Hydrogels derived from demineralized and decellularized bone – novel materials for bone regeneration

Lisa J White 1, Stephen F Badylak 2, Kevin M Shakesheff 1

1 School of Pharmacy, University of Nottingham, Nottingham, UK 2 McGowan Institute for Regenerative Medicine, University of Pittsburgh, USA.

Introduction

The extracellular matrix (ECM) of mammalian tissues has been isolated, decellularized and utilized as a scaffold to facilitate the repair and reconstruction of numerous tissues. Recent studies have suggested that superior function and complex tissue formation occurred when ECM scaffolds were derived from site specific homologous tissues compared to heterologous tissues. Musculoskeletal conditions are the most common cause of severe long term pain and physical disability worldwide [1]. Treatment of bone defects often involves the use of bone graft substitutes, such as demineralized bone matrix (DBM). Current delivery of DBM particles requires incorporation within a carrier liquid. However, differences in osteogenic activity, inflammation and nephrotoxicity have been reported with various carrier liquids. Thus, the objectives of this study were to produce hydrogel forms of DBM and ECM from bovine bone, denoted bDBM and bECM, and to thoroughly characterize their mechanical and biological properties and determine their suitability for use in bone regeneration applications.

Materials and Methods

Bovine femurs were treated with in-house developed demineralization and decellularization protocols as described in [2]. A pepsin digestion and solubilization technique was then applied. Gelation was induced by neutralizing the salt concentration and pH of the pepsin digests or a collagen solution, at 4°C followed by warming to 37°C. In vitro cell proliferation of mouse primary calvarial cells (mPCs) on the surface of hydrogels was assessed using the CellTiter 96® MTS colorimetric assay and tested for normality and statistically compared using a Tukey-Kramer multiple comparisons test. Significance for all statistical analyses was defined as p<0.001. Osteogenic differentiation of human mesenchymal stem cells (hMSCs) on the hydrogels was assessed by immunostaining for Runx2, cadherin-11, osteopontin and osteocalcin expression.

Results

Enhanced proliferation of mouse primary calvarial cells was achieved on ECM hydrogels, compared to collagen type I and DBM hydrogels as shown in Figure 1. HMSCs undergoing osteogenic differentiation had distinct differences in morphology (Figure 2) and protein expression when seeded upon bDBM hydrogels compared to bECM hydrogels.

Discussion and Conclusions

The bDBM and bECM hydrogels possess distinct structural, mechanical and biological properties, including osteogenic functionality. These novel hydrogels have significant potential for clinical delivery in bone regeneration without the need for carrier liquids.

References List


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