Amniotic fluid stem cells versus bone marrow mesenchymal stem cells for bone tissue engineering

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Introduction

In tissue engineering (TE), the ideal cell source to be used in a wide spectrum of applications is yet to be found. Bone marrow mesenchymal stem cells (BMSCs) have been widely studied, indicating that BMSCs can be differentiated into cells of the osteogenic lineage. Thus, BMSCs have become the gold standard for studies in orthopaedic TE. However, novel stem cell sources, such as amniotic fluid stem cells (AFSCs) have been identified, showing important and unique features that may allow successful applications in the regeneration of bone tissue.

This study was designed to compare the osteogenic potential of both BMSCs and AFSCS under distinct culture environments in order to determine whether the osteogenic differentiation process of both types of stem cells is related to the origin of the cells. Osteogenic differentiation was carried out in two and three dimensions (3D) using a culture treated plate and by seeding the cells onto microfibrous SPCL scaffolds (a blend of starch and poly-caprolactone), respectively.

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. hAFSCs were isolated as described previously[1], and cultured in basic hAFCs 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.

hBMSCs and hAFSCs 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 and 21 days). To study 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) produced by fiber bonding[2] 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 in both 2D and 3D substrates, and for both cells, it was detected the presence of osteogenic markers and mineralized matrix formation. 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

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