Hybrid Scaffolds for the Delivery of Multiple Proteins in Bone Tissue Engineering

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Statement of Purpose: Growth factors clearly play important roles in controlling cellular functions in tissue regeneration. An appropriate matrix able to deliver gradually bioactive factors at right dose and time, according to the needs of the normal regeneration process, is still a major challenge in regenerative medicine. Calcium phosphate (CaP) coatings have shown excellent osteoconductive ability and high affinity for proteins, which makes them ideal carriers for the delivery of osteoinductive agents. In this context, biodegradable scaffolds coated with biomimetic CaP layers and incorporating osteogenic growth factors, may constitute an effective way to provide osteoconductive as well as osteoinductive properties in a single material. Therefore, we propose a multifunction hybrid scaffold, consisting of a biodegradable polymeric fibre-mesh, to provide a 3D structure for cell adhesion and proliferation, and coated with CaP layers to deliver bioactive factors that can regulate cell behaviour.

Methods: Fibre-mesh scaffolds were produced by wet-spinning using a starch/poly-ethylene-vinyl alcohol blend (SEVA-C, 50/50 wt%). The scaffold degradation was studied in PBS solution containing 160 U/L α-amylase and assessed by determining the percentage weight loss and quantification of reducing sugars released in to the solution from starch hydrolysis. Scaffolds morphology was analysed by SEM and μCT. The fibre-mesh scaffolds were coated with CaP layers using the biomimetic methodology developed by *Kokubo et al. [1]*. Metabolic activity and proliferation of osteoblasts (SaOs-2), onto uncoated and coated scaffolds, were assessed by MTS assay and DNA quantification, respectively. Labeled proteins were incorporated at different stages during CaP formation. Bovine serum albumin (BSA) was conjugated with fluorescent dyes (fluorescein isothiocyanate, FITC and Rhodamine B isothiocyanate, Rhod) before incorporation into the coating. The distribution of the labeled-protein within the coating was visualized by confocal laser scanning microscopy (CLSM). Release studies were performed in TRIS buffer and the amount of released protein measured using fluorescence microplate reader.

Results: Using wet-spinning technique, we were able to fabricate a 3D fiber-mesh structure with high porosity and surface area (Fig. 1a,b) and interesting mechanical properties (elastic behaviour in the wet state). The effect of α-amylase enzyme on the scaffolds degradation was evident with 40% of weight loss after 12 weeks (Fig. 1c). The presence of increasing concentration of sugars in solution confirms the degradation of the starch polymer in the scaffold. In order to consider the use of the scaffolds in bone tissue engineering, the biocompatibility of the scaffolds in regards to cell viability and proliferation was evaluated. Cell viability and proliferation increased with culturing time and were higher for CaP coated scaffolds (Fig. 2a,b).

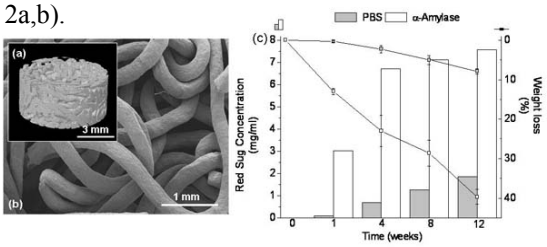

Figure 1- SEM (a) and μCT (b) images of SEVA-C fibre meshes scaffolds (Cylindrical form: ∅ = 5.5 ± 0.4 mm, e = 3.8 ± 0.3 mm) and their degradation behaviour (c) in presence of α-amylase enzyme.

Furthermore, MTS assay proved that cells remain viable, showing an increased metabolic activity, confirmed by an enhancement in cell number for the CaP coated scaffolds. We next investigated the carrier potential of CaP coating for the dual release of proteins. CLSM (Fig. 2c) shows a combination of green and red fluorescence, confirming the incorporation and homogeneous distribution of FITC- and Rhod-BSA in different layers of the coating. The release studies (Fig. 2d) indicate that the protein incorporated in the outer layers (Rhod-BSA) is released at faster rates, whereas the protein present in the inner layers (FITC-BSA) shows a more sustained release.

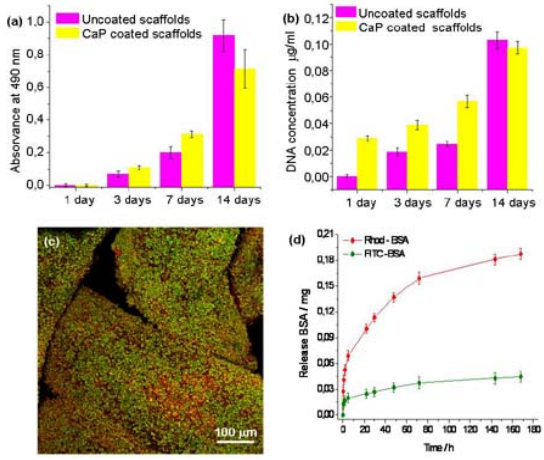

Fig. 2- Viability (a) and proliferation (b) of SaOs-2 cells seeded onto uncoated and CaP coated scaffolds. CLSM images of FITC- and Rhod-BSA within the CaP coating (c) and their cumulative release from the coating (d). Rhod-BSA (red), FITC-BSA (green).

Conclusions: A new hybrid scaffold, with a highly porous structure to support cell seeding and cell infiltration, has been developed while allowing the incorporation and sustained release of proteins that can enhance the function of seeded cells.

References: 1. Abe, Y et al., *J Mater Sci Mater Med*, 1, 233, 1990.

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