Nano-structured Biodegradable Ceramics for the Prevention and Treatment of Bone Cancer

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ABSTRACT

In the present study, functionalized bioresorbable nano-materials (less than 50 nm in size) were formulated to specifically attach to cancerous (not healthy) bone to form an implant used to treat bone cancer. After attachment, sustained release of anti-cancer agents occurred at targeted sites. Specifically, inorganic biodegradable nanomaterials (including ceramics like hydroxyapatite or HA) were functionalized with anti-cancer drugs (such as doxorubicin using covalent chemical attachment). The outer coating of the embedded nanoparticle systems will also be created to have different biodegradation rates for the controlled release of anticancer agents to the target site. In this study, we provide evidence of synthesizing highly degradable nano-amorphous calcium phosphate particles and slowly degradable nano-crystalline HA particles as drug delivery carriers to treat bone cancer.

Keywords: Bone cancer, Drug delivery, Hydroxyapatite

INTRODUCTION

Skeletal complications resulting from bone cancer are an important healthcare problem. Although bone cancer has been studied for a number of years, no current effective prevention and treatment methods exist for this disease. The response assessment of bone cancer to therapy is difficult and it takes several months for bone lytic lesions to show signs of recovery. Also, complete response of bone lesions to treatment is very rare. Further, drug delivery is still considered a weak link in drug development, with more than 95% of all new potential therapeutics having poor pharmacokinetics. The difficulties associated with oral delivery of complex anti-cancer formulations presents an even greater obstacle when treating localized or specific indications, such as bone cancer, as compared with systemic indications.

This opens the need for an orthopedic implant device to properly return function lost by missing bone to the patient. Beforehand an intelligent drug targeting system is essential to avoid complications associated with the bone implantation materials. The potential for the use of nanoparticles in the task of drug delivery to the target site is huge and may change the way of currently used drug delivery systems. Many anti-cancer agents can be incorporated into biocompatible materials to form the implant. Among the anti-cancer agents available today, the most effective for treating bone cancer is doxorubicin [1].

The implant can be used for targeted chemotherapy. The implant can be surgically placed at or around the tumor site or within the bone directly adjacent to the tumor. Moreover, these drug delivery devices can be chemically functionalized to specifically attach to cancerous (not healthy) bone. After attachment to cancerous bone, the anti-cancer agents are released continuously or periodically from the implant at a desired rate. Periodical release can be achieved with nanoparticles and nanoparticles loaded with anti-cancer agents. The anti-cancer molecules then approach the tumor cells preferentially as a result of their proximity. The release rate and release profile can be tailored by the morphologies of the biocompatible implant. To achieve a fast resorption rate, amorphous calcium phosphate and nanocrystalline HA drug delivery carriers are excellent candidates [2]. The objective of the present study was to create nanoparticle systems that have different biodegradation rates for the controlled release of embedded bioactive agents to the target site.

METHODS

The process used to synthesize the calcium phosphate-based particles is outlined below in equation 1. Specifically, HA was precipitated through a well-established wet chemical process [3]. Concentrated ammonium hydroxide was used to maintain the reaction mixture at a pH of 10 throughout the reaction. 0.6 M ammonium phosphate and 1.0 M calcium nitrate were also added slowly (3.6ml/min). Calcium phosphate precipitation occurred while stirring for 20 hours at room temperature.

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \longrightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 20NH_4NO_3 \quad (1)$$

For the preparation of nano amorphous calcium phosphate, the above mentioned precipitated particles were washed with deionized water and treated hydrothermally (Parr Instrument) at 70°C for 20 hours [3]. The white powder thus obtained was dried under vacuum prior to pressing into compacts.

For the preparation of nano crystalline HA, the above mentioned precipitated particles were processed hydrothermally at 200°C for 20 hrs. During hydrothermal treatment, high crystallization can be achieved at relatively low temperatures but under a higher pressure than

atmospheric. As a result, nano-sized crystalline HA can be obtained.

The resulting particles (both nano-amorphous calcium phosphate and nano-crystalline HA) were then separately crushed and pressed into compacts (approximately 10 mm in diameter and 1–1.5 mm in height) via a uniaxial pressing cycle that reached up to 1 GPa over a 10 min period. For the preparation of conventional HA, the pressed nano amorphous calcium phosphate compacts were sintered at 1100^0C in air for 2 hrs (with a kiln ramp rate of 22^0C/min).

RESULTS AND DISCUSSION

Material properties of the nano-amorphous calcium phosphate, nano-crystalline HA, and conventional HA particles are summarized in Table 1. Specifically, X-ray diffraction (XRD) 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 nanocrystalline 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 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).

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]	142.11	62.165	0.26
(Particle or grain size[nm])	(13)	(31)	(7400)
Agglomerate size [μm]	8.78	4.84	120
(Median [μm])	(8.84)	(5.21)	(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.

When the aforementioned particles were compacted, SEM analysis provided evidence of increased nanometer surface roughness for the nano amorphous calcium phosphate compared to nano crystalline HA; compacts of nano-crystalline HA appeared more rough than conventional HA compacts (Figure 1). The specific surface area of nano-amorphous calcium phosphate synthesized and processed at low temperatures (Figure 1a) was significantly larger than that of nano-crystalline HA (Figure 1b). Figure 1c revealed that significant morphological transformations occurred when nano-crystalline HA was sintered to conventional HA.

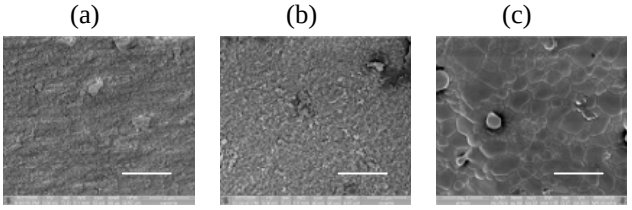

Figure 1. Scanning Electron Microscopy Images of Calcium Phosphate-based Compacts. Increased compact surface roughness was observed on (a) nano-amorphous calcium phosphate compared to (b) nano-crystalline HA and (c) conventional HA. Bars = 10 μM.

CONCLUSIONS

The results of this study provided evidence of the synthesis of calcium phosphate-based nanoparticles with different nanometer sizes and degradation properties. Studies have also provided evidence of the ability to immobilize chemical groups on such particles. Further studies involve how these nano-sized materials will have specific properties which make them competitive drug carriers for the musculoskeletal system, particularly to treat bone cancer.

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