CRYOPRESERVATION OF TISSUE ENGINEERED CONSTRUCTS FOR BONE: RETENTION OF CELL VIABILITY AND MINERALIZATION IN BIODEGRADABLE POLYMER MATRICES

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INTRODUCTION

Critical size defects are a challenge for the orthopaedic surgeon. Autografts, the primary treatment, are often limited by the quantity and quality of bone for harvesting. To circumvent the problems associated with existing biological grafts, bone tissue engineering has emerged as an alternative approach in the formation of viable bone grafting systems. Large scale clinical use of tissue engineered bone will require provisions for its mass availability and accessibility. This involves the ability to bank tissue engineered bone, which would allow for its immediate procurement upon an orthopaedic surgeon’s request.

Understanding the effects of cryopreservation on tissue engineered bone is imperative because the process of freezing and thawing may inflict structural and metabolic damage to the cells, mainly due to the intracellular ice crystal formation and dehydration during deep freezing.1 In this preliminary investigation, we examined the ability of mineralized primary rabbit osteoblasts, cultured on a poly(lactide-co-glycolide) (PLAGA) matrix, to withstand the physiological stresses associated with low temperature tissue banking. We hypothesized that in the presence of dimethyl sulfoxide (DMSO) freezing medium and under optimal culturing conditions, osteoblasts on a polymer scaffold would retain cell viability and mineralization structures following cryopreservation at -196°C.

MATERIALS & METHODS

Rectangular thin films were fabricated from (85:15) poly(lactide-co-glycolide) using a traditional solvent-casting method.2 In this process, the polymer was first dissolved in methylene chloride, then poured into a Teflon-coated dish. The dish was then placed in a -20°C freezer to allow solvent evaporation. The thin film matrices were subsequently cut into 1 x 2 cm rectangles. Scaffolds were UV sterilized for 10 minutes prior to cell culture.

The ulna of New Zealand White Rabbits (3.5 kg) was isolated, cleaned of adherent tissue, and collagenase digested to obtain primary osteoblasts. The cells were grown to confluency, then seeded on PLAGA thin films at a density of 25,000 cells/scaffold. The cells were cultured on the polymer for 21, 28, and 35 days in DMEM supplemented with 10% FBS, 1% P/S, 1% 116 mM CaCl2, 0.05 g L-Ascorbic Acid and 0.324 g β-glycerol phosphate at 37°C and 5% CO2. At the appropriate time point, cells were transferred to cryogenic vials containing 1.5 ml DMSO freezing medium (Fisher Scientific). All samples were cooled at a rate of -1°C/min to -70°C, stored at -70°C for 3 days, submerged in liquid nitrogen (-196°C) for 35 days, rapidly thawed to 37°C in a water bath, then cultured for 3 days under the incubation conditions previously stated.

Cell morphology and mineral formation were examined using scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDXA). Confirmation of cell viability following cryopreservation was obtained using a colorimetric assay, CellTiter 96 (Promega). A quantitative calcium mineralization measurement was performed following an assay by Jacobs, et al.3

RESULTS & DISCUSSION

Our results indicate that mineralized osteoblasts grown on a biodegradable PLAGA scaffold for 21, 28, and 35 days, were able to withstand the stresses associated with low temperature tissue banking. SEM results indicated the osteoblasts remained adhered to the scaffold and mineralization structures intact (Figure 1). EDXA detected the presence of phosphorus and calcium, confirming the presence of mineralized structures. Cell viability measurements were obtained by monitoring a reaction specific to metabolically active cells. Our results indicated this metabolic activity is retained by the cells following low temperature banking (Figure 2).

Furthermore, quantitative Alizarin Red S calcium mineralization levels (Figure 3) before and after cryopreservation indicate retention of mineralization by the tissue engineered bone for initial culture periods of 28 and 35 days. The lower levels of mineralization following cryopreservation indicate retention of the formation of mineralized matrix.

REFERENCES