Type I Collagen-Alginate Composite Hydrogel Promotes Chondrogenesis of Murine Adipose Derived Stromal Cells

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INTRODUCTION

Alginate hydrogel is an advantageous scaffold material for cartilage tissue engineering because it encapsulates cells homogenously and provides intrinsic porosity. Blending alginate with cell adhesive molecules such as type I collagen to make composite hydrogels can further promote chondrogenenesis of mesenchymal stem cells.[1,2] However, in these conventional composites, the collagen presumably maintained a soluble monomer form since no steps were taken to allow the monomeric collagen to reassemble into fibrils. A method introduced by Gillette et al.[3] induced the formation of native-like collagen fibrils as well as a 3D collagen network in alginate matrix. Since the reassembled collagen fibrils are known to support chondrogenesis of chondrocytes,[4] we postulated such composite hydrogel containing native-like collagen fibrils might promote the proliferation as well as chondrogenesis of adipose derived stromal cells (ASCs).

METHODS

Cells: mASCs were isolated from male FVB mice at 3-4 weeks of age in accordance with the Notre Dame Institutional Animal Care and Use Committee approved protocol. Cells were expanded in medium consisting of DMEM, 10% heat-inactivated FBS, and 1% Pen/Strep.

Cell-seeded scaffold fabrication: The collagen-alginate composite hydrolgel was prepared following a published method.[3] Alginate (Novamatrix, Philadelphia, PA) solutions at concentration of 1.2, 2.4, and 4.8 wt% in 0.9% NaCl were prepared as stocks. Soluble type I collagen solution of 0.32 wt% was neutralized with 10x PBS and NaOH before mixing with alginate solutions. The collagen and alginate was mixed at an appropriate volume ratio to obtain final concentration of 1.2 wt% alginate and 0, 0.16 and 0.24 wt% collagen. Then it was mixed well with cells, and 100 µl of suspension was pipetted into a mold which was custom-modified from a 96-well plate. The cell-seeded hydrogels were incubated at 37 ^{o}C for 30 min to allow the gelation of collagen in the alginate solution. Subsequently, the samples were exposed to 102 mM $CaCl_2$ for 20 min to polymerize the alginate before demolding, followed by additional 10 min soaking in 102 mM $CaCl_2$ to complete the polymerization. For the pure alginate samples, 1 wt % $CaSO_4$ was added to induce preliminary gelation which facilitates the subsequent step of complete gelation. Cells were encapsulated at $6x10^6$ cells/ml. The encapsulated cells were rinsed twice in 0.9% NaCl and once in DMEM before being transferred to culture plates.

Chondrogenic differentiation: The differentiation medium consisted of the growth medium supplemented with 1% ITS+, 50 ug/ml ascorbic acid and 100 ng/ml BMP-6.

Evaluation: Cell proliferation in the hydrogels was examined by measuring dsDNA content (PicoGreen™, Invitrogen) after culturing them for 0, 3 and 7 days in the expansion medium. Expression of chondrogenic genes SOX9, ACAN and COL2A1 and hypertrophic gene COL10A1 was assessed via real time RT-PCR. Immunostaining for aggrecan and collagen type II was examined on cryosections.

Statistics. Two-way or one-way ANOVA was performed to determine statistical significance ($p<0.05$).

RESULTS

Significantly higher dsDNA content was measured in two composite hydrogels after 7 d in comparison to the alginate control, though a decrease in cell number was observed after 3 d culture in all the three groups. Two-way ANOVA showed collagen and culture time had significant effect on cell growth, but interaction between collagen and time factor was not significant (data not shown).

Real time RT-PCR showed that the chondrogenic genes were all upregulated in the composites in comparison to pure alginate (Fig.1), and that this effect was larger in the composites with a higher collagen concentration ($p<0.05$). The same trend was also seen in the expression of hypertrophic gene type X collagen.

Immunohistochemical staining showed both aggrecan and type II collagen was significantly expressed in all the cultures (Fig. 2).

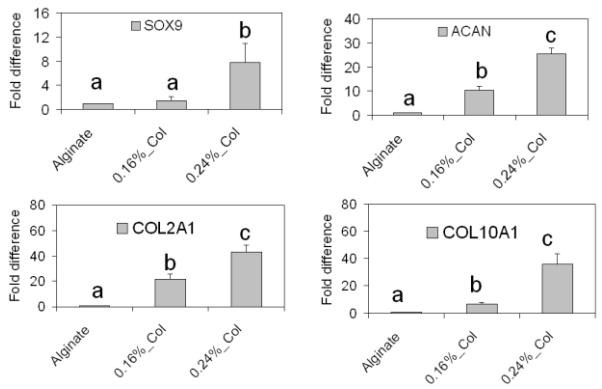

Fig. 1. The relative mRNA level of chondrogenic and hypertrophic genes for the chondrogenic differentiation culture after 14 d. One-way ANOVA analysis was performed ($p<0.05$).

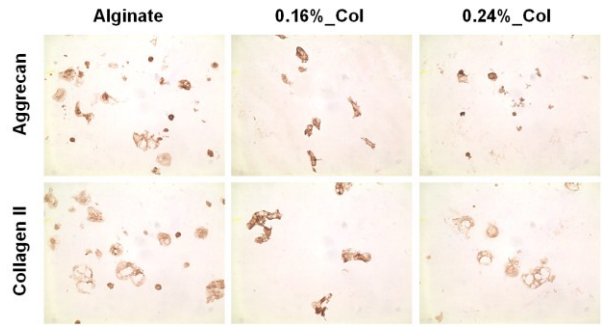

Fig. 2 Immunostaining for aggrecan and type II collagen for the chondrogenic differentiation culture after 14 d.

DISCUSSION

The present method[3] allows soluble type I collagen monomers to reassemble to fibrils in the alginate hydrogel matrix. The incorporation of collagen fibrils provides cell anchorage which is not available in pure alginate. After 7 d, the mASCs population became significantly higher in composites than in the alginate control. Also, the composite hydrogels significantly promoted chondrogenic differentiation of mASCs, and the effect is positively correlated to the collagen concentration. In addition, the composite hydrogels did not show any discernable volume contraction during the culture period, in direct contrast to pure collagen hydrogels which are well known for their contraction upon cell seeding. Thus, the collagen-alginate composite shows great promise for cartilage tissue engineering.

SIGNIFICANCE:

The present study demonstrates that reassembled type I collagen fibrils in alginate hydrogel may promote chondrogenesis of mASCs. The composite has direct impact on cartilage tissue engineering where a biologically more active alginate-based hydrogel is desired.

REFERENCES

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