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#44 Design and characterization of a three-layer collagen-based scaffold to modulate BMSC behaviour for enthesis regeneration

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Introduction

The enthesis is a specialised tissue interface between tendon and bone, essential for adequate force transmission. Following injuries and surgical repair, the enthesis is often not re-established and traditionally used tissue substitutes have lacked to reproduce the complexity of the native tissue. We hypothesised that a collagen-based three-layer scaffold that mimic the composition of the enthesis, in combination with bioactive molecules, will enhance the functional regeneration of the tissue.

Materials and Methods

A three-layer sponge composed of a tendon-like layer (collagen I), a cartilage-like layer (collagen II) and a bone-like layer (collagen I and hydroxyapatite) was fabricated. Bone-marrow stem cells (BMSCs) were seeded on the scaffolds and cultured in differentiation media (chondrogenic, tenogenic and osteogenic). Alizarin Red and Alcian Blue were performed to evaluate BMSC differentiation towards osteogenic and chondrogenic lineage. Tenogenic differentiation of BMSCs was evaluated through expression of collagen I and tenascin by immunofluorescence staining. Subsequently, the cartilage-like layer was functionalized with insulin growth factor 1 (IGF-1) and the analysis repeated.

Results

The scaffolds promoted osteogenic differentiation of BMSC selectively in the bone-like layer in scaffolds cultured in basal and osteogenic media. Alcian blue staining revealed the presence of proteoglycans selectively in the cartilage-like layer in scaffolds cultured in chondrogenic media but not in basal media. Increased expression of the tenogenic markers collagen I and tenascin was observed in the tendon-like layer of scaffolds cultured in tenogenic but not in basal media (**Fig.1**). The presence of IGF-1 increased osteogenic and chondrogenic differentiation of BMSCs.

Conclusion

The collagen composition of the non-functionalized 3-layer sponge was able to regulate BMSC differentiation in a localized manner within the scaffold. The functionalization with IGF-1 accelerated chondrogenic and osteogenic BMSC differentiation. Overall, functionalization of the 3-layer scaffolds holds promising potential in developing novel and more efficient strategies towards enthesis regeneration.

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