INTRODUCTION

Genetic modification of chondrocytes is a promising adjunct for improving cell-based strategies of cartilage repair, such as autologous chondrocyte transplantation. Questions still remain, though, as to how to evaluate the repair tissue formed in these experiments. It is not clear if the newly-formed matrix integrates into the articular cartilage substrate or if the collagen and proteoglycan content and distribution in the new tissue is similar to native articular cartilage. The technique of Fourier transform infrared imaging (FT-IRI) has recently been used to evaluate matrix properties in other connective tissues (1). This methodology permits characterization of quantity, quality and distribution of collagen, proteoglycan (PG) and mineralized phases in a 400 X 400 micron region of a histological specimen at 7 microns spatial resolution. In the current study, bovine chondrocytes treated with an adenovirus (Ad) vector encoding bone morphogenetic protein-7 (AdBMP-7) were transplanted onto bovine cartilage explants in vitro and the matrix evaluated by FT-IRI.

METHODS

Adenovirus (Ad) vectors used were E1-, partial E3- based on the Ad5 serotype with the expression cassette in the E1 position under the CMV promoter. Ad vector encoding human BMP-7 (AdBMP-7) was constructed as previously described (2). Briefly, the cDNA for human BMP-7 was cloned into a shuttle plasmid and co-transfected with an Ad backbone plasmid pM17 into HEK 293 cells. Bovine chondrocytes (BC) were isolated by overnight digestion of minced mature bovine articular cartilage in 0.1% collagenase. BC were maintained in M-199 media supplemented with 10% fetal bovine serum and 50ug/ml ascorbate. BC were transfected by incubation with 5000 particle units (pu)/cell of Ad vector for 2 hr at 37°C in monolayer culture. For each experiment BC were transfected with AdBMP-7, a control virus (Adβ-gal, encoding the e.coli β-galactosidase gene) or mock transfected (no virus). After transfection BC were trypsinized and micromass cultures (approximately 10^6 cells/ 10^6 µl) were transplanted onto the cut surface of 5 mm diameter explant cartilage. This new information will assist in design of new materials for cartilage repair, specifically with respect to the quantities of type II collagen and proteoglycan content showed.

RESULTS

FT-IR images based on PG content showed a distinct interface between the bovine AC and the transplanted chondrocytes (Figure 1). It is apparent from these images that the AdBMP-7 transfected chondrocyte matrix produced more PG compared to both naïve chondrocyte matrix, and to native bovine AC. This was confirmed by comparison of the average values for the tissues, where PG content in AdBMP-7 (0.45 ± 0.28) was significantly greater than that of naïve (0.16 ± 0.04) and of AC (0.24 ± 0.06). These results parallel the mRNA analysis previously done (data not shown) that found an approximately 5-fold increase in aggrecan message in the AdBMP-7 transfected chondrocytes compared to naïve chondrocytes. In contrast, there was significantly less type II collagen in both AdBMP-7 compared to AC (9.56 ± 2.02 vs. 46.6 ± 11.6) and in naïve chondrocyte matrix compared to AC (8.06 ± 2.19 vs. 27.3 ± 0.96). Interestingly, the AdBMP-7 chondrocyte matrix showed an uneven type II collagen distribution, with some regions having a higher type II collagen content compared to the naïve chondrocyte matrix (Figure 2).

DISCUSSION

FT-IRI is a powerful new technique which can assess matrix quality in a variety of connective tissues. In this study, FT-IR images enabled visualization and quantitation of the components of repair tissues in an in vitro model of gene-modified autologous chondrocyte transplantation. Although the average type II collagen content appeared similar in the repair tissue formed by naïve and AdBMP-7-transfected chondrocytes, it was obvious from the tissue images that the distribution was different. Proteoglycan content was also significantly higher in the repair tissue formed by the AdBMP-7 transfected chondrocytes than in tissue formed by naïve chondrocytes and in the explant cartilage tissue. This new information will assist in design of new materials for cartilage repair, specifically with respect to the quantities of type II collagen and proteoglycan required.

REFERENCES:

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