

Dual Compartmentalized Functionality of TGF- β in the Synovial Joint

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INTRODUCTION: The health of the synovial joint is highly dependent on the activity of the anabolic signaling molecule transforming growth factor beta (TGF- β). The major regulatory feature of TGF- β stems from its presence in an inactive latent complex (LTGF- β), whereby the active TGF- β signaling peptide is surrounded by a sequestering propeptide shell. LTGF- β is present at high levels in synovial fluid (SF) in a soluble form and in articular cartilage, bound to the tissue ECM. In both configurations, in order for TGF- β to elicit a biological response, the signaling peptide must first undergo release from this complex (termed TGF- β activation) and subsequently reach cell receptors. Recent discoveries have provided considerable insights into the mechanistic details of this process, including: 1) the role of physiologic mechanical deformation in inducing LTGF- β activation (e.g., activation of soluble LTGF- β in SF via physiologic fluid shear [1] and activation of ECM-bound LTGF- β via tissue strain or integrin traction [2]), and 2) the characterization of the remarkably high binding affinity between the active TGF- β signaling peptide and the cartilage ECM, which substantially inhibits activated TGF- β 's transport through the tissue [3].

Overall, these discoveries point to the existence of a unique compartmentalized functionality of TGF- β activity in the synovial joint, whereby: 1) Soluble LTGF- β in SF can be activated via load-induced fluid shear and is unable to penetrate deep into articular cartilage, establishing a synovial compartment (Fig 1) of SF TGF- β that acts predominantly on SF-peripheral cells (superficial zone [SZ] chondrocytes & synoviocytes), and 2) ECM-bound LTGF- β can be activated by tissue strain or cell integrins and acts locally, establishing an ECM compartment (Fig 1) that acts on surrounding chondrocytes.

Here, we assess the functionality of these dual compartments in the synovial joint using a controlled cartilage explant model. Initially, we confirm the degree of TGF- β activity compartmentalization by assessing the penetration of activated TGF- β from SF into live articular cartilage. Secondly, given the established effect of large TGF- β doses on enhancing boundary lubricant secretion by SF-peripheral cells [4], we examine the effect of TGF- β activated in SF on regulating boundary lubricant levels. Here we focus on active TGF- β doses associated with physiologic shear-induced activation (~ 1 ng/mL shear-activated TGF- β per hour for 1-4 hours of shear activity [joint exercise] per day [1]). Lastly, we assess the functionality of the ECM compartment in maintaining the integrity of the cartilage ECM by assessing the effect of inhibition of the endogenous activity of ECM-bound LTGF- β on the integrity of cartilage explants over long-term culture.

METHODS: Cylindrical articular cartilage explants were procured from femoral condyles of bovine calves (n=5) and human specimen autopsy donors (NDRI; n=4; HS1: 51, HS2: 58, HS3: 58, HS4: 60 years old). **Penetration of activated TGF- β into articular cartilage:** Live $\varnothing 6 \times 3$ mm bovine explants were cultured in medium supplemented with 10 ng/mL exogenous active TGF- $\beta 3$ (aTGF- $\beta 3$). After 1 and 4 weeks, explants were sub-punched ($\varnothing 3$ mm), axially sectioned, and analyzed for their exogenous aTGF- $\beta 3$ content via ELISA [3]. **Synovial Compartment Functionality:** The effect of shear-activated levels of TGF- β on the secretion of the critical synovial boundary lubricant, PRG4, by SZ cartilage explants was assessed. Here, explants were exposed to aTGF- $\beta 3$ at a range of doses (0, 0.1, 0.3, 1, 3 ng/mL) and daily durations (1, 4 or 24 h) that encompass those associated with shear-induced activation from physiologic joint exercise. Subsequently, conditioned medium was collected and analyzed for secreted PRG4 via ELISA [4]. **ECM Compartment Functionality:** The effect of endogenous activity of ECM-bound LTGF- β on articular cartilage integrity was assessed. Here, explants ($\varnothing 2 \times 2$ mm) were maintained in DMEM for 1 month while in the absence or presence of a TGF- β receptor kinase inhibitor LY364947 (LY; n=10 bovine explants per group; n=8 explants per human specimen per group). After culture, explants were analyzed for their compressive Young's modulus (E_y) and GAG content.

RESULTS: Penetration of activated TGF- β : Exogenous aTGF- $\beta 3$ exhibited substantial gradients in cartilage explants, even for up to 4 weeks of exposure, with aTGF- $\beta 3$ unable to reach beyond the topmost 400 μ m of the tissue (Fig 2). **Synovial Compartment:** aTGF- $\beta 3$ induced significant enhancements of explant PRG4 secretion, reaching up to 10-fold enhancements at aTGF- $\beta 3$ levels shown to be activated by physiologic SF shearing (~ 1 ng/mL). PRG4 enhancements were comparable for all aTGF- β exposure durations (1, 4, 24h). **ECM Compartment:** LY induced a significant decrease in E_y of bovine explants (67% decrease; $p < 0.05$) and 3 out of 4 human specimens (34% on avg; $p < 0.05$). Further, LY induced a significant decrease in GAG content of bovine explants (33% decrease; $p < 0.05$) and one of the human specimens (H1) (22% decrease; $p < 0.05$). GAG contents have not yet been measured for H3 and H4. No viability loss was observed for any tested specimen (results not shown).

DISCUSSION: The results of this study point to TGF- β 's dual compartmentalized role in regulating both boundary lubrication and the integrity of the articular cartilage ECM in the synovial joint (Fig 1). This compartmentalized role is supported by severe penetration limitations of SF-activated TGF- β into articular cartilage. Due to a combination of ECM binding interactions [3] and cell-mediated internalization [5], aTGF- β is unable to penetrate beyond the topmost 400 μ m of the tissue (Fig 2), supporting the notion that TGF- β activated in SF acts exclusively on SF-peripheral cells (i.e., synoviocytes and SZ chondrocytes). This synovial compartment appears to predominantly regulate SF boundary lubricants, as TGF- β activation levels associated with physiologic joint activity (1 ng/mL for 1 or 4 hr daily exposure) can give rise to substantial enhancements in the secretion rate of the critical boundary lubricant, PRG4 (Fig 3A). An additional implication of the limited penetration of SF-activated TGF- β is the understanding that deeper zone chondrocytes alternatively rely exclusively on TGF- β activity from the local stores of ECM-bound LTGF- β in the cartilage tissue (i.e., ECM compartment). Here we perform a novel examination of the inhibition of endogenous ECM-bound TGF- β activity on the long-term integrity of articular cartilage. Results demonstrate that inhibition of ECM-bound TGF- β can greatly diminish the integrity of cartilage, as marked by significant decreases in E_y in both immature bovine and aging human (51-60 yr) specimens. It is important to note that, given the ability of mechanical loading to activate ECM-bound LTGF- β [2], the functional role of this compartment in maintaining cartilage may be far more considerable in response to mechanical loading, a hypothesis that will be examined in future investigations.

SIGNIFICANCE: This study advances a novel understanding of how TGF- β is compartmentally regulated to maintain synovial joint health and sets the foundation for the development of targeted therapeutics that can reverse the course of TGF- β regulatory break-down during joint degeneration.

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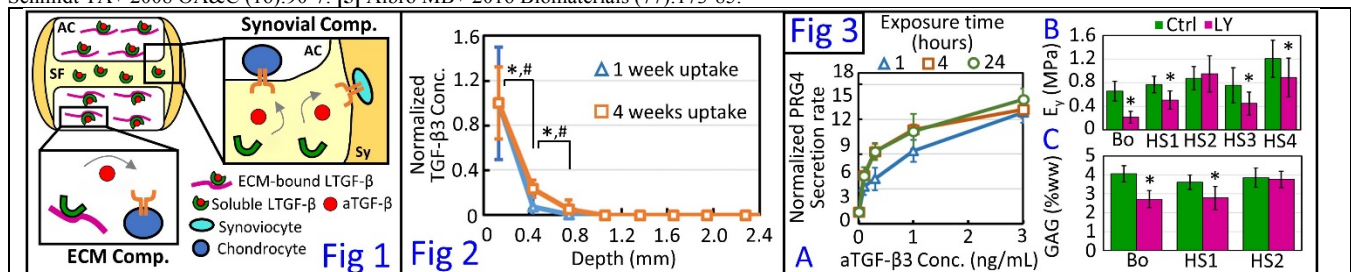


Fig 1: Schematic of dual compartmentalized regulation of TGF- β activity in the articular cartilage (AC) and synovium (Sy) of the synovial joint: 1) Soluble LTGF- β activated in SF acts exclusively on SF-peripheral cells (SZ chondrocytes/synoviocytes), enhancing PRG4 secretion rates. 2) ECM-bound LTGF- β activated in cartilage acts locally on chondrocytes, regulating cartilage ECM integrity. **Fig 2:** Gradients of exogenous aTGF- β through depth of articular cartilage, normalized to surface concentration. **Fig 3:** (A) PRG4 secretion rate (normalized to TGF- β -free rate) from SZ cartilage explants in response to varying exposure doses and durations of aTGF- β . (B) E_y and (C) GAG of bovine explants (Bo) and human explants (HS1, HS2, HS3, HS4) after 1-month culture with TGF- β receptor kinase inhibitor (LY). * $p < 0.05$: significant decrease below corresponding ctrl.