

Response of Adult Human Articular Chondrocytes to a Non-fouling RGD-peptide Modified Surface

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INTRODUCTION:

Prior to implantation to induce repair of cartilage defects [1], chondrocytes are typically expanded in tissue culture treated polystyrene (TCPS) to increase their number. However, during expansion, cells de-differentiate and their re-differentiation capacity is often limited. Since chondrocyte phenotype strongly depends on the interaction with RGD [2], here we hypothesized that a RGD-peptide modified surface (i) supports chondrocyte attachment and growth, and (ii) enhances the post-expansion ability of cells to re-differentiate and form cartilaginous tissues.

METHODS:

Adult Human Articular Chondrocytes (AHAC) from cartilage biopsies of three donors were isolated and expanded for two passages [3] on TCPS coated with RGD-functionalized PLL-g-PEG (RGD) or – as controls – on TCPS coated with non-functionalized PLL-g-PEG (PEG) [4] or on non-modified TCPS. AHAC were assessed for the capacity to attach and proliferate on the different substrates. Cell morphology was analyzed by confocal laser scanning microscopy (CLSM). The phenotype of expanded cells was determined by RT-PCR quantification of mRNA expression of collagen types I, II and X. Expanded AHAC were induced to re-differentiate by culture as 3D pellets in serum free medium containing TGFβ1. Resulting tissues were assessed histologically (Safranin O stain for glycosaminoglycans, GAG), immunohisto-chemically (stain for type II collagen), biochemically (content of GAG/DNA) and by RT-PCR [3]. Mann-Whitney tests were used to determine statistically significant differences.

RESULTS:

AHAC attached and proliferated comparably on TCPS and RGD, whereas attachment on PEG was significantly reduced (5-fold). As compared to cells expanded in TCPS, chondrocytes grown in RGD expressed significantly higher (5-fold) levels of collagen type II mRNA, a marker of chondrocyte differentiation, and formed more

filopodia (Fig. 1). Expression of types I and X collagen were unchanged. Following 3D pellet culture, tissues generated by cells expanded on RGD contained 20% higher amounts of GAG/DNA than those expanded on TCPS. However, expression levels for type II collagen mRNA were not statistically different and only small differences were observed following tissue stain for GAG and type II collagen.

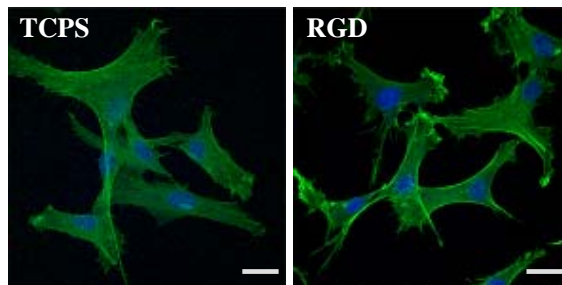


Fig. 1: CLSM images of fixed and fluorescently labeled AHAC. AHAC on RGD formed more filopodia than on TCPS. Green: actin filaments, blue: nuclei. Scale bars: 20 μm.

DISCUSSION & CONCLUSIONS: Our findings indicate that the bioligand RGD, presented using PLL-g-PEG chemistry, supports AHAC attachment and proliferation. In addition, chondrocytes expanded in RGD better maintained the differentiated phenotype, likely by specific integrin interactions. However, following transfer in a 3D environment, differences in cells expanded in the different substrates were less marked. This suggests that effects mediated by specific chondrocyte-RGD interactions are reversible, and prompts for the use of scaffolds with a RGD-modified surface for 3D culture of chondrocytes and engineering of cartilage grafts.

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