Novel Porous Nanostructured Calcium Phosphate Cement Based Scaffolds for Bone Tissue Engineering

A. Roy[1], Hong Wu[2], Hsu-Feng Ko[1], Fatima Syed-Picard[2] C. Sfeir[2], and P. N. Kumta[3].
[1]Department of Bioengineering University of Pittsburgh, Pittsburgh, PA, 15261, USA.
[2]Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA, 15261, USA.
[3]Department of Bioengineering, Chemical Engineering, Mechanical Engineering and Materials Science, and Oral Biology, University of Pittsburgh, PA, 15261, USA.

Statement of Purpose: Various ceramics based scaffolds have been studied for bone tissue regeneration. Most of these scaffolds comprise hydroxyapatite (HA) based ceramics and calcium phosphate cements (CPC). CPCs are moldable, putty like compounds that can be easily introduced into the defect site. Furthermore, they set within a well-defined time at body temperatures resulting in a mechanically stable, osteoinductive material. Additionally, CPCs convert into natural bone like calcium-deficient hydroxyapatite (CDHA) *in vivo* making them attractive for bone tissue engineering. CPCs could be more attractive for clinical applications if they would exhibit better resorption-degradation characteristics *in vivo*. This could be achieved by introduction of pores (macro-, meso- and micro-pores) within the CPC and also better exploit the precursor reaction chemistry to induce conversion of the CPC into nano-crystalline CDHA. The presence of pores accelerates bone tissue colonization with the increased exposed area leading to faster kinetics of resorption. Presence of nano-crystalline CDHA may further enhance the natural bone growth due to improved cell response. The present work describes the synthesis of a novel CPC that serves not only as a scaffold exhibiting excellent cell and host tissue biocompatibility but also converts to high surface area nano-crystalline CDHA.

Methods: Dry cement powder (CC-CPC) was prepared using tri-calcium phosphate based precursors. Colloidal nano HA containing solutions was used as the liquid for the cement forming reaction. 40% (by weight) of polyol was used as a pore former in the CC-CPC. The dried cements samples were stored under ambient conditions. Some of these samples were also soaked in phosphate buffer saline (PBS-1x, Lonza) for 3, 6, and 15days. The cements were characterized for structure, morphology, surface area, and porosity using x-ray diffraction (XRD), scanning electron microscopy (SEM), N_2 adsorption, and mercury porosimetry. *In vitro* MC3T3-cell proliferation was assessed using the nontoxic Alamar Blue dye. *In vivo* studies were conducted in the segmental rabbit ulnae defect of critical size 1.5 cm (n=6).

Results: The initial and final setting times of these cements were 8 (± 1) min and 18 (± 1) min, respectively, at 298K. The cohesion time of these cements were ~ 4 ± 1 min in PBS, and the injectability was observed to be in the 60-70% range. The cements were also found to be neutral, maintaining a pH of ~7.4 over a period of 15 days, and are thus observed to be non-toxic to cells. XRD phase analyses of these cements clearly demonstrate complete conversion of the cements to CDHA after 15.0 and 6 days, respectively. The SEM studies also show a complete change in morphology of the initial particles and preferential growth of CDHA nano-whiskers on the surface of the initial particles. Furthermore, the SEM images of PCC-CPC clearly show the formation of macro- and micro-pores after dissolution of mannitol. The pore size distribution of cements were further studied using mercury-porosimetry, which clearly demonstrate that PCC-CPC contains large numbers of macro-pores (between 100 and 1 μm) compared to CC-CPC. However, both of these cements exhibit significant micro and meso porosity. The BET surface area of the as prepared CC-CPC was ~26 m^2/g and the surface area increased to ~55 m^2/g after 6 days of soaking in PBS decreasing to ~35 m^2/g after 15 days. The BET surface area of the PCC-CPC on the other hand, was ~ 11, 59, and 42 m^2/g for the as prepared, 6 day, and 15 day PBS soaked samples, respectively. These surface area data together with the XRD and SEM results prove that the cements after aging in PBS converted into nano-crystalline CDHA. Proliferation results using Alamar Blue show that the cells grow well in both these cements, however, the PCC-CPC displays the best cell growth compared to other cements. Post implant 12 weeks *in vivo* results on PCC-CPC scaffolds (Fig. 1) using radiographical, micro-CT and histological assessment clearly demonstrate the formation of new bone on the surface and bulk of the porous scaffold thus indicating rapid dissolution of the cement.

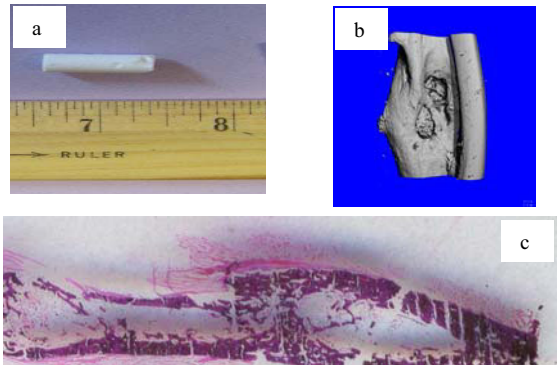

Figure 1: (a) As-prepared cement scaffolds before implantation; (b) Micro-CT of the implanted cement after 12 weeks; (c) Histological assessment showing new bone formation.

Conclusions: The present study describes the development of a novel porous biocompatible CPC (PCC-CPC) at neutral pH. The setting and handling characteristics of this cement are highly suitable for use in clinical setting. Presence of large fraction of macro-porosities together with the formation of nano-crystalline CDHA dramatically improves the *in vivo* dissolution rate. The formation of *de nova* bone further highlights the potential of these CPCs for bone regeneration.