Biomimetic Mineral Stability on 3D PLGA Scaffolds Immersed in Different Media

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Statement of Purpose: The bone-like carbonate apatite (BLCA) coatings have a great potential to apply in orthopaedic and dental implants due to their excellent biocompatibility and biodegradability. BLCA layer can be coated biomimetically in the polymer surfaces by soaking in the simulated body fluid (SBF) [1]. This SBF contains similar ionic constituents to human blood plasma. The deposition of mineral on substrates using this biomimetic method provides many advantages over conventional methods which utilize higher temperatures and pressures. Advantage of this biomimetic method is that biologically active molecules can be co-precipitated with inorganic components, such as carbonate apatite to form organic-inorganic matrix due to gentle conditions throughout the process. Therefore, these matrices can be used as carriers for organic molecules such as protein, drug or growth factors. In addition, BLCA coated polymer scaffolds can be used as templates for bone tissue engineering to enhance the osteoblast function. In order to use BLCA for therapeutic applications, BLCA stability in physiological media is important. In present study, we accelerate BLCA mineral process using 5x SBF and study the mineral stability at 37ºC for 21 days in physiological media and cell culture media.

Methods: Micro-porous 3D poly(lactic-co-glycolic acid) PLGA scaffolds were fabricated by the solvent casting/salt leaching technique using chloroform to dissolve the polymer. In this method, sieved sodium chloride particles (250-425 μm) were placed in the glass vials and then polymer/solvent solution was cast into the vials to make a scaffold. The BLCA layer was deposited soaking the scaffolds in 5x SBF (Table I) for 2 days with prior surface treatments of NaOH. Samples were analyzed by scanning electron microscopy (SEM), fourier transform infra-red (FTIR). The scaffolds coated with BLCA layer were placed in the 24 well plates containing 2 ml of media, such as Tris Buffered Saline-pH 7.4, cell culture media containing αMEM supplemented with 10% FBS, and 1% penicillin-streptomycin and incubated at 37°C for 21 days. In order to provide the exact physiological environment similar to the body, mineralized scaffolds were not vortexed using the stir bars on the vials.

Table I: Ion Concentrations (in mM) of SBF

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	SO_4^{2-}
1x SBF	145.2	5.0	1.5	2.5	152.0	4.2	1.0	0.5
5x SBF	726.0	25.0	7.5	12.5	760.0	21.0	5.0	2.5

Results / Discussion: The open-pore structure of 3D PLGA (85/15) scaffolds was analyzed by SEM (Fig. 1a) and the pore size of these scaffolds is in the range of 250-425 μm, which was well established for osteoblast infiltration into a porous material. The BLCA layer can be deposited soaking the scaffolds in 1x SBF for more than 16 days. We accelerated the deposition of mineral on scaffolds for 1 day, modifying the mineralization process using surface treatments and 5x SBF. The BLCA layer was coated biomimetically, in the surfaces of the PLGA scaffolds after 1 day incubation at 37°C with 5x SBF (Fig. 1b and 1c). SEM images of mineralized PLGA scaffolds immersed in both Tris saline (Fig. 1d), and culture media (Fig. 1e) at 37°C for 21days exhibits existence of mineral in the surfaces of scaffold. Incorporation of biomolecules into BLCA by co-precipitation provides interaction with crystal lattice in the mineral instead of surface adsorption [2]. Therefore, release of biological agents from the mineral occurs upon dissolving the mineral layer in the implant. Some studies have shown that biomimetic mineral coating can stimulate cellular activity and positively influence proliferation and differentiation of marrow stem cells [3].

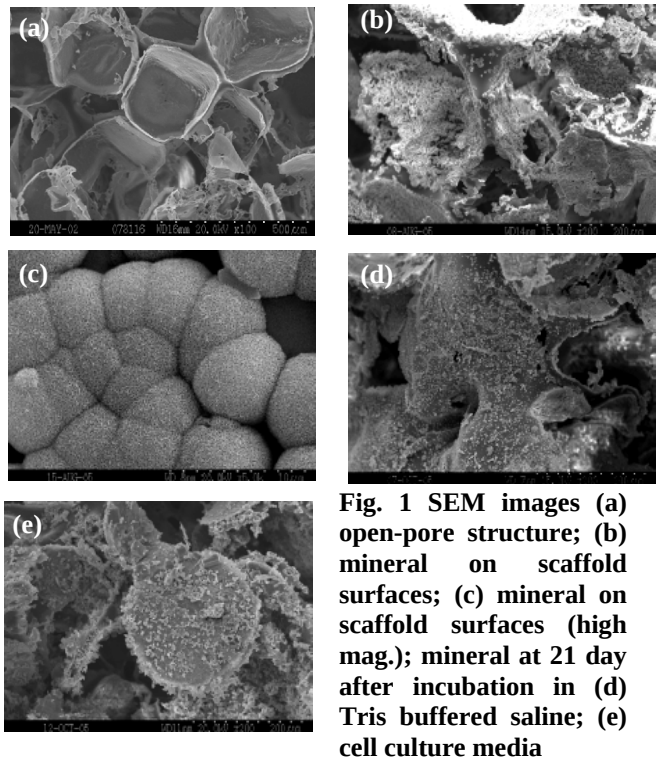

Fig. 1 SEM images (a) open-pore structure; (b) mineral on scaffold surfaces; (c) mineral on scaffold surfaces (high mag.); mineral at 21 day after incubation in (d) Tris buffered saline; (e) cell culture media

Conclusions: The BLCA layer was coated *in-vitro* in the surfaces of 3D PLGA scaffolds using hydrolysis techniques and 5x SBF, within 1 day at normal pressure and temperature. The BLCA layer on surfaces of scaffold was stable even after 21 days immersed in Tris Buffered Saline and culture media. This study suggests that BLCA are stable for at least 3 weeks in the media, and therefore, mineral has a potential to use as a carrier for biological molecules for localized release applications.

References: (1). Abe Y, J Mat Sci Mater Med 1990; (1): 233. (2). Liu Y, Biomat 2003; 24: 65. (3) Christine L, J Biomed Mater Res, 2000; 49: 423.