Nano-Structured Biodegradable Ceramics for the Treatment of Osteoporosis

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ABSTRACT

In this study, nanotechnology (or the design of materials with 10^{-9} m dimensions) was used to develop novel drug-carrying systems that specifically attach to osteoporotic (not healthy) bone. Some of these novel drug carrying systems distribute pharmaceutical agents locally to quickly increase bone mass. These efforts focus on the prolonged release of bioactive agents (specifically, bone morphogenetic protein-2 (BMP-2)) to efficiently regenerate enough bone for the patient to return to a normal active lifestyle. Particularly calcium phosphate-based nanomaterials were functionalized with bioactive chemicals (such as RGD, a model peptide known to increase bone cell function). The outer coating of the embedded nanoparticle systems were also created to have different biodegradation rates for the controlled release of embedded bioactive agents to the target site. In this manner, ceramic nanoparticle drug delivery systems were developed for fighting osteoporosis.

Keywords: Nanomaterial, Osteoporosis, Hydroxyapatite

INTRODUCTION

Skeletal complications resulting from osteoporosis are an important healthcare problem. Although osteoporosis has been studied for a number of years, no current effective prevention and treatment methods exist for this disease (1). There are several major barriers that exist for the use of any pharmaceutical agents to stimulate new bone formation. In this study, nanotechnology (or the design of materials with 10^{-9}m dimensions) was used to develop novel drug-carrying systems that specifically attach to osteoporotic (not healthy) bone. Moreover, some of these novel drug carrying systems distribute pharmaceutical agents locally to quickly increase bone mass. These efforts focus on the prolonged release of bioactive agents (specifically, bone morphogenetic protein-2 (BMP-2)) to efficiently regenerate enough bone for the patient to return to a normal active lifestyle (2). Particularly, inorganic biodegradable nanomaterials (including ceramics like hydroxyapatite or HA) were functionalized in this study with bioactive chemicals (such as RGD, a model peptide known to increase bone cell function). Such bioactive groups were placed on the outer surface of the nanoparticle systems using various techniques resulting in covalent chemical attachment. The outer coating of the embedded nanoparticle systems were created to have different biodegradation rates for the controlled release of embedded bioactive agents to the target site.

METHODS

Nano-amorphous calcium phosphate and nano-crystalline HA were synthesized by a wet chemical process followed by hydrothermal treatment at 70^0C and 200^0C, respectively (3). High crystallization was achieved at relatively low temperatures but under a higher pressure than atmospheric. Conventional HA was also synthesized by sintering the above particles at 1100^0C.

For the attachment of bioactive compounds to HA and amorphous calcium phosphate nanomaterials, we used aminophase chemistry (Fig. 1). Aminophase chemistry is a widely used technique primarily because it has been utilized to functionalize several biological molecules to promote cell adhesion [3].

Each step of the bioactive functionalization was confirmed by a novel CBQCA (3-4-carboxybenzoyl quinoline-2-carboxaldehyde) fluorescence method (3). CBQCA is inherently a non-fluorescent molecule but fluoresces well when attached to amine groups that arise from the aminated surfaces and the amines from bioactive group moieties.

Compacts were sterilized under UV light for 4 h prior to cell experiments. Human osteoblasts (bone-forming cells; CRL-11372 American Type Culture Collection, population numbers 7–8) in DMEM supplemented with 10% fetal bovine serum (Hyclone) and 1% Penicillin/Streptomycin (Hyclone) were seeded at a density of 3500 cells/ cm^2 onto the compacts of interest and were then placed in standard cell culture conditions for 4 h. After the prescribed time period, substrates were rinsed in phosphate buffered saline to remove any non-adherent cells. The remaining cells were fixed with formaldehyde, stained with Hoescht 33258 dye, and counted under a fluorescence microscope. Five random fields were counted per compact. All experiments were run in triplicate and repeated at least three separate times. Standard t-tests were used to check statistical significance between means.

RESULTS AND DISCUSSION

Material properties of the nanoamorphous calcium phosphate, nanocrystalline HA, and conventional HA particles are summarized in Table 1. Specifically, X-ray diffraction (XRD) provided evidence of only one material phase in both nanocrystalline HA and conventional HA,

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[a]	High (3mg/day)	Low (2.14mg/day)	Very Low (0.29mg/day)

[a]Degradation taken between 14 and 21 days of immersion in cell culture media.

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 13nm particle sizes, respectively, while conventional HA possessed a particle size of 7400nm. All particle types significantly agglomerated into micron sizes; specifically, nano-crystalline HA and nano-amorphous calcium phosphate agglomeration sizes were 5.21 and 8.84μm, 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 placed in DMEM cell culture media showed that nano-amorphous calcium phosphate had a greater degradation profile (3mg/day) compared to nano-crystalline HA (2.14 mg/day), while conventional HA had a very low degradation profile compared to the other compacts (0.29mg/day).

HA-peptide functionalization was characterized by the CBQCA method. Overall the results indicated the ability to functionalize bioactive groups (such as RGD in this study) onto the nanophase HA and calcium phosphate materials; critical criteria to allow attachment of other drug molecules, particularly BMP-2 and agents that will direct nanoparticles to osteoporotic bone.

Cell adhesion experiments showed greater adhesion of osteoblasts to calcium phosphate-based compacts functionalized with immobilized RGD peptides compared to either respective compacts functionalized with RGE or the non-functionalized (plain) compacts (Fig. 1).

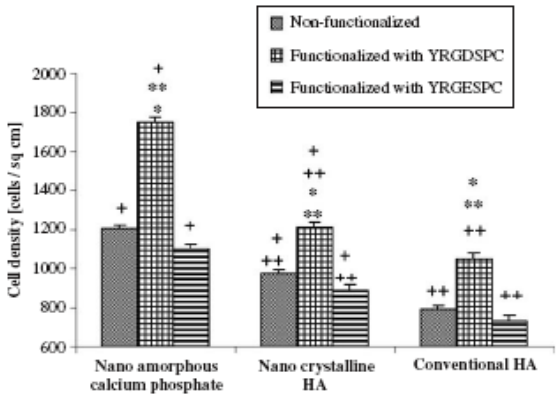

Figure 1: Greatest osteoblast adhesion on amorphous calcium phosphate compact functionalized with YRGDSPC. Values are mean ± SEM; n=3; *p<0.01 compared to respective non-functionalized compacts, **p<0.01 compared to respective compacts functionalized with YRGESPC (negative control), +p<0.01 compared to respective conventional HA functionalization, and ++p<0.01 compared to respective nano amorphous calcium phosphate functionalization.

Strikingly, osteoblast adhesion on conventional HA functionalized with RGD is statistically similar to unfunctionalized nano-amorphous calcium phosphate. In this manner, this study provided the first evidence that the benefits of immobilizing RGD on conventional HA can be matched by using non-functionalized nano-amorphous calcium phosphate. In addition, results provided evidence of increased osteoblast adhesion on nano-amorphous calcium phosphate compared to nano-crystalline HA and conventional HA. Increased osteoblast adhesion on nano-crystalline compared to conventional HA confirmed that of previous studies [4]. However, this may be the first time increased osteoblast adhesion was observed on nano-amorphous calcium phosphate compared to nano-crystalline HA.

CONCLUSIONS

The results of this study provided evidence of the synthesis of HA nanoparticles with different nanometer sizes and degradation properties. In addition, results show the ability to functionalize peptide 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 for numerous applications. In particular, the cell adhesion peptide (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. Each reaction step was characterized by a novel, versatile CBQCA approach. Osteoblast cell experiments provided the first evidence that the benefits of immobilizing RGD on conventional HA can be matched by using non-functionalized nano-amorphous calcium phosphate. Also, this may be the first time increased osteoblast adhesion was observed on nano-amorphous calcium phosphate compared to nano crystalline HA.

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REFERENCES

[1]. Barbucci R. Integrated Biomaterials Science. 2002, New York: Kluwer Academic Publishers, p 189-689.
[2]. Rengachary SE. Neurosurg Focus. 2002, 13, 1-6.
[3]. Balasundaram G, Sato M, Webster TJ. Biomaterials. 2006, 27, 2798–2805.
[4]. Webster T.J, Siegel R.W, Bizios R. Biomaterials. 2000, 21, 1803-1810.