Avascular Meniscus Healing by Stem Cell Recruitment: Effect of Dose and Release Rate of CTGF and TGFβ3

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ABSTRACT INTRODUCTION: Meniscus injuries are one of the most important contributing factors of osteoarthritis (OA), with over one million Americans undergoing meniscus repair each year. Tears in the inner avascular region hardly heal due to its poor intrinsic healing capacity thus frequently extended into the middle-third region, followed by meniscus deterioration. Recently, we have reported that a timely controlled application of connective tissue growth factor (CTGF) and transforming tissue growth factor beta 3 (TGFβ3) successfully improved healing of avascular meniscus tears by inducing recruitment and step-wise fibrocartilaginous differentiation of mesenchymal stem/progenitor cells (MSCs). Moreover, a single application of CTGF-loaded fibrin glue (transient release) mixed with PLGA microspheres (µS)-encapsulating TGFβ3 (sustained release) successfully recruited synovial MSCs into the defect sites, followed by integrated healing with fibrocartilaginous tissue. In this study, we investigated effects of CTGF dose and release rate of TGFβ3 on avascular meniscus healing in our existing explant model. Our hypothesis is that doses and release rates of CTGF and TGFβ3 are contributing factors for functional outcome in avascular meniscus healing by stem cell recruitment.

METHODS: Following our established method, a meniscus explant model was used to study in vitro healing of avascular meniscus tears. Menisci isolated from skeletally mature bovine knee joints were prepared and longitudinal incisions were made in the inner third zone. Then fibrin glue loaded with 100 ng/ml (low dose) or 1,000 ng/ml (high dose) CTGF and TGFβ3-µS was applied to the incised site. Briefly, 50 mg/mL fibrinogen and 50 U/mL thrombin with or without CTGF and 10 mg of PLGA µS-encapsulating TGFβ3 were co-injected in between the incised tissue surfaces using FibrilJet® dual-injector with a blending applicator (Nordson Micromedics, Westlake, OH). PLGA µS-encapsulating TGFβ3 (2.5 µg per 250 mg PLGA) were prepared by the double-emulsion technique as per our prior works, and in vitro release kinetics of TGFβ3 were measured by ELISA. Different release rate of TGFβ3 were applied by using different compositions of PLGA with fast and slow degradation rates. The meniscus explants were then cultured on top of monolayer-cultured human synovial MSCs (syMSCs) for 8 wks with fibrous and/or chondrogenic supplements. All the harvested explants were analyzed for healing of avascular tears using histology, biochemical assays, and mechanical testing. Recruited and engrafted human syMSCs were labeled by human nucleus antigen, and collagen fibers orientation in the healing zone was quantitatively analyzed using our automatic digital imaging analysis with polarized images.

RESULTS SECTION: Consistently with our previous reports, a short-term release of CTGF (~6 days) and sustained release of TGFβ3 (>35 days) successfully induced integrative healing of avascular meniscus tears in dose and release rate-dependent manner (Fig. 1A). Histologically, high CTGF dose and slow TGFβ3 release appeared to show better integration of incised menisci in comparison with low CTGF dose and fast release of TGFβ3 (Fig. 1A). Slow release of TGFβ3 showed denser fibrocartilaginous matrix in the healing region as compared to the fast release (Fig. 1A). Consistently, total COL and GAG contents were significantly higher in the high dose CTGF and the slow TGFβ3 release than the low dose and the fast release (Fig. 1B). In addition, the high dose CTGF and slow release of TGFβ3 resulted in highly aligned collagen fibrils in the healing zone as compared to the other groups (Fig. 2A). Quantitatively, the angular deviation (AD) of collagen fibrils was lower in the high dose of CTGF and slow release of TGFβ3 as compared to the other groups (Fig. 2B). Human nucleus staining showed that significantly more syMSCs were recruited and engrafted in the healing zone with high dose of CTGF as compared to the low dose (data not shown; n = 5 per group, p<0.05). Tensile modulus and ultimate strength of the healed tissue were significantly higher in the high dose of CTGF and the slow release of TGFβ3 (data not shown; n = 5 per group, p<0.05).

DISCUSSION: Our previous work demonstrated that CTGF induces stem cells recruitment and formation of intermediate fibrous integration, whereas TGFβ3 leads to fibrocartilaginous remodeling for healing of avascular meniscus tears. The present findings suggest that the quality of meniscus healing is affected by the dose of CTGF and the release rate of TGFβ3. Despite the likely clear role of CTGF from our previous work, the dose effect of CTGF on the quality of meniscus healing was dependent on the release rate of TGFβ3. With fast TGFβ3 release, high dose of CTGF resulted in less COL than low dose and no difference in the GAG contents. With slow TGFβ3 release, however, the high CTGF dose yielded less GAG than the low dose. It is thus suggested that dose-effect of CTGF and the role of TGFβ3 release rate are complimentary to each other. From the multiple combinations of CTGF doses and TGFβ3 release rates, we found that high dose (1,000 ng/mL) of CTGF and slow release rate of TGFβ3 (from PLGA 85:15) are the most effective in avascular meniscus healing. Collectively, this study may represent an important step to develop a novel therapeutics to induce seamless healing of inner meniscus tears by stem cell recruitment.

SIGNIFICANCE: Our novel strategy to induce inner meniscus healing may overcome limitations of the current treatments of meniscus injuries.

IMAGES AND TABLES:

![Fig. 1. Effect of CTGF dose and TGFβ3 release rate on induced healing of avascular meniscus tears: (A) Macrosopic and histological analysis with H&E, Picrisirus Red (PR), and Alcan Blue (AB) staining (scale = 100 µm). (B) Quantitative analysis of COL and GAG in the healing zone of meniscus (n=5 per group; *p<0.01, #p<0.05 compared to native).](image1)

![Fig. 2. Polarized images of PR stained slides (A) showing collagen fibers orientation (scale = 50 µm) and angular deviation (AD) analyzed by automated imaging processing (B) (n = 5 per group; *p<0.01 compared to low dose, #p<0.01 compared between fast and slow).](image2)