Biomimetic Citrate-Presenting Osteoinductive Composites

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Statement of Purpose: A prevailing strategy to improve the mechanical integrity and bioactivity of orthopedic materials has been to incorporate calcium phosphate ceramics to better mimic the native composition of bone.[1] However, prior research has shown that bone is not only composed of a nanocomposite of collagen and inorganic minerals, but is also rich in citrate content. Citrate, the ionic conjugate base of citric acid, is historically known as an intermediate of the Kreb's cycle, but studies have shown that a majority of the body's citrate content is located in skeletal tissues playing large roles in metabolism, calcium chelation, hydroxyapatite (HA) formation, and regulating the thickness of bone apatite structure.[2-3] Surprisingly, citrate has not been mentioned in most of the literature related to orthopedic biomaterials in the past 30 years. The natural existence of citrate in bone hints that citrate should be considered in bone biomaterial design, and motivates us to develop the next generation of biomimetic citrate-presenting hydroxyapatite composites, which can match the native composition of bone, provide adequate mechanical support, minimize inflammatory responses, quickly induce bone regeneration, and fully integrate with the surrounding tissue.

Methods: Citrate-based polymer blend hydroxyapatite (CBPBHA) composites were first fabricated by synthesizing and blending together two separate previously developed citrate containing polymers, poly (octanediol) citrate (POC) [4] and crosslinked urethane-doped polyester (CUPE) [5], to provide free pendant citrate moieties for HA binding and increased composite strength, respectively. POC and CUPE were dissolved in 1,4-dioxane and combined with 65-wt.% HA to form various polymer blend ratios composited with HA. Following solvent evaporation, the clay-like mixture was inserted into machined cylindrical metal molds and compressed into rod shaped samples. The resulting cylindrical composites were then post-polymerized for 5 days at 80 °C followed by 120 °C under 2 Pa vacuum for 1 day. Differential scanning calorimetry measurements were conducted to characterize the thermal properties and homogeneity of the polymer blends. Unconfined compression tests, *in vitro* degradation, and *in vitro* mineralization studies were also performed to evaluate the composites. To test the cytocompatibility, osteoblast differentiation of C2C12 cells was evaluated on CBPBHA composites by determining osterix (OSX) and alkaline phosphatase (ALP) gene expression levels. The role of citrate supplementation in culture medium for stem cells and osteoblastic was also investigated. The *in vivo* performance of the CBPBHA composites was evaluated in a rabbit lateral femoral condyle model. Computer tomography analysis was used to determine the extent of osseointegration. Samples were harvested after 6 weeks of implantation and the inflammatory response was characterized using histological and immunohistochemical staining analysis.

Results: CBPBHA composites consist of the newly developed osteoinductive citrate-based polymers, CUPE and POC composited with HA. The results showed that a 10 wt.-% addition of POC into the mechanically viable CUPE/HA network produced materials with a compressive strength of 116.23 ± 5.37 MPa, which falls into the range of human cortical bone (100-230MPa). CBPBHA composites promoted *in vitro* mineralization, and greatly amplified C2C12 OSX gene and ALP gene expression (Fig. 1A). Citrate media supplementation *in vitro* both increased ALP expression in the MG-63 osteoblasts and promoted calcium matrix formation in bone marrow stromal cells (BMSC) in a dose-dependent manner. After 6-weeks implantation in a rabbit lateral femoral condyle defect model, CBPBHA composites demonstrated osseointegration absent of fibrous tissue encapsulation (Fig. 1B).

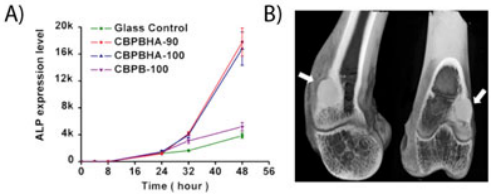

Figure 1. A) ALP gene expression levels of C2C12 cultured on CBPBHA and B) 2D micro-CT images of the CBPBHA implants with surrounding bone of the lateral femoral condyle at 6 weeks of post-implantation.

Conclusions: We have developed a new class of osteoinductive biodegradable citrate-based polymer blend HA (CBPBHA) composites based on our newly developed CUPE, POC, and HA. The discovery of CBPBHA composites bridges the gap in previous bone biomaterial designs in that the role of citrate molecules was inadvertently overlooked. Future studies will focus on further understanding the role of citrate in culture medium for bone stem cell differentiation and optimizing the citrate-content in polymer/HA composites for orthopedic applications. CBPBHA composites represent a new generation of bone biomaterials that address the critical issues such as inflammation, osteoinductivity, and osteointegration. The preliminary understanding on the role of citrates in culture medium and biomaterials is instrumental and it opens new avenues for future bone stem cell culture and bone biomaterial designs.

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References:

[1] Rezwan K. Biomaterials. 2006;27:3413-31.
[2] Eastoe JE. Biochem J. 1954;57:453-9.
[3] Hu YY. Proc Natl Acad Sci USA. 2010;107:22425-9.
[4] Yang J. Biomaterials. 2006;27:1889-98.
[5] Dey J. Biomaterials. 2008;29:4637-49.