A COLLAGEN GLYCOSAMINOGLYCAN SCAFFOLD SUPPORTS ADULT RAT MESENCHYMAL STEM CELL DIFFERENTIATION ALONG THE OSTEOGENIC AND CHONDROGENIC ROUTES

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Introduction

Adult mesenchymal stem cells have the proclivity to differentiate along multiple lineages giving rise to new bone, cartilage, muscle or fat. Collagen, a normal constituent of bone, provides strength and structural stability and is therefore a potential candidate for use as a substrate on which to engineer bone and cartilage from their respective mesenchymal-derived precursors. In this study, a collagen-glycosaminoglycan (collagen-GAG) scaffold was used to provide a suitable 3-dimensional (3-D) environment on which to culture adult rat mesenchymal stem cells (MSCs) and induce differentiation along the osteogenic and chondrogenic lineages.

Methods

MSCs were extracted from the tibial and femoral bone marrow of adult Wistar rats and maintained in culture in 2-D and were seeded onto 3-D collagen-GAG scaffolds. Immunostaining for Endoglin, a putative stem cell marker, verified the presence of MSCs *in vitro*. MSCs were treated with osteoinductive and chondro-inductive factors for 2 and 3 weeks. Collagen I and osteocalcin immuno-fluorescence were used to verify osteogenesis. Histological analysis (Von Kossa and Alizarin Red S) was carried out to ascertain matrix mineralisation. Collagen II immunofluorescence was used to assess chondrogenesis. The role of extracellular signal regulated kinase (ERK) in osteogenesis was assessed using the selective inhibitor U0126, in conjunction with western immunoblotting and immunocytochemistry.

Results

The results demonstrate that adult rat MSCs can undergo osteogenesis when grown on the collagen-GAG scaffold and stimulated with osteogenic factors, as evaluated by the temporal induction of the bone-specific proteins, collagen I and osteocalcin, and subsequent matrix mineralization. The osteogenic factors were coupled to activation of the extracellular-regulated protein kinase (ERK) and this kinase was found to play a role in the osteogenic process ($P<0.01$ students t test ± SEM n=6 and $P<0.05$, one way ANOVA ± SEM n=8). As well as supporting osteogenesis, when the cell seeded scaffold was exposed to chondrogenic factors, collagen II immunoreactivity was increased, providing evidence that the scaffold can also provide a suitable 3-dimensional environment with which to support chondrogenesis.

Conclusions

The data demonstrates the suitability of collagen-GAG scaffolds in bone and cartilage regeneration and also the requirement for ERK activation in the osteogenic differentiation of adult MSCs. A better understanding of the mechanisms behind such processes could lead to the successful manufacture of tissue engineered 3-D constructs for use in the treatment of musculoskeletal disorders.

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