Effect of Calcium Coating on Tyrosine-Derived Polycarbonate Scaffold Architecture

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Statement of Purpose: Degradable scaffolds fabricated with osteoconductive calcium coatings may serve as a viable alternative to the use of allografts and autografts for bone regeneration. Although various reports have shown the benefits of coupling calcium coatings with scaffolds, there still exists a gap regarding the effects of the coating process on scaffold porosity [1]. It is hypothesized that this coating process, as well as the concentration of ions, will affect the coating produced and in turn scaffold architecture. Towards this end, tyrosine-derived polycarbonate scaffolds have been fabricated with various calcium coatings and characterized via micro-computed tomography (micro-CT), scanning electron microscopy (SEM), and x-ray diffraction (XRD).

Methods: Tyrosine-derived polymers were synthesized by condensation reaction of triphosgene and tyrosine-derived diphenol [2]. Scaffolds were fabricated using a combination of solvent casting/ porogen leaching/ phase separation techniques [3]. Calcium coatings were produced by using a precipitation method. The ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, and F^-) and temperature during coating process were varied. Architecture was investigated using a Skyscan 1172 micro-CT, the settings for the scans included sample rotation without a filter during acquisition at 40 kV and 250 µA. SEM was also employed for architecture analysis (Amray 1830I, 20 kV). ImageJ (v1.42q, NIH) was utilized for pore diameter measurements. Aside from micro-CT, porosity was measured according to the following equation [4]:

$$\text{Porosity} = (1 - V_g / V_A) \times 100\%, \qquad (1)$$

where V_g is the volume of scaffold material, and V_A is the scaffold volume. Calcium coatings were analyzed with XRD (Philips X'Pert). Scans were performed between 2θ values of 10° and 80° at a rate of 0.5° /min.

Results: Scaffolds were fabricated and initially characterized without calcium coatings. Macro and micropores were produced (Figure 1 A). Pore diameter from SEM/ ImageJ measurements averaged 15 µm for the micropores, and 400 µm for the macropores (Table 1). When compared to the total porosity obtained from micro CT, there is a difference of 6%. This difference could be attributed to the measurement technique; micro CT uses a reconstructed 3-dimensional image which is then analyzed. Total porosity includes closed pores as well as interconnected pores. In the case of the aforementioned formula, these parameters are not considered. Additional parameters obtained from micro CT include closed porosity as well as total area of pore space. Closed porosity cannot be calculated with other conventional methods of porosimetry, i.e. mercury and pycnometry [4]. This parameter is of significance since a scaffold having a high percentage of closed pores will obstruct cell migration, nutrient flow, and angiogenesis. Total pore space is related to the total porosity and accounts for both open and closed pores.

SEM and XRD analysis (data not shown) was performed to characterize the precipitated coating formed on and within the scaffolds. XRD analysis of bare scaffolds showed an amorphous curve, indicating the absence of a crystal coating. Calcium coated scaffolds were also scanned; diffractograms indicate a change in height of the hydroxyapatite (HA) peak as well as the phases present with variations of coating recipe and process. The pore architecture following the coating process indicates that precipitation occurs within both macro and micro pores. Crystal morphology was also investigated. Coatings performed at room temperature produced plate-like crystals in the micron scale, and were observed to occlude scaffold pores. However, by reducing the salt concentration, coatings were produced which were considerably thinner, thus retaining open porosity (data not shown).

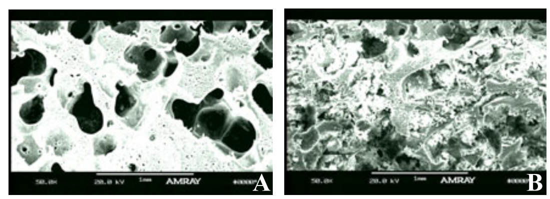

Figure 1. SEM micrograph of uncoated scaffold (A) and calcium coated scaffold (B) (scale bar = 1 mm).

Table 1: Architectural parameters of uncoated scaffolds.

Parameter	Average value	Analytical method
Pore diameter	15 µm (micro) 400 µm (macro)	SEM
Total porosity	80%	Theoretical calculation
Total porosity	86%	Micro CT
Closed porosity	.7%	Micro CT
Total area of pore space	17 mm^2	Micro CT

Conclusions: Tyrosine-derived polycarbonate scaffolds with and without calcium coatings were fabricated and characterized. The calcium coating process was found to affect scaffold architecture. This data indicates that the coating process can be fine tuned to produce calcium phosphate coating while preserving scaffold architecture.

References:

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