Effect of Pore Size on In Vitro Biocompatibility of Porous Poly(Lactide-co-Glycolide)/Calcium Phosphate Scaffolds for Bone Tissue Engineering

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Statement of Purpose: The combination of cells with porous scaffolds to produce 3D hybrid osteogenic constructs is a common subject in bone tissue engineering research. A scaffold composed of poly(lactide-co-glycolide) (PLGA) and calcium phosphate (CaP) presents improved mechanical properties and enhanced in vivo biocompatibility with bone tissue (Lickorish D. Biomaterials, 2007,28:1495-1502). Recently, we have showed that osteoblastic cells grown on this scaffold surface (Beloti MM. J Biomater Appl. 2008, DOI: 10.1177/0885328208094082). However, the effect of pore size on in vitro biocompatibility of PLGA/CaP scaffolds has not been evaluated. Then, this study aimed at evaluating the osteoblastic cell responses to PLGA/CaP scaffolds presenting three different interconnected pore size ranges.

Methods: Bone marrow cells were flushed out of rat femora and cultured in osteogenic medium composed of alpha-MEM (Gibco, Grand Island, NY, USA) supplemented with 15% fetal bovine serum (Gibco), 50 μg ml^{-1} vancomycin (Acros Organics, Gell, Belgium), 20 μg ml^{-1} ampicillin (USB Corporation, Cleveland, OH, USA), 0.3 μg ml^{-1} fungizone (Gibco), 10^{-7} M dexamethazone (Sigma, St Louis, MO, USA), 5 μg ml^{-1} ascorbic acid (Gibco), and 7 mM β-glycerophosphate (Sigma). First passage cells were seeded at a cell density of $2x10^4$ on PLGA/CaP scaffolds presenting 85% of interconnected porosity and three different pore size ranges, 470-590 μm (S1), 590-850 μm (S2) and 850-1200 μm (S3), and cultured for 10 days. Cells were grown at 37° C in a humidified atmosphere of 5% CO_2 and 95% air, and the medium was changed every 3 or 4 days. At day 10 cell growth was determined by MTT (Sigma) assay, alkaline phosphatase (ALP) activity by the release of thymolphthalein from thymolphthalein monophosphate using a commercial kit (Labtest Diagnostica SA, MG, Brazil), and gene expression of the osteoblast markers runt-related transcription factor 2 (Runx2), osterix (OSX), collagen type I (COL), Msh homeobox 2 (Msx2), ALP, osteocalcin (OC), and bone sialoprotein (BSP) by real-time RT-PCR. Gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method and GADPH expression as reference. MTT and ALP activity assays were carried out in quintuplicates (n=5), and real-time RT-PCR in triplicates (n=3). Data were compared by Kruskal-Wallis test followed by the Fischer test based on rank (level of significance: 5%).

Results: All results are showed in Table 1. Cell growth and ALP activity were not affected by scaffold pore size. Gene expression of all evaluated osteoblast markers was affected by pore size in the following way: Runx2 - S1<S2<S3; OSX - S1<S2=S3; COL - S1=S2<S3; Msx2 - S1<S2<S3; ALP - S1<S2<S3; OC - S1<S2<S3; BSP - S1<S2<S3.

Table 1. Cell growth (MTT), ALP activity and gene expression of osteoblast markers in cells cultured on PLGA/CaP scaffolds with different pore sizes for 10 days. Data are presented as mean±SD.

	Scaffold pore size (μm)		
	470-590 (S1)	590-850 (S2)	850-1200 (S3)
MTT	0.47±0.06	0.46±0.02	0.36±0.07
ALP activity	7.1±1.5	7.3±1.0	7.6±1.5
Runx2	0.29±0.04	1.55±0.21	3.54±0.60
OSX	0.56±0.03	0.97±0.10	0.86±0.10
COL	0.26±0.02	0.24±0.002	0.63±0.01
Msx2	0.84±0.55	9.39±1.47	27.22±8.12
ALP	0.16±0.05	0.38±0.11	0.73±0.05
OC	0.35±0.08	1.59±0.05	1.84±0.14
BSP	0.32±0.004	0.91±0.02	1.35±0.07

Conclusions: Irrespective of the pore sizes evaluated, PLGA/CaP scaffolds are biocompatible as cells grown and differentiated on them. Based on gene expression analysis, an increase in pore size range corresponds to an increase in the expression of osteoblast markers with scaffolds presenting pore size ranging from 850 to 1200 μm allowing the highest osteoblastic phenotype expression. Thus, PLGA/CaP scaffolds with pore sizes varying between 850 and 1200 μm seems to be more suitable for bone tissue engineering purposes. From now, further experiments are needed to evaluate the effect of pore size on in vivo bone response to this scaffold.

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