Response behaviors study of human bone marrow-derived mesenchymal stem cells and endothelial cells to calcium phosphate scaffolds

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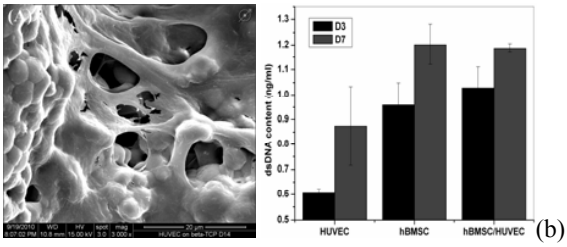
Introduction: A fast and successful vascularization of synthetic bone scaffolds after implantation is essential for new bone formation, and remains one of the main challenges in bone tissue engineering. It is well-established that the process of new blood vessel formation is coupled to the osteogenesis of bone marrow stem cells and the development and maturation of bone. Bone regeneration and repairs depend on complex interactions between bone-forming cells and vascular endothelial cells. Pre-seeding a scaffold with cells could improve angiogenesis and bone development through this complex crosstalk between endothelial cells and bone-forming cells. However, this angiogenetic and co-culture studies were often performed in gel-like matrices using 2-D Matrigel or in collagen-based 3-D matrices. Knowledge about the response of endothelial cells and their co-culture with bone-forming cells to beta-tricalcium phosphate (β-TCP) scaffolds in a 3D interconnected porous architecture is limited. Therefore, the aim of this study is to investigate the response behaviors of endothelial cells on a β-TCP scaffold. Meanwhile, we also investigated the proliferation behaviors of human bone marrow mesenchymal stem cells and endothelial cells co-cultured on the porous 3D β-TCP scaffold.

Methods: β-TCP scaffolds with completely interconnected pores were prepared by template-casting method. Scaffolds (5-6 mm height, 8mm diameter) with interconnected pores of approximately 350-500 μm were used in these experiments. Human bone marrow-derived mesenchymal cells (hBMSC) were cultured in complete media (CM) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, and human umbilical vein endothelial cells (HUVEC) constitutively expressing green fluorescent protein (GFP) were maintained in endothelial basal medium (EBM-2; Lonza) and supplemented with endothelial growth BulletKit (Lonza). Mono-culture of the hBMSC or HUVEC and co-culture were performed on β-TCP scaffolds. β-TCP scaffolds seeded with HUVEC or HUVEC/hBMSC were observed by scanning electron microscopy (SEM). Endothelial cells alone or in co-culture with hBMSC cells on β-TCP scaffolds were fixed using 4% paraformaldehyde and incubation with primary antibody(PECAM, CD31), and were washed and labeled with the corresponding anti-mouse (Alexa 594). Finally, constructs were mounted and images were captured by a laser scanning confocal microscopy.

Results: In mono-culture, both the hBMSC and HUVEC attached, spread, and proliferated well on the β-TCP scaffolds. HBMSC showed spindle-like morphology, a characteristic feature of hBMSC, and HUVEC exhibited typical cobblestone-like morphology and grew well in a monolayer. Fluorescent images and SEM result indicated a typical flattened morphology of HUVEC seeded on the scaffold (Figure a). In both mono-cultures and co-culture, cells attached to the β-TCP scaffold and proliferated well in the inner pore and on the struts of the scaffold. Fluorescent images showed intact cytoskeletons and nuclei of mono-culture and co-culture cells on the scaffolds. DNA content results indicated the proliferation of monoculture of hBMSC and HUVEC and co-culture (Figure b). Laser confocal microscopy images indicated both mono-cultures of HUVEC and co-culture of HUVEC with hBMSC presented positive PECAM-1 immunostaining.



(b)

Figure. SEM image of HUVEC cells (a) and proliferation profile of mono-culture and co-culture (b).

Conclusions: In this study, we clearly demonstrate that β-TCP scaffolds support cell attachment and proliferation of hBMSC and HUVEC cells in mono-cultured and co-cultured case. This three-dimensional scaffold could provide a basis for further development of complex bone constructs with a potential regeneration and vascularization capacity.

References

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Acknowledgements

We would like to acknowledge the supports from March of Dimes Birth Defect Foundation, Airlift Research Foundation, Wallace H. Coulter Foundation, DOD W81XWH-10-1-0966, and NIH R01AR057837.