EFECTOS DE FIBRIN GLUE COMPONENTS ON CHONDROCYTE GROWTH AND MATRIX FORMATION

Introduction

Fibrin glue has been considered for use in a variety of orthopaedic procedures, including fixation of chondral fragments, chondral chips, and osteochondral fragments [1]. Fibrin preparations have also been considered as a material for delivering chondrocytes or growing cartilaginous tissue for cartilage repair procedures [2–4]. Chondrocytes may also interact with fibrin glue in the autologous chondrocyte transplantation procedure, when injected chondrocytes are sealed within cartilage defects by application of fibrin glue to overlying periosteum [5]. However, chondrocyte growth and matrix production vary between fibrin preparations [6]. The concentration of fibrinogen, thrombin, and fibrinolytics inhibitors can affect clot formation and dissolution [7], and may also affect chondrocyte growth. Thus, the objectives of this study were to characterize chondrocyte proliferation and matrix production in well-defined fibrin glue preparations, and to compare these parameters to those of chondrocyte monolayer and alginate cultures.

Methods

Chondrocytes were isolated from full-thickness articular cartilage of the femoral condyles of skeletally mature bovines by sequential enzymatic digestion with pronase and collagenase. The cells were either (1) plated as high (200,000 cells/cm²) density monolayers, (2) formed into alginate beads (4 million cells/ml), or (3) cast into fibrin gels (also at 4 million cells/ml), made from a commercially-available fibrin sealant preparation (Tisseel®, Baxter). These preparations were mixtures of fibrinogen (F, 1X=75-115 mg/ml fibrinogen and 3000 KIU/ml aprotinin) and thrombin (T, 1X=500 IU/ml), in particular: (A) F/1, T/1; (B) F/2, T/2; (C) F/5, T/25; (D) F/16.5, T/16.5; and (E) F/1, T/100. The cell-fibrin constructs were cast between glass slides, separated by 3 mm, and punched into multiple 5mm diameter disks. Samples were incubated with medium (DMEM including 10% FBS and 25 µg/ml ascorbate) and either analyzed after 1 day or 2 weeks, with changes of medium (3 ml/million cells) 3 times per week. The conditioned medium was saved for analysis. At the end of the culture period, the constructs were weighted wet. Then, the cultured cells and also the fibrin-based cartilaginous constructs were solubilized, and analyzed for DNA with Hoechst 33258 and for sulfated glycosaminoglycan (GAG) using DMMB. The conditioned medium was also analyzed for GAG.

Statistical Analysis The effects of culture methodology (construct type on DNA content (relative to that at day 0), GAG content in construct, medium, and total (relative to DNA content at day 0) were analyzed by ANOVA with Tukey post-hoc tests. Cells from each animal were distributed evenly amongst the different culture groups, so that animal was considered a random variable in the ANOVAs. Data are expressed as mean±SEM, n=6 samples per condition.

Results

The analysis of DNA content indicated that seeding of cells in fibrin glue preparations was similarly efficient (Fig. 1A), and that cell proliferation occurred between day 1 and day 14 under all conditions (Fig. 1B). This resulted in a robust ~4- to 8-fold expansion of cell number. The change in DNA content varied significantly between culture conditions (p<0.001), being greater in monolayer culture than all other conditions (p<0.05). However, the change in DNA content was indistinguishable between fibrin preparations and alginate culture, except for a slight decrease (p<0.05) in the F/5, T/25 group. GAG content in the cell and medium fractions and overall culture also varied significantly between culture conditions (each, p<0.001, Fig. 2A). Relative to the number of cells (DNA) present at Day 1, the GAG content in the alginate beads and monolayer of cells were similar. However, the GAG content in fibrin constructs was up to ~50% greater than that of monolayer cultures (each p<0.01) and also slightly greater for the F/2, T/2 and F/16, T/16 groups than that of alginate culture (each p<0.05). The similarity between alginate and monolayer culture was also true of the GAG secreted into the medium. The difference between these cultures and fibrin cultures was also evident, with the latter secreting less GAG into the medium (each, p<0.001 versus monolayer or alginate cultures). Thus, the overall effect of fibrin encapsulation of chondrocytes was a decrease in overall GAG formation (each, p<0.05), but an increase in efficiency and overall retention of GAG in the construct.

Discussion

These results have implications for cartilage repair, using fibrin-derived materials. Here, chondrocytes proliferated and deposited matrix within fibrin glue preparations with a pattern that is more like chondrocytes in alginate than chondrocytes in monolayer. Since chondrocytes proliferated more in monolayer than in alginate culture (Fig. 1B), the similar overall deposition of GAG (Fig. 2A) can be interpreted as chondrocytes exhibiting a rate of GAG deposition on a per cell basis (at the end of the culture period) that is markedly greater in alginate than monolayer culture. In this regard, encapsulation of chondrocytes in fibrin glue preparations appears to have a similar effect as alginate, and indeed also promotes better GAG retention within the construct than does alginate beads (Fig. 2A).

These results further support the idea that fibrin-derived materials may be useful as delivery vehicles for chondrocytes [4,6]. The chondrocytes could either be expanded in number in vitro, or delivered to the defect and allowed to expand in vivo. In cartilage repair applications, certain fibrin compositions may be advantageous not only in supporting cell proliferation and GAG production, but also in allowing easy handling. Even the full-strength fibrin glue preparation facilitated cell proliferation and matrix deposition.

References


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