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Growth factor supplementation improves native and engineered meniscus repair in vitro

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ABSTRACT

Few therapeutic options exist for meniscus repair after injury. Local delivery of growth factors may stimulate repair and create a favorable environment for engineered replacement materials. In this study we assessed the effect of basic fibroblast growth factor (bFGF) (a pro-mitotic agent) and transforming growth factor β3 (TGF-β3) (a pro-matrix formation agent) on meniscus repair and the integration/maturation of electrospun poly(ε-caprolactone) (PCL) scaffolds for meniscus tissue engineering. Circular meniscus repair constructs were formed and refilled with either native tissue or scaffolds. Repair constructs were cultured in serum-containing medium for 4 and 8 weeks with various growth factor formulations, and assessed for mechanical strength, biochemical content, and histological appearance. Results showed that either short-term delivery of bFGF or sustained delivery of TGF-β3 increased integration strength for both juvenile and adult bovine tissue, with similar findings for engineered materials. While TGF-β3 increased proteoglycan content in the explants, bFGF did not increase DNA content after 8 weeks of culture. This work suggests that in vivo delivery of bFGF or TGF-β3 may stimulate meniscus repair, but that the time course of delivery will strongly influence success. Further, this study demonstrates that electrospun scaffolds are a promising material for meniscus tissue engineering, achieving comparable or superior integration compared with native tissue.

1. Introduction

The meniscus is a C-shaped fibrocartilage in the knee that transmits load from the femur to the tibia [1,2]. The unique architecture and composition of the meniscus, consisting of aligned collagen bundles and centrally located proteoglycan, allows it to withstand both tensile and compressive forces in order to transfer loads and maintain joint stability during movement [3]. Due to the high stresses imparted on the tissue [4] both acute and degenerative tears are common, and the natural repair capacity is limited, especially in the inner avascular regions [5]. Of the 850,000 meniscus surgeries performed annually in the USA [6] resection is the most common technique to alleviate symptoms associated with meniscal tears. However, this procedure can result in joint incongruency and significant stresses on the adjacent cartilage, which can lead to premature degeneration (i.e. osteoarthritis) [7,8]. Few procedures exist to repair the meniscus, and those that are performed do not restore native tissue structure and function. Thus there is a need for novel strategies to improve meniscus repair.

Delivery of biological factors may stimulate tissue repair either alone or in combination with mechanical stabilization. Early work in this area delivered vascular endothelial growth factor (VEGF) from sutures to stimulate blood vessel formation in the damaged region [9]. However, delivery of VEGF from sutures failed to improve healing in vivo in a number of studies, perhaps due to suboptimal time courses of delivery [10,11]. Rather than modulating the vascular supply, another approach is to alter biosynthesis and matrix assembly at the repair site. During repair, new matrix must be formed by nearby cells to bridge the wound gap, creating a mechanically stable interface. Increasing the amount of matrix deposited by each cell or increasing the overall number of cells (or a combination of the two) may improve repair. One of the most potent stimulators of matrix deposition in meniscal cells is transforming growth factor β3 (TGF-β3) [12–16], although other growth factors such as bFGF, PDGF-AB, IGF-1 and EGF can all increase matrix production [17]. Basic fibroblast growth factor (bFGF) strongly stimulates the proliferation of meniscus cells in monolayer culture as well as in tissue engineered constructs [17–20]. For this reason both TGF-β3 and bFGF were identified as potential meniscus repair factors by Kasemkijwattana et al. [21], and Imler et al. showed that TGF-β3 stimulated protein and proteoglycan deposition to a greater extent than bFGF in meniscus explants [16]. Due to the ability of these growth factors to stimulate matrix deposition and increase cell number, they are promising candidates for promoting repair of avascular meniscus tears as well as the maturation and integration of engineered materials in vivo.

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Another important parameter in repair is the time course of delivery of biological factors. Many growth factors function during a very specific window and at precise doses, and may work in concert with other cues [22]. Clinically, growth factors could be delivered with a bolus injection or via sustained release from a biomaterial over a given period of time, however, continual delivery of a growth factor over very long periods of time is challenging. Fortunately, recent work suggests that short-term exposure to growth factors may actually have superior efficacy compared with continual delivery [23]. These findings suggest that delivery of a biological factor to enhance meniscus repair need not occur continuously, but rather might need only be applied in the appropriate time frame to exert maximal impact.

Chemical cues alone may not be sufficient to restore meniscus function in situations where repair is not possible, as is the case when the tissue is severely disrupted or degraded. To address this issue, tissue engineering generates structures that recapitulate native tissue architecture and behavior [24]. Recently biodegradable scaffolds composed of porous collagen (Menaflex) or porous polyurethane (ActiFit) were introduced into clinical practice to replace regions of resected meniscus [25]. Other pre-clinical materials and scaffolds under investigation include subintestinal submucosa, anatomically shaped alginate hydrogels, and hyaluronic acid–based constructs, to name but a few [26–28]. Our laboratory has focused on the use of electrospun scaffolds, which are fabricated by collecting nanosized synthetic and biological polymeric fibers on electrically charged surfaces [29]. These scaffolds are amenable to cell attachment, proliferation and infiltration [30–32] and can be functionalized to release biological agents and growth factors [33–35].

While these nanofibrous scaffolds have shown promise in vitro, additional work is required to evaluate how these scaffolds are colonized by cells from the native tissue, to determine how the scaffolds integrate mechanically with the native tissue, and the key modulators in this integration process. Because large animal studies of meniscus repair are quite costly, smaller in vitro experiments may be beneficial for assessing the potential of new therapies [36]. Early work demonstrated that meniscus tissue remains viable when cultured under proper medium conditions [37]. Later, concentric explants were used to test the influence of inflammatory cytokines and matrix metalloproteinases on meniscus integration [38]. More recently we have used this model to demonstrate that, in accordance with observations made clinically [39], immature meniscus undergoes self-repair to a greater extent than mature meniscus in vitro [30] and that TGF-β3 bolsters this integration process [30,38]. Further, we explored how electrospun scaffolds in annular meniscus defects are colonized by native tissue cells with time in culture [30].

To further these lines of inquiry, this study evaluated the impact of TGF-β3 and bFGF delivery for short (1 week) and sustained (8 weeks) periods, alone and in combination, and assessed the mechanical integration strength of the repair, as well as the biochemical content of the repair material. This work was carried out in both juvenile and adult bovine meniscus defects (meniscus to meniscus integration), as well as in meniscus defects "repaired" with electrospun scaffold. We hypothesized that bFGF would improve integration by increasing the cell density in the tissue interface while TGF would act to increase matrix density at the repair interface, and that short-term delivery of growth factors would elicit comparable integration to continual delivery.

2. Materials and methods

2.1. Evaluation of meniscus to meniscus repair with growth factor supplementation

Menisci were dissected from the knee joints of juvenile (0–3 months old) and adult (skeletally mature, >2 years old) bovine limbs in a sterile manner. Cylinders (8 mm diameter × 3 mm thick) were excised centrally in the axial direction using a dermal punch (Miltex, Plainsboro, NJ), as shown in Fig. 1A. To simulate an acute meniscus tear, a full thickness inner columnar defect (4 mm diameter) was made and the core reinserted with care to maintain the fiber alignment, as shown in Fig. 1B and C.

Meniscus repair constructs were cultured in control medium (Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin/streptomysin/fungizone (PSF), 50 μg ml^{-1} ascorbate-2-phosphate) supplemented with five different growth factor regimens: continual 50 ng ml^{-1} bFGF, 1 week of bFGF, continual 10 ng ml^{-1} TGF-β3, combined continual bFGF and TGF-β3, combined 1 week of bFGF and continual TGF-β3, or 1 week of TGF-β3 (Fig. 1D). Explants were incubated in 6-well plates covered completely in medium, which was replaced twice weekly. After 4 and 8 weeks of culture, the mechanical integration strength was evaluated using a custom testing device [30]. Briefly, an Instron 5848 was fitted with a 3.5 mm diameter indenter in series with a 50 N load cell. This indenter was placed above a plate with a 5 mm diameter through hole. The meniscus sample was placed on the plate, and the indenter pressed through the defect site at a rate of 0.0833 mm s^{-1}. The integration strength was calculated as:

$$\text{integration strength} = \frac{\text{maximum force } (N)}{2\pi r \cdot h}$$

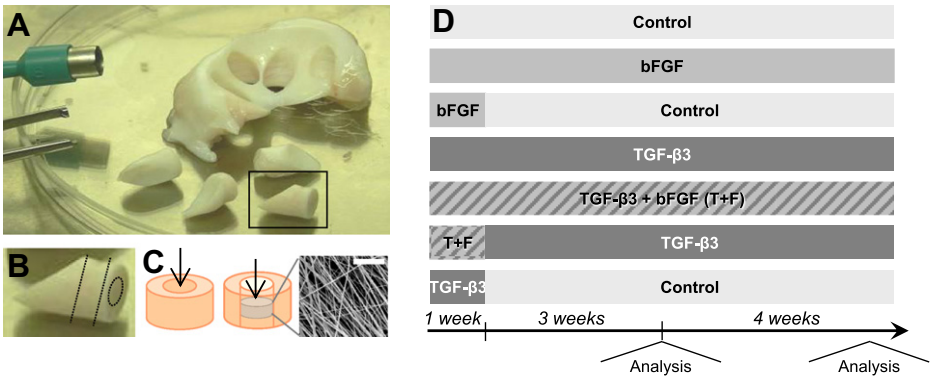

Fig. 1. Experimental set-up and study design. (A) 8 mm cylinders were excised from bovine meniscus. (B) The tissue cylinders were flattened and a smaller biopsy punch was used to remove a 4 mm core. (C) This tissue core was either reinserted back into the tissue (left), or replaced with a disc of electrospun PCL (right). Inlay scale 20 μm. (D) Schematic of media formulations and temporal exposure regimens and testing time points over 8 weeks.

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