BMSCs function on 3D porous PLGA scaffold coated with bone like minerals through rapid mineralization

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Statement of Purpose:

The bone-like mineral (BLM) coatings have a great potential to apply in orthopedic and dental implants due to their excellent biocompatibility and biodegradability[1-3]. The BLM can be coated biomimetically in the polymer surfaces by soaking in the simulated body fluid (SBF) [1, 4-5]. This SBF contains similar ionic constituents to human blood plasma. We accelerated deposition of minerals on 3D poly(lactic-co-glycolic acid) PLGA scaffolds from 16 days to 1-2 days using 5x SBF instead of 1x SBF. BLM coated PLGA scaffolds can be used to deliver protein, growth factors, and drugs. We incorporated insulin like growth factor (IGF-I) in to BLM coated surfaces of 3D PLGA scaffolds and studied release kinetic.

Methods:

Micro-porous 3D PLGA (85/15) scaffolds were fabricated by the solvent casting / salt leaching technique using chloroform to dissolve the polymer. SBF contains similar ionic constituents to human blood plasma. Minerals were coated in the 3D polymers by soaking in simulated body fluid (SBF) for 48 hrs.

Table 1: Ion Concentrations (in mM) of Blood Plasma and Simulated Body Fluids

Ions	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	$H2PO_4^-$	SO_4^{2-}	pH
Blood Plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	7.2-7.4
1xSBF	145.2	5.0	1.5	2.5	152.0	4.2	1.0	0.5	7.4
5xSBF	726.0	25.0	7.5	12.5	760.0	21.0	5.0	2.5	6.8

The BLM layer coated 3 D PLGA scaffolds were analyzed by Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red (FTIR) and X-ray Diffraction (XRD).The 3D PLGA scaffolds were also coated biomimetically with SBF incorporated growth factor (IGF-I),then we studied the release kinetic of the IGF-I in phosphate buffer saline(PBS) at 37^0C for 30 days. Murine Bone marrow stromal cells (BMSC) were harvested using femur and tibia of the mice. These BMSC were cultured using osteogenic media (αMEM, 10% FBS, 50 µg/ml L-ascorbic acid, 10nm dexamethasone,1% penicillin Streptomycin, 10 mM β-glycerol phosphate) in T-75 flasks. The cultures were maintained at 37^0C in a humidified 5% CO_2 / 95% air atmosphere. Each scaffold was seeded with 1ml of cell suspension containing 2.3 x 10^5 cells and incubated for 2h, 4h, 6h, 18h and 24h different time points in 24 well plates. Each time point contains mineralized scaffolds and control (non-mineralized) scaffolds (n=3). After each time point, we calculated number of cells attached with the scaffolds using Micro BCA assay test.

Results/Discussion:

XRD patterns of BLM coated scaffolds with different incubation time in 5x SBF, from 2θ = 10 to 60 degrees were shown relative to human bone mineral sample in Fig. 1 .XRD spectra shows that mineral deposition increases with incubation time. FTIR spectra (data not shown here) also show that the peak intensity of Carbonate ($CO3^{2-}$) & phosphate ($PO4^{3-}$) increases with incubation time.

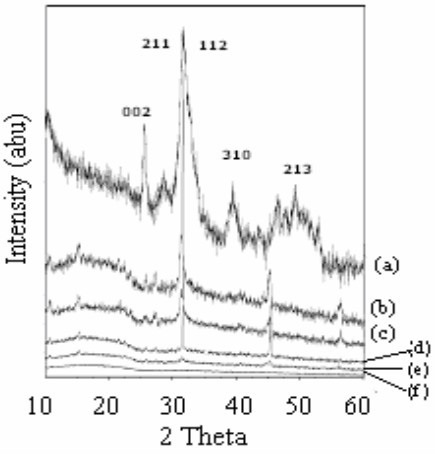
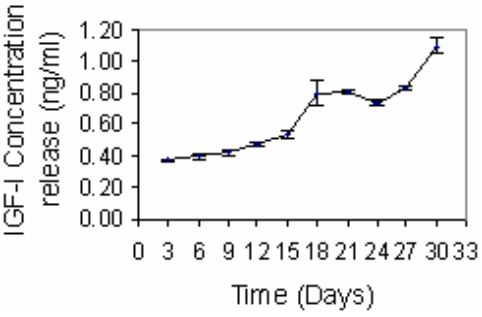
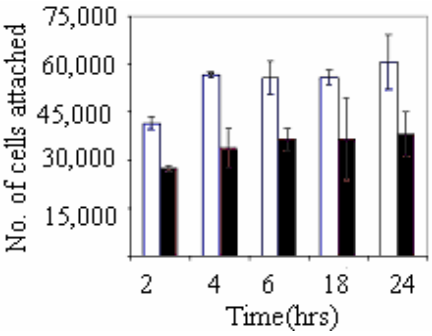


Fig: 1 XRD spectra for (a) Human bone (b) Min-48 hr (c) Min-36 hr (d) Min-24 hr (e) Min-12 hr (f) PLGA



IGF-I incorporated 3D PLGA scaffolds exhibit sustain release instead of burst release with time in Fig: 2.

Fig: 2 Kinetic release of IFG-I vs. Time



Mineralized scaffolds enhance the BMSCs attachment compare with control scaffolds in Fig: 3.

Fig: 3 Cell attachment graph

Conclusion:

This study suggests that by increasing mineral concentration and adjusting pH of SBF, accelerates the BLM coating on 3D PLGA scaffolds in less time. The BLM coated PLGA scaffold has a potential to use as a drug delivery carrier. The BLM coated PLGA scaffold also enhance the cell attachment activity of BMSC.

References:

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