Latent TGF-β Contents of Synovial Joint Tissues

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INTRODUCTION: The health of synovial joint tissues (e.g., cartilage, meniscus, tendons) requires the well-regulated activity of the anabolic signaling molecule transforming growth factor beta (TGF-β). It has long been understood that the major regulatory feature of TGF-β stems from its presence in the synovial joint in an inactive latent complex (latent TGF-β [LTGF-β]), whereby the active TGF-β signaling peptide is surrounded by a unique propeptide shell, which initially acts to sequester it from cell receptors [1]. In recent years, a series of hallmark discoveries have elucidated the critical role the mechanical loading environment plays in inducing the activation of LTGF-β in the synovial joint: 1) Activation of soluble LTGF-β in synovial fluid (SF) can be induced by physiologic fluid shearing [2], and 2) Activation of LTGF-β bound to the tissue ECM can be induced by matrix strain and integrin traction [3]. Overall, this has led to an evolved understanding of the importance of LTGF-β stores in serving as a critical reservoir of growth factor activity that can be mechanically triggered, thus providing the tissue a degree of protection when faced with its intense mechanical environment.

While it has generally been surmised that the synovial joint possesses abundant supplies of LTGF-β, it is intriguing that there exists limited data on LTGF-β measurements in synovial joint tissues. In regards to measurements of ECM-bound LTGF-β, originally, Morales et al. reported levels as high as 300ng/mL LTGF-β (all isoforms) in the ECM of immature bovine cartilage [4]. Our work has subsequently reported a range of 100-200ng/mL LTGF-β1 in similar tissues [5]. However, remarkably, we still possess a limited understanding of how LTGF-β levels may vary across critical factors such as tissue regions, aging and maturation, and species. In this investigation, we attempt to advance our understanding of the regulatory potential of LTGF-β in the synovial joint by perform novel assessments of its content in human articular cartilage specimens and how its content varies by: 1) synovial joint tissue type (cartilage, meniscus, tendon) and 2) tissue age (fetal, skeletally immature, skeletally mature). In these assessments, we measure two critical regulatory pools of LTGF-β: 1) LTGF-β bound to the tissue ECM and 2) LTGF-β that is continuously secreted by cells into the surrounding SF. Further, in consideration of the pronounced autoinduction behavior of TGF-β, we assess LTGF-β secretion rates in the absence and presence of physiologic active TGF-β activity.

METHODS: **Tissue Sources**: Cylindrical articular cartilage explants (∅3×2mm) were harvested from the femoral condyles of fetal, skeletally-immature (2 month), and skeletally-mature (2 & 5-year-old) bovine specimens (Animal Technologies). Meniscal explants were harvested from the 2-month bovine inner meniscus. Tendon explants (1mm thick sections) were harvested from the 2-month bovine patellar tendon. Human femoral condyle cartilage explants (∅3×2mm) were procured from autopsy donors via the NDRI (n=4 donors; ages 58, 51, 60, 58). **ECM-bound LTGF-β Concentration**: TGF-β was extracted from explants (n=8 explants per specimen) via 48h treatment of 4M guanidine hydrochloride (which activates and extracts all LTGF-β) and subsequently measured via a TGF-β1 isoform-specific ELISA (R&D Systems) [5]. The DNA content of paired specimens were measured via Pico Green. LTGF-β1 contents were reported as levels normalized by the tissue volume (LTGF-$\beta1_{vol}$) and levels normalized to tissue cell quantity (LTGF-$\beta1_{cell}$). **LTGF-β Secretion Rate**: Immature bovine cartilage, bovine meniscal cartilage, and human cartilage explants (n=5 explants per specimen) were maintained in high glucose DMEM supplemented with PSAM, L-proline, BSA, and ascorbate (±1ng/mL active TGF-β3). Conditioned medium was collected every 48h for 2 weeks and assayed for the LTGF-β1 secretion rate [5]. LTGF-β secretion rates were similarly reported as levels normalized by the tissue volume (LTGF-$\beta1_{vol}$ secretion rate) and levels normalized to tissue cell quantity (LTGF-$\beta1_{cell}$ secretion rate).

RESULTS: **Tissue Type Dependence**: LTGF-$\beta1_{vol}$ levels varied significantly with tissue type, decreasing in order of cartilage>meniscus>tendon (Fig 1A; $p<0.05$). However, LTGF-$\beta1_{cell}$ levels did not significantly vary (Fig 1B). LTGF-β1 secretion rate levels increased with active TGF-β exposure and were generally similar for cartilage and meniscus specimens. **Age Dependence**: While LTGF-$\beta1_{vol}$ levels significantly decreased with tissue age (Fig 2A; $p<0.05$), LTGF-$\beta1_{cell}$ levels did not significantly vary (Fig 2B). **LTGF-β in Human Cartilage**: LTGF-$\beta1_{vol}$ levels of 3 of 4 human specimens were significantly elevated above bovine levels ($p<0.05$; 2.9-fold above on avg; Fig 3A). For LTGF-$\beta1_{cell}$ measurements, levels increased even further above bovine levels (11.6-fold above on avg; Fig 3B). All human specimens exhibited a significant increase in LTGF-β1 secretion rate when exposed to active TGF-β ($p<0.05$). While LTGF-$\beta1_{vol}$ secretion rates were significantly below bovine levels, LTGF-$\beta1_{cell}$ secretion rates were significantly above bovine levels.

DISCUSSION: This study represents one of the first characterizations of the variation of LTGF-β content of synovial joint tissues. Intriguingly, results demonstrate the while LTGF-β levels per tissue volume can vary considerably in the synovial joint by tissue type (Fig 1A) and specimen age (Fig 2A), LTGF-β levels per cell quantity remain fairly uniform (Figs 1B, 2B). This finding highlights that LTGF-β can serve as an important reservoir of anabolic growth factor activity for a wide range of synovial joint cells. Given the mechanically-labile nature of LTGF-β and the powerful biosynthetic effect of activated TGF-β, these reservoirs can considerably support tissue maintenance and repair in response to the characteristic intense, dynamic mechanical environment of the synovial joint. Further, it is important to highlight the remarkably high levels of LTGF-β_{cell} measured in human cartilage specimens, yielding near 10-fold enhancements above bovine levels in ECM-bound LTGF-β content (Fig 3B) and near 6-fold enhancements in LTGF-β secretion rates (Fig 3D). This finding is particularly intriguing given the advanced age of the human specimens analyzed in this study (51-60 age range), suggesting that, in contrast to evidence in animal tissue systems [6], TGF-β activity likely plays an important role in continuing to protect the aging human synovial joint. This notion is further supported by the observed strong biosynthetic enhancements of human cartilage in response to 1ng/mL TGF-β activity (Fig 3C, D).

SIGNIFICANCE: This study advances a novel understanding of the critical role of LTGF-β reservoirs in supporting the homeostasis of the connective tissues of the synovial joint with particular emphasis on its potential importance in the protection of aging human articular cartilage.

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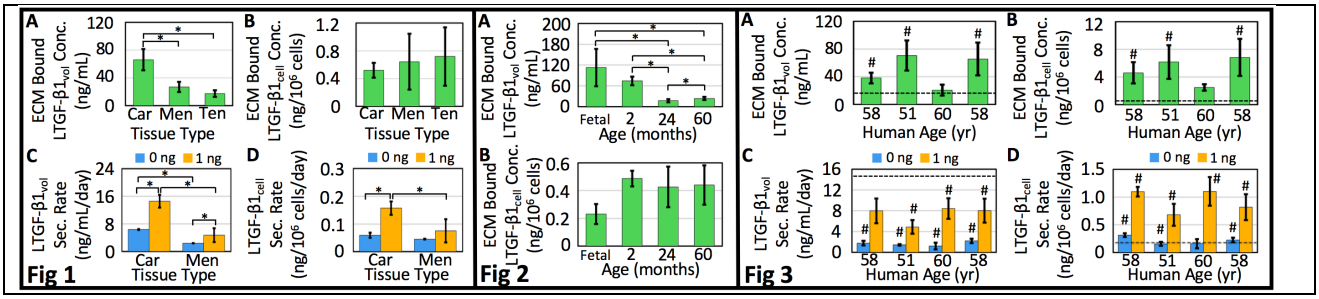

Fig 1: Levels of ECM-bound LTGF-β1 in immature bovine explants of cartilage (Car), meniscus (Men), and tendon (Ten) normalized by (A) tissue volume and (B) cell quantity. LTGF-β1 secretion rate from explants normalized by (C) tissue volume and (D) cell quantity. *$p<0.05$: significant difference between groups. **Fig 2:** Levels of ECM-bound LTGF-β1 in aging bovine cartilage explants normalized by (A) tissue volume and (B) cell quantity. *$p<0.05$: significant difference between groups **Fig 3:** Levels of ECM-bound LTGF-β1 in human cartilage explants normalized to (A) tissue volume and (B) cell quantity. Dashed line represents adult bovine value. LTGF-β1 secretion rate from explants normalized by (C) tissue volume and (D) cell quantity. Dashed line represents immature bovine level (1ng/mL active TGF-β3 exposed). #$p<0.05$: significant difference from corresponding bovine level.