Universidade do Minho
Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

AMNIOTIC FLUID STEM CELLS VERSUS BONE MARROW MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING

Márcia T. Rodrigues[1,2,3] , Sang J. Lee[3], Anthony Atala[3], James Y. Yoo[3], Manuela E. Gomes[1,2], Rui L. Reis[1,2]

[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimaraes, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimaraes, Portugal; [3] Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, North Carolina
mrodrigues@dep.uminho.pt

KEYWORDS

Bone marrow stem cells, amniotic fluid stem cells, osteogenic differentiation, natural-based biomaterials, bone tissue engineering

ABSTRACT

This study aims to compare the osteogenic potential of bone marrow stem cells (BMSCs) and amniotic fluid stem cells (AFSCs) towards bone tissue engineering (TE) strategies. For this purpose, both cell types were cultured in in distinct conditions (2D and 3D)in order to assess the origin-related response of stem cells to different external environments during the osteogenic process. Thus, the osteogenic differentiation was assessed and evaluated on 2D and 3D conditions, provided by standard tissue culture plates, and by seeding/culturing the cells onto microfibrous SPCL scaffolds (a blend of starch and poly-caprolactone), respectively, in osteogenic supplemented media for up to 3 weeks.
Although both BMSCs and AFSCs were successfully differentiated into the osteogenic lineage, as indicated by the presence of mineralized matrix together with other bone related markers, results indicated that the progression of the osteogenic phenotype is related to cell origin and the culturing environment, as bone related markers are differently expressed by AFSCs and BMSCs.

INTRODUCTION

In TE, the ideal cell source to be used in a wide spectrum of applications is yet to be found. BMSCs have been widely studied, and consequently became the gold standard for studies in orthopaedic TE. Amniotic fluid is also arising as a promissing stem cell source, as AFSCs evidence important unique features such as an extensive ability for expansion and differentiation into functional cells with potential in future approaches towards the regeneration of bone tissue.
This study was designed to compare the osteogenic potential of BMSCs and AFSCs under distinct culture environments to determine if the osteogenic differentiation process of both cell types is related to cell origin. Osteogenic differentiation was carried out in 2 or 3 dimensions (3D) using a culture treated plate or by seeding the cells onto microfibrous SPCL scaffolds (a blend of starch and poly-caprolactone), respectively. SPCL scaffolds, obtained by a fiber melt extrusion/fiber bonding process were selected as 3D environment as these scaffolds have been extensively studied and characterized for bone TE strategies (Gomes. 2006). BMSCs and AFSCs were successfully differentiated into the osteogenic phenotype, and developed mineralized extracellular matrix. Nevertheless, cells presented different expression patterns of bone-related markers as well as different timings of differentiation, indicating that both cell origin and the culturing environment have a significant impact in the progression of the osteogenic phenotype in AFSCs and BMSCs.

MATERIALS AND METHODS

Human BMSCs, purchased from Lonza®, were expanded in basal BMSCs medium: α-MEM, 10% embryonic screened-FBS (ES-FBS, HyClone) and 1% penicillin /streptavidin solution. Human AFSCs were isolated as described previously (DeCoppi. 2007), and cultured in basic AFSCs medium (BAFC) composed of α-MEM (HyClone), 18% Chang B (Irvine Scientific), 1% Chang C (Irvine Scientific) media, 2% L-glutamine (HyClone) and 15% ES-FBS.

Semana da Escola de Engenharia
October 24 - 27, 2011

Both BMSCs and AFSCs were seeded onto tissue culture plates (2D culture) at passage 5 and 24, respectively, with 30,000 cells/well. Cells were cultured for 3 days in basal medium, and then exchanged to osteogenic medium, composed of DMEM with 10% FBS (HyClone), 100 nM dexamethasone (Sigma), 50 µM L-ascorbic acid (Sigma) and 10 mM glycerol 2-phosphate disodium salt hydrate (Sigma) for up to 3 weeks (0, 7, 14 or 21 days). To analyse the behavior of hBMSCs and hAFSCs in a 3D milieu, both type of cells were seeded onto SPCL scaffolds (7 mm x 4 mm cylinders) at a concentration of $1.2x10^6$ cells/scaffold. Similarly to 2D culture, cells were cultured in basal medium for 3 days and then in osteogenic media for up to 3 weeks.

Retrieved samples were characterized for cellular viability with Calcein AM and for the presence of osteogenic markers and matrix formation by alkaline phosphatase (ALP) and Alizarin Red (AR) stainings as well as the presence of RunX-2 and collagen I in the matrix by immunofluorescence. Cell morphology and matrix formation in the 3D environment, were also assessed by scanning electronic microscopy (SEM).

RESULTS AND DISCUSSION

AFSCs and BMSCs proliferated and colonized both 2D and 3D substrates, and for both cell types, the presence of osteogenic markers and mineralized matrix formation was detected. Nevertheless, AFSCs showed higher proliferation rate and enhanced mineralization of the ECM in 2D cultures, when compared to BMSCs.

In a 3D environment, ECM mineralization was observed at 14 and 21 days for BMSCs and AFSCs, respectively, and changes in the expression of bone related markers from 2D to 3D cultures were cell origin related, indicating that culture environments also play an important role in cellular response during osteogenic differentiation. Furthermore, the collagen fibers covering the scaffolds seem to be aligned, showing some degree of organization.

Despite similar viability and RunX2 levels during the experimental study, as well as collagen I levels after 21 days in osteo culture, BMSCs and AFSCs showed a different behavior in terms of mineralization; not only mineralization occurs latter in AFSCs constructs but BMSCs also produced more mineralized matrix, when seeded onto SPCL scaffolds. The continuous expression of RunX-2 of BMSCs in SPCL scaffolds also indicates that osteoblast differentiation process is likely to continue in time, reinforcing the ECM production and maturation.

CONCLUSIONS

BMSCs and AFSCs were successfully differentiated into the osteogenic lineage with production of mineralized ECM. However the two cell types presented different expression patterns of bone-related markers, and different timings of differentiation, indicating that both cell origin and the culture environment have a significant impact on the differentiation of stem cells into the osteogenic phenotype.

REFERENCES

DeCoppi P. *et al*. 2007. "Amniotic stem cell lines with potential for therapy." *Nature Biotechnology*, 25, No1, 100-106.

Gomes M.E. *et al*. 2006. "In vitro localization of bone growth factors in constructs of biodegradable scaffolds seeded with marrow stromal cells and cultured in a flow perfusion bioreactor." *Tissue Engineering*, 12 No1, 177-188.

ACKNOWLEDGMENTS

M. T. Rodrigues thanks the Portuguese Foundation for Science and Technology (FCT) for providing a PhD scholarship (SFRH/BD/30745/2006). This study was supported, in part, by Telemedicine and Advanced Technology Research Center (TATRC) at the U.S. Army Medical Research.

AUTHORS' BIOGRAPHIES

MÁRCIA T. RODRIGUES was born in Porto, Portugal and went to the University of Minho where she obtained a BSc degree in Applied Biology in 2004. She is currently writing her PhD Thesis entitled "Development of tissue engineered strategies combining stem cells and scaffolds aimed to regenerate bone and osteochondral interfaces". Her PhD was performed at University of Minho in a collaboration with Wake Forrest Institute for Regenerative Medicine in USA. Her email address is mtsrodrigues@dep.uminho.pt and her detailed CV can be found at http://www.3bs.uminho.pt.