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Comparative Evaluation of Mesenchymal Stromal Cell Growth and Differentiation on a Novel Polycaprolactone Scaffold for Regenerating Large Tissue Trauma Defects

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INTRODUCTION

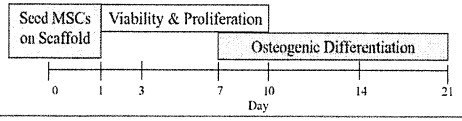
Trauma can result in large tissue defects, which pose a significant challenge to healing, especially for injuries of irregular shape and size that occur in the military and civilian settings. While autologous grafts remain the gold standard, their disadvantages include long inpatient care, donor site morbidity, and difficulty in matching defect geometry. Cell-based therapy offers a promising alternative. Here, human mesenchymal stromal cells (MSC) derived from adipose tissue (ADSC), amniotic tissue (AMSC), bone marrow (BMSC), chorionic tissue (CHSC), and umbilical cord tissue (UCSC) were evaluated for their viability and osteogenic differentiation potential on a novel polydopamine-coated poly(ε-caprolactone) (PCL) scaffold. These scaffolds are highly porous (65-75%), which is critical for cellular infiltration and blood vessel formation, while maintaining a high stiffness. Importantly, the scaffolds can be fabricated in custom geometries to fit the defect.

OBJECTIVE

The objective of this study was to determine the optimal MSC source that has the highest viability and potential for osteogenic differentiation on a scaffold suitable for bone healing.

METHODS

- MSC viability: MSC viability was evaluated in PCL scaffolds for 1, 3, 7, or 10 days with calcein AM and ethidium homodimer-1, labeling live and dead cells, respectively.
- MSC proliferation: Proliferation was determined at day 1, 3, 7, and 10 by measuring the activity of dehydrogenases with a CCK-8 assay.
- Differentiation: Osteogenesis was induced with osteogenic media and differentiation was confirmed by calcium deposition via alizarin red staining and alkaline phosphatase (ALP) activity with an ALP assay.
- Scaffold structure: BMSCs, differentiated for 14 days, were imaged via μCT (SkyScan 1172). The elastic modulus and hardness (EM/H) were measured via Oliver-Pharr approach, using a nanoindenter (iNano®, Nanomechanics).
- Statistical Analysis: Proliferation, differentiation, and EM/H were analyzed with: one-way repeated measures ANOVA/Dunnett, one-way ANOVA/Bonferroni, and Mann-Whitney, respectively (α=0.05).



RESULTS: VIABILITY AND PROLIFERATION

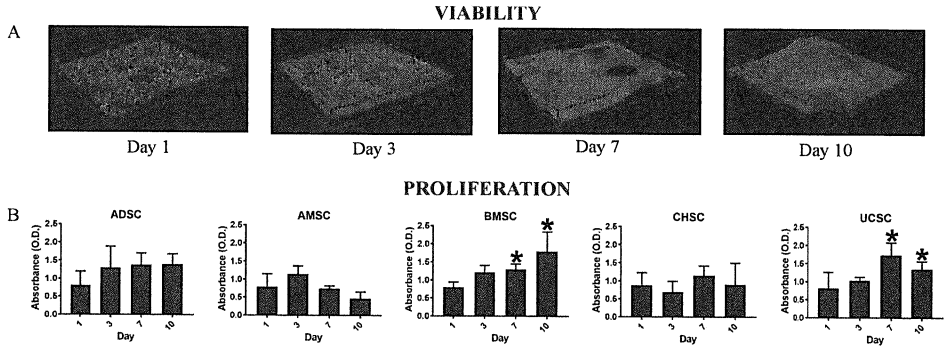


Figure 1: Viability and proliferation. (A) Representative image of MSC viability at day 1, 3, 7, and 10. PCL scaffolds support MSC viability as indicated by the live cells (green) and few dead cells (red). (B) Cell proliferation was determined by the CCK-8 assay for each cell type at day 1, 3, 7, and 10. Data are plotted as mean absorbance±SD. Significant differences within each cell type are compared to day 1 and are denoted by asterisks.

RESULTS: OSTEOGENIC DIFFERENTIATION

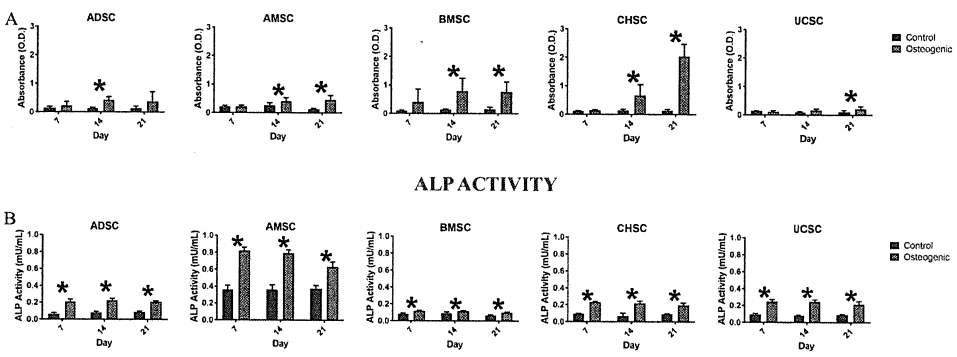


Figure 2: Osteogenic differentiation. Differentiation for each cell type was analyzed via (A) calcium deposition as quantified by alizarin red extraction and (B) ALP activity after 7, 14, or 21 days in control or osteogenic differentiation media. Data are plotted as mean absorbance or ALP activity±SD. Significant differences between groups for each time point are denoted by asterisks.

RESULTS: SCAFFOLD STRUCTURE

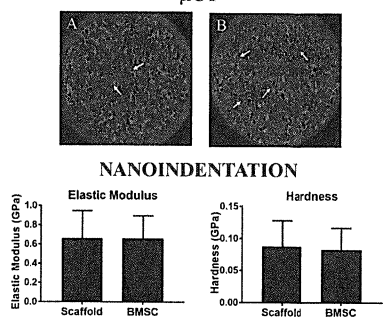


Figure 3: Scaffold structure. μCT images of a scaffold (A) without or (B) with BMSCs differentiated for 14 days. White and yellow arrows show the scaffold pores and radio opaque material produced from BMSCs, respectively. BMSCs do not affect the elastic modulus or hardness after 14 days (mean±SD).

SUMMARY

Major Findings

- Polydopamine-coated PCL scaffolds support ADSC, AMSC, BMSC, CHSC, and UCSC viability. Significant differences in proliferation were noted for BMSCs and UCSCs.
- BMSCs and CHSCs showed the most consistent osteogenic differentiation based on calcium deposition and ALP activity.
- BMSCs and their calcium deposition observed in the μCT images indicated that the cells are differentiating to osteoblasts.
- This study provides supportive insight on the optimal cell sources for bone healing within a PCL scaffold.

Future Studies

- Select the top three performing cell types to evaluate bone healing in a critical size craniomaxillofacial bone defect model.

DISCLAIMER

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