ENGINEERING A REPLACEMENT BONE TISSUE WITH ADULT ADIPOSE-DERIVED STEM CELLS

Abstract

by

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The current gold standard for bone repair, autologous bone graft, is inherently costly and destructive to the site from which the tissue is harvested. Moreover, the amount of available donor tissue is limited, so it is not always a viable option. The aim of this work was to investigate tissue engineering as an alternative to autograft-based bone repair through the combination of donor cells and three-dimensional cell carriers. Constructs were designed to stimulate either intramembranous or endochondral bone formation upon implantation *in vivo*, and subsequent tissue analysis was driven by the desire to improve the understanding of qualities necessary for a successful engineered bone replacement.

To evaluate the intramembranous bone forming capacity of human adipose-derived stem cells, novel collagen scaffolds with or without hydroxyapatite reinforcement were loaded with cells and treated with growth or an osteogenic differentiation medium. Upon implantation, the cell-scaffold constructs produced varying amounts of immature bone characterized by highly vascularized osteocalcin and osteopontin-positive matrix and an increase in mineralized tissue

volume as assessed by μCT. Hydroxyapatite reinforcement and the particular pre-treatment of the cells were identified as key factors influencing tissue formation *in vivo*.

In an effort to determine important characteristics of an engineered construct capable of inducing an endochondral response *in vivo*, an ectopic model of endochondral ossification was developed with pre-chondrogenic murine cell line ATDC5. When coupled with an osteoinductive scaffold *in vivo*, cell pellets that had been pre-treated in an optimized chondrogenic differentiation medium underwent further chondrogenic maturation and then partial ossification over the course of 8 weeks *in vivo*. This effect was dependent on the treatment medium applied as well as a three-dimensional culture environment.

With these findings in mind, chondrogenic treatment was applied to human adipose-derived stem cells seeded in high density pellet culture or encapsulated in alginate beads to investigate the potential for endochondral bone formation in a clinically-relevant cell type. Despite identical pre-treatment, the two culture systems induced significantly different volumes of mineralized tissue formation *in vivo*. Moreover, the location of mineralized tissue was distinct for each culture type. Cell pellets resulted in a greater amount of mineralized tissue which accumulated around the exterior of the implants, while mineralized tissue deposits were confined to regions within cell-seeded alginate bead constructs. These findings further highlighted the importance of pre-treatment and culture methods with respect to the *in vivo* response induced by tissue engineered constructs.