated surfaces of HA. It is expected that the surface energy of biomaterials can be controlled using the attachment site (e.g., silane), spacer (e.g., alkane), and end group (e.g., amine). It is important to note that although aminophase chemistry has been used for glass, little to no reports are in the literature for its use on HA. The amine treated substrates can be treated with hetero-bifunctional cross-linker, N-succinimidyl-3-maleimido-propionate (SMP), in N,N-dimethyl formamide (DMF), resulting in substrates with exposed maleimide groups. Finally bioactive molecules can be attached through the covalent addition of the cysteine thiol (-SH) group to the maleimide group.

Peptide functionalization of calcium phosphate-based materials was characterized by a well established CBQCA (3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde) method [3]. Inherently CBQCA is a non-fluorescence molecule but upon reaction with amine groups in the presence of cyanide or thiols, it fluoresces well.

III. RESULTS

A. Synthesis of Magnetic Nanoparticles of Calcium Phosphate-based Materials

Material properties of the nano-amorphous calcium phosphate, nano-crystalline HA, and conventional HA particles are summarized in Table 1. Specifically, X-ray diffraction provided evidence of only one material phase in both nano-crystalline HA and conventional HA, while no crystalline phases were determined for the nano-amorphous calcium phosphate particles. BET provided evidence that nano-crystalline HA and nano- amorphous calcium phosphate had 31 and 13 nm particle sizes, respectively, while conventional HA possessed a particle size of 7400 nm. All particle types significantly agglomerated into micron sizes; specifically, nano-crystalline HA and nano-amorphous calcium phosphate agglomeration sizes were 5.21 and 8.84 μm, respectively, while conventional HA agglomerated to 169 μm. Lastly, nano-crystalline HA and nano- amorphous calcium phosphate particle shapes were irregular while conventional HA possessed cylindrical shapes. Degradation experiments for all compacts in cell culture media showed that nano-amorphous calcium phosphate displayed a higher degradation property compared to nano-crystalline HA, while conventional HA displayed a very low degradation property compared to other compacts.

Table 1. *Summary of material properties of nano-amorphous calcium phosphate, nano-crystalline HA and conventional HA.*

Characterization	**Nano Amorphous Calcium Phosphate**	**Nano Crystalline HA**	**Conventional HA**
Crystalline phase	--------	HA	HA
Ca/P ratio	1.66	1.61	1.63
BET surface area [m^2/g] (Particle or grain size [nm])	142.11 (13)	62.165 (31)	0.26 (7400)
Agglomerate size [μm] (Median [μm])	8.78 (8.84)	4.84 (5.21)	120 (169)
Particle morphology	Irregular shape	Irregular shape	Cylindrical
Degradation	High	Low	Very low

Particles made from iron oxide usually behave differently in magnetic fields depending on their size. To check the magnetic property of the synthesized nanoparticles, magnetic fields were applied externally. Interestingly, both magnetically coated nano-amorphous calcium phosphate and nano-crystalline showed strong magnetic properties (Figure 1).

(a) (b)

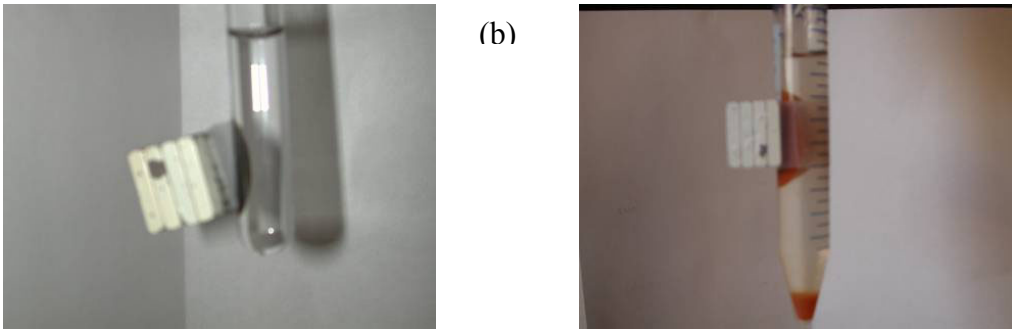

Figure 1. Images of magnetic nanoparticles of calcium phosphate-based materials upon applying external magnetic fields. Pictured here are the external magnets with magnetic (a) nano-amorphous calcium phosphate and (b) nano-crystalline HA particles in solution.

B. Chemical Functionalization of Calcium Phosphate-based Nanoparticles

The model bioactive compound chosen for the biofunctionalization was YRGDSPC peptide. Overall, results of the present study demonstrated the ability to functionalize these amino acid groups on the nano amorphous calcium phosphate, nano-crystalline HA and conventional HA (Figure 2). Specifically, in the absence of the CBQCA, APTES treated HA showed no fluorescence (2a1, 2b1, 2c1). In contrast, in the presence of CBQCA, the same materials showed very good fluorescence (2a2, 2b2, 2c2). As expected, SMP treated materials showed no fluorescence (2a3, 2b3, 2c3). Finally, peptide attached surfaces showed strong fluorescence due to the presence of amine molecules in the peptide (2a4, 2b4, 2c4). This preliminary experiment confirmed the ability to successfully functionalize HA and calcium phosphate-based nanoparticles using aminosilane chemistry followed by a model RGD peptide.

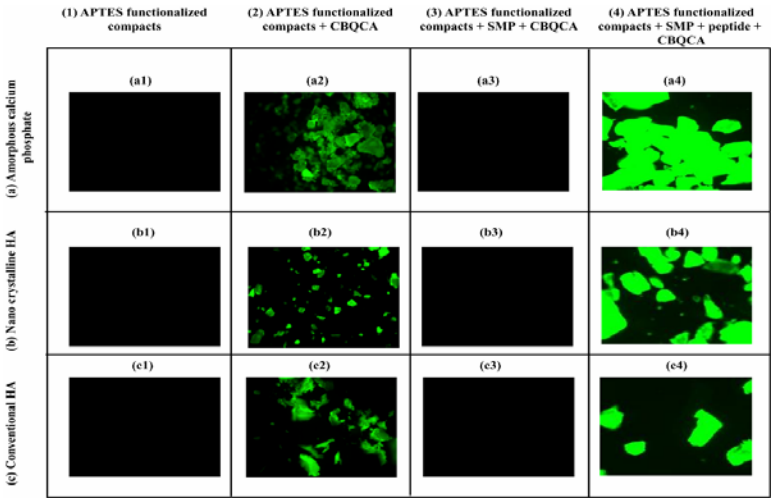

Figure 2. Fluorescent images of (a) nano amorphous calcium phosphate, (b) nano crystalline hydroxyapatite and (c) conventional hydroxyapatite functionalization; (1) HA+APTES, (2) HA+APTES+CBQCA, (3) HA+APTES+SMP+CBQCA, and (4) HA+RGD peptide functionalized. APTES=3-Aminopropyltriethoxysilane; CBQCA=3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde. Magnification = 10X.

IV. DISCUSSION

The results of this study provided evidence of the synthesis of nano-amorphous calcium phosphate and nano-crystalline HA coated magnetic particles. In addition, results show the ability to functionalize amino groups not only on conventional HA but also on the nanophase HA and calcium phosphate compacts; critical criteria to allow attachment of other bioactive molecules that will be directed to osteoporotic bone to rebuild bone mass. In particular, RGD was used as a model peptide in this study and was immobilized on the calcium phosphate-based compacts via aminosilane chemistry followed by a maleimide cross-linker molecule. By fabricating highly degradable nano-amorphous calcium phosphate and slowly degradable nano-crystalline HA, materials that can provide for a wide range of drug release profiles have been created. Next steps in this research are to determine attachment efficiency to osteoporotic compared to healthy bone, imbed bone building agents into the nanoparticles, determine release profiles once attached to weak bone, and determine bone regeneration.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

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