Computational and Experimental Characterizations of the Spatiotemporal Activity and Functional Role of TGF-β in the Synovial Joint

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Abstract

TGF-β is a prominent anabolic signaling molecule associated with synovial joint health. Recent work has uncovered mechanochemical mechanisms that activate the latent form of TGF-β (LTGF-β) in the synovial joint—synovial fluid (SF) shearing or cartilage compression—pointing to mechanobiological phenomena, whereby enhanced TGF-β activity occurs during joint stimulation. Here, we implement computational and experimental models to better understand the role of mechanochemical-activated TGF-β (aTGF-β) in regulating the functional biosynthetic activities of synovial joint tissues. Reaction-diffusion models describe the pronounced role of extracellular chemical reactions—load-induced activation, reversible ECM-binding, and cell-mediated internalization—in modulating the spatiotemporal distribution of aTGF-β in joint tissues. Of note, aTGF-β from SF shearing predominantly acts on peripheral tissue regions (superficial zone [SZ] cartilage and synovium) and aTGF-β from cartilage compression predominantly acts on chondrocytes through all cartilage layers. Further, ECM reversible binding sites in cartilage act to modulate the temporal delivery of aTGF-β to cells, creating a dynamic where short durations of joint activity give rise to extended periods of aTGF-β exposure at moderated doses. *Ex vivo* tissue models were subsequently utilized to characterize the influence of physiologic aTGF-β activity regimens in regulating functional biosynthetic activities. Physiologic exposure regimens of aTGF-β in SF induce strong 4-fold to 9-fold enhancements in secretion rate of the synovial biolubricant, PRG4, from SZ cartilage and synovium explants. Further, aTGF-β inhibition in cartilage over 1-month culture leads to a pronounced loss of GAG content (30-35% decrease) and tissue softening (60-65% E_Y reduction). Overall, this work advances a novel perspective on the regulation of TGF-β in the synovial joint and its role in maintaining synovial joint health.

1. Introduction

Articular cartilage is a low-friction, load-bearing connective tissue that allows for smooth, pain-free motion at synovial joint interfaces. Notably, tissue composition is optimized for its mechanical performance, consisting of: 1) a dense extracellular matrix (ECM) of glycosaminoglycans (GAG) interspersed within a type-II collagen fibril network that supports mechanical loads and promotes interstitial fluid pressurization during loading, yielding the tissue's low friction properties (Krishnan et al., 2004), and 2) the presence of lubricious biomolecules concentrated at the cartilage surface (e.g. hyaluronan and proteoglycan-4 [PRG4]) (Jay, 1992; Schumacher et al., 1994) that serve as boundary lubricants to further improve tribological properties (Jay et al., 2007; Schmidt et al., 2007). The mechanical functionality of cartilage is dependent on the coordinated biological activity of specialized cells throughout the synovial joint. Articular chondrocytes through the cartilage depth secrete GAG and collagen to maintain tissue composition, structure, and mechanical properties. Chondrocytes in the cartilage surficial zone (SZ) and fibroblasts in the joint lining synovium secrete hyaluronan and PRG4 to replenish their supply in synovial fluid (SF). The long-term health of cartilage depends on mechanochemical feedback mechanisms that coordinate functional activities in response to the synovial joint's mechanical environment.

Transforming growth factor β (TGF-β) is a prominent anabolic signaling molecule associated with synovial joint health (Barnard et al., 1990). TGF-β regulation is essential as TGF-β knockout (Serra et al., 1997; Chen et al., 2012) and TGF-β excesses (Fava et al., 1989; van Beuningen et al., 1994; Bakker et al., 2001) are both associated with the initiation of pronounced joint pathology. TGF-β strongly enhances the secretion of biolubricants (Jones and Flannery, 2007; Niikura and Reddi, 2007; Lee et al., 2008; Schmidt et al., 2008) and ECM structural proteins (Hiraki et al., 1988; Morales and Roberts, 1988; Redini et al., 1988; Grimaud et al., 2002; Zhen and Cao, 2014), pointing to a physiologic role in promoting the major functional activities of synovial joint cells. However, the mechanistic details by which TGF-β regulates these activities remains poorly understand due, in part, to the presence of a complex assortment of molecular interactions associated with TGF-β in the extracellular environment, which can significantly influence its spatiotemporal activity in the synovial joint.

Notably, TGF-β is present in the synovial joint's extracellular environment in a unique, inactive latent complex, consisting of the TGF-β signaling peptide surrounded by a pro-peptide shell (latency associated peptide [LAP]), together constituting the latent TGF-β (LTGF-β) complex (Hyytiainen et al., 2004). In order for the TGF-β ligand to bind to cell receptors and elicit a biological response, it must first undergo release from the latent complex—a process termed TGF-β

activation (Gleizes et al., 1997). LTGF-β is present in the synovial joint in distinct endogenous pools: 1) in SF, where it resides in a soluble form (Fava, 1989; Lotz et al., 1990; Albro et al., 2012) and 2) in articular cartilage, where it is bound to the ECM via covalent tethering through latent TGF-β binding proteins (Morales et al., 1991). Recent work has uncovered a host of physiologic mechanochemical activation pathways in synovial joint tissues, including activation of SF LTGF-β by fluid shearing in response to physiologic joint motion (Albro, 2012), and activation of ECM-bound LTGF-β in cartilage by physiologic cartilage compression (Madej et al., 2014; Madej et al., 2016; Tang et al., 2018) and cell-secreted enzymes (Boyan et al., 1994; Pedrozo et al., 1999; D'Angelo et al., 2001; Maeda et al., 2001) (Fig.1A). Together, these pathways point to the existence of mechanobiological feedback mechanisms, whereby physiologic synovial joint loading may upregulate TGF-β activity, in turn promoting the biosynthesis of boundary lubricants and structural ECM constituents from synovial joint cells. Adding to the complexity of extracellular TGF-β regulation is the capability of the active TGF-β (aTGF-β) peptide to undergo a host of additional molecular interactions after release from the latent complex, including pronounced binding to cartilage ECM constituents (Albro et al., 2013) and internalization by chondrocytes (Albro et al., 2016). The impact of these interactions on the activity and distribution of aTGF-β may be significant, potentially acting to sequester aTGF-β in specific tissue regions or modulate its temporal presentation to cells. In healthy synovial joint tissues, steady-state levels of aTGF-β are low—greater than 98% of TGF-β resides in its latent complex (Albro et al., 2013), suggesting that activation, matrix binding, and internalization may act in coordination to tightly regulate TGF-β's spatiotemporal activity.

Here, we implement a series of computational and experimental models to better understand the role of endogenous LTGF-β in regulating the functional activities of synovial joint tissues. A computational reaction-diffusion framework is developed describing the spatiotemporal distribution of physiologically activated TGF-β in joint tissue regions while accounting for the salient chemical reactions that act on TGF-β in the extracellular realm— LTGF-β activation, aTGF-β-to-ECM reversible binding , and aTGF-β cell-mediated internalization. Subsequently, *ex vivo* experimental models are utilized to characterize the influence of distinct pools of endogenous LTGF-β on the functionality of synovial joint tissues, including the role of activated LTGF-β in SF on regulating biolubricant secretion and the role of activated LTGF-β in cartilage on regulating cartilage tissue properties.

2. Materials and Methods

2.1. Materials

Live cartilage explants were sterilely harvested from the femoral condyles of immature (2-month-old) or mature (5-year-old) bovines. Live human femoral condyle explants were obtained from five autopsy donors (Do 1-5) via the NDRI within 48 hours postmortem: (age/sex: Do1: 51/♂, Do2: 58/♀, Do3: 58/♀, Do4: 60/♀ and Do5: 74/♂). For all cartilage explants, a portion of their deep zone was excised, yielding cylindrical disks of desired thickness with their articular surface intact. Different culture media were selected for each study based on their capacity to maintain each tissue type at homeostasis *in vitro* (Supplementary Material). Live immature bovine synovium specimens were sterilely harvested from the knee joint for synoviocyte monolayer studies.

2.2. Reaction-Diffusion Modeling

A reaction-diffusion framework and FEA solver (Fig.1B) was implemented to model the spatiotemporal distribution of aTGF-β in synovial joint tissues in response to: 1) mechanochemical activation of each latent pool (soluble LTGF-β in SF, ECM-bound LTGF-β in cartilage), and 2) chemical reactions acting on the activated TGF-β molecule. Chemical reactions of load-induced activation of LTGF-β, reversible binding of aTGF-β to the ECM, and cell-mediated internalization of aTGF-β were described using first order elementary reaction kinetics and prescribed for each synovial joint tissue region—i.e., SF, synovium, cartilage SZ (topmost 0.23mm), cartilage MZ (0.23-0.70mm), and cartilage DZ (0.70-2.00mm) (Raub et al., 2012) (Fig.1C-D). Binding and internalization chemical reaction rate constants were prescribed based on a combination of previously established and newly performed experimental measures (Supplementary Materials). LTGF-β activation rates were applied to mimic daily regimens of physiologic dynamic loading-induced mechanobiological activation of each LTGF-β pool, consisting of one hour of joint loading (activation phase), followed by a subsequent 23 hours without loading (resting phase) (Fig.1E)—this regimen mimics an extreme physiologic case where activation occurs exclusively during a 1-hour exercise regimen. Activation chemical reaction rate constants were prescribed based on our prior *in vitro* measures (Supplementary Materials). Model predictions were used to parametrically describe: 1) spatiotemporal distribution of different states of activated TGF-β—free, ECM-bound, and cell-internalized aTGF-β—in each synovial joint tissue region, and 2) the contribution of different LTGF-β pools (SF LTGF-β, cartilage LTGF-β) to tissue region-specific aTGF-β supply.

2.3. Reaction-Diffusion Model Validation

The accuracy of our reaction-diffusion framework was examined by comparing the experimental uptake of exogenous aTGF-β in cartilage explants to model predictions. Immature bovine explants (∅6mm×3mm) were exposed continuously to medium-supplemented exogeneous aTGF-β3 for one or four weeks at 0.1ng/mL or 1ng/mL, representing a dose range associated with SF shear-induced activation (Albro, 2012). The impact of extracellular chemical reactions on TGF-β uptake was differentially assessed by measuring TGF-β uptake in either live explants, where both reversible binding and chondrocyte-internalization reactions occur, or devitalized explants (achieved via a freeze-thaw cycle), where only reversible binding occurs. At culture completion, the depth-dependent distribution of exogenous aTGF-β3 in explants was analyzed via ELISA and compared to model predictions (Supplementary Materials).

2.4. SF LTGF-β Pool Activation on PRG4 Secretion

PRG4 secretion from immature bovine cartilage explants, human cartilage explants, and bovine synoviocyte monolayers was monitored in response to exogenous aTGF-β (human recombinant active TGF-β, R&D Systems) solutions, which serve as surrogates for TGF-β activated *in situ* via physiologic shearing. Explants were exposed to solutions of prescribed doses and exposure durations to mimic physiologic exposure regimens (0.1-1.0ng/mL per hour under 1-4 hours daily activity) and beyond. For immature bovine explants (∅5mm×2mm), PRG4 secretion was measured daily following 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300ng/mL aTGF-β1 (n=3 explants per dose) administered for 1 hour daily, 4 hours daily, or continuously over 5 days. For synoviocytes, PRG4 secretion was measured daily following 0, 0.3, 1, 3, 10 ng/mL aTGF-β1 (n=3 explants per dose) administered for the aforementioned durations. For human explants (∅2mm×2mm; donors 1-4 [n=12 per donor]), PRG4 secretion rates were measured every 2 days over 12 days during continuous administration of aTGF-β3 or aTGF-β1 at 0, 0.1, 1, or 10ng/mL doses (n=3 explants per group per donor). Varied TGF-β exposure durations were not performed on human explants. PRG4 secretion measures are reported as the PRG4 secretion enhancement, relative to TGF-β-free secretion (Supplementary Materials).

2.5. ECM-bound LTGF-β Pool Activation on Cartilage Properties

The influence of activated TGF-β from the cartilage LTGF-β pool on tissue composition and property regulation was assessed in an *ex vivo* model. This initial characterization was performed in the absence of mechanical loading, whereby TGF-β activation may be induced via biochemical means, albeit at a lower rate. Properties of cartilage explants were

monitored in culture while inhibiting the activity of endogenous activated TGF-β in the cartilage ECM. Activity inhibition was achieved via media supplementation of the TGF-β type-I receptor kinase inhibitor, LY364947, which exhibits high specificity for TGF-β-mediated chondrocyte-ECM suppression (Supplementary Materials). Immature bovine, mature bovine, and human explants were maintained in the absence (Ctrl) or presence (Inh) of LY364947 at 5μg/mL (n=8 explants per group per donor) over 1 month of culture. Subsequently, explants were analyzed for their full-thickness and depth-dependent Young's modulus (Supplementary Materials), GAG content (dimethylmethalyne blue assay), and viability (Live/Dead^{TM} imaging).

3. Results

3.1. Reaction-Diffusion Model Validation

Exogenous aTGF-β exhibited pronounced gradients through the cartilage depth (Fig.2A). After 1 week, for devitalized explants, aTGF-β was concentrated at the cartilage surface, marked by a 125-fold concentration increase above the bath level at the surface region and limited penetration into deeper regions due to the presence of high levels of non-specific aTGF-β binding sites which act to delay diffusive uptake (Fig.2B). For live explants, accumulation at the tissue surface was less pronounced, marked by a 56-fold enhancement above bath levels (Fig.2B), and penetration was further limited; statistically significant aTGF-β levels were only present in the topmost 375μm. After 4 weeks, uptake gradients for live explants were largely maintained due to the balance between cell-mediated internalization and diffusive influx of aTGF-β (Fig.2C). Similar behavior was observed for uptake with the 0.1ng/mL aTGF-β bath concentration (Fig.S3). For all experimental groups, model simulations exhibited strong agreement, (R^2=0.58-0.99).

3.2. Spatiotemporal Activity of Physiologically Activated TGF-β in the Synovial Joint

The spatiotemporal distribution of activated TGF-β from each LTGF-β pool is described separately (Fig.3&4). For the cartilage LTGF-β source, one hour of physiologic compression initiates a supply of 170pg/mL of aTGF-β throughout all cartilage zones (Fig.3A). Upon activation, TGF-β is rapidly re-sequestered in the ECM via binding interactions. After activation cessation, over the subsequent 23-hour resting phase, bound aTGF-β undergoes release from the ECM, freeing it for internalization by surrounding chondrocytes in each cartilage zone. As aTGF-β transitions from bound to internalized, free aTGF-β levels do not exceed 4pg/mL. For the SF LTGF-β source, one hour of physiologic shearing initiates a supply

of 0.08ng/mL of aTGF-β within SF (Fig.4). Upon activation, this TGF-β is similarly re-sequestered at ECM binding sites within the SF-lining tissue regions (SZ cartilage and synovium). During the activation phase, bound aTGF-β progressively accumulates in these regions, reaching levels of 140pg/mL and 32pg/mL at the surface of each region, respectively. However, aTGF-β is unable to achieve significant penetration into the deeper MZ and DZ regions of articular cartilage due to hindered uptake from binding and internalization reactions. After activation cessation, during the resting phase, bound aTGF-β undergoes release from the ECM, freeing it for internalization by SZ chondrocytes and synoviocytes within the respective tissue regions (Fig.4B-D). The relative contribution of each pool of LTGF-β to the supply of free, bound, and internalized TGF-β to synovial joint cells differs significantly by tissue region, a disparity that depends on the region's proximity to the SF LTGF-β source (Fig.5). On average, synoviocytes and chondrocytes from the SZ, MZ, and DZ respectively obtain 29%, 57%, 89%, and 99%, of their daily internalized dose of TGF-β from the cartilage LTGF-β pool and 71%, 43%, 11%, and 1% from the SF LTGF-β pool.

3.3. SF LTGF-β Pool Activation on PRG4 Secretion

Physiologic aTGF-β exposure doses (0.1-1ng/mL) and durations (1 or 4 hours daily) induced strong dose-dependent enhancements of PRG4 secretion in bovine cartilage, ranging from 4-fold enhancements at 0.1ng/mL to 7-fold enhancements at 1ng/mL (Fig.6A). Within this dosage range, the continuous application of aTGF-β only led to modest further increases in the enhancement ratio, increasing by and additional 14% at 0.1ng/mL and 22% at 1ng/mL. Over a broader range of supraphysiologic aTGF-β doses, the bovine cartilage PRG4 secretion rate exhibited a biphasic dose-dependent response, reaching 15-fold enhancements at 10ng/mL but decaying to only 3-fold enhancements at 300ng/mL—this biphasic response is consistent with prior dose-dependent characterization of TGF-β on cell biosynthesis (Niikura, 2007). Bovine synoviocytes exhibited a similar increase in PRG4 secretion rate for physiologic exposure durations (1h or 4h) reaching a 7-fold enhancement at 0.3ng/mL aTGF-β exposure—no significant difference was observed between exposure doses ($p>0.05$) or durations ($p>0.75$) (Fig.6B). In response to continuous aTGF-β exposure in the physiologic dose range (0.1-1ng/mL), explants from each human donor exhibited similar enhancements, marked by 3-fold to 6-fold enhancements for aTGF-β3 and 5-fold to 10-fold enhancements for aTGF-β1 (Fig.6C&D).

3.4. ECM-bound LTGF-β Pool Activation on Cartilage Properties

Inhibition of ECM-bound LTGF-β activity in cartilage explants led to decreases in mechanical properties and loss of GAG content. For immature and mature bovine cartilage explants, one-month TGF-β inhibition induced 65% and 60% decreases in E_Y, respectively (Fig.7A;$p<0.05$), and 30% and 35% decreases in GAG, respectively (Fig.7B;$p<0.05$), relative to control levels. Spatial modulus maps depict an inhibition-induced stiffness loss at all depth regions of the tissue (Fig.7C&D). For human cartilage explants, one-month TGF-β inhibition induced significant decreases in E_Y and GAG for 4 out of 5 donors ($p<0.05$), marked by an average decrease of 35% for E_Y (Fig.7E) and 20% for GAG (Fig.7F), relative to control levels. No viability loss was observed for explants (Fig.7G).

4. Discussion

This study advances a novel perspective on the regulation of TGF-β in the synovial joint and its role in promoting the functional activities of synovial joint tissues. The combined implementation of reaction-diffusion modeling and experimental *ex vivo* tissue characterizations allows for a comprehensive exploration of the spatiotemporal distribution of mechanochemically activated LTGF-β and its impact on regulating synovial joint biolubricant secretion and cartilage mechanical properties in response to physiologic mechanical loading.

Our reaction-diffusion framework provides an opportunity to explore the spatiotemporal distribution of physiologically-activated TGF-β in the synovial joint in response to a complex array of chemical reactions. Models illustrate the role of chemical reactions in creating a unique temporal interplay between different states of aTGF-β in tissue: free, ECM-bound, and cell-internalized (Fig.8A). Here, we observe that upon activation from the latent complex, aTGF-β is rapidly re-sequestered in the ECM due to interactions with reversible binding sites. Upon activation cessation, aTGF-β undergoes a slow, sustained release from binding sites, freeing it to be internalized by nearby cells (chondrocytes or synoviocytes). A consequence of this dynamic is that cells are exposed to free aTGF-β at a dose that is at only a small fraction of the activated concentration—at any given time, less than 2% of aTGF-β resides in its free state in the tissue. Further, this dynamic allows cells to be exposed to activated free TGF-β for a duration far exceeding that of the initial activation pulse—approximately 24 hours of continued exposure results from an initial 1-hour load-induced activation phase. The physical basis for this behavior arises from the differential magnitudes of chemical reaction rates in the tissue. ECM binding sites for aTGF-β in cartilage are present at concentrations that far exceed aTGF-β levels ($>4\times10^5$ ng/mL (Albro, 2013)), allowing for aTGF-β to be rapidly re-sequestered at binding sites upon activation. However, despite these high concentrations, ECM binding sites exhibit a relatively low affinity for aTGF-β (~25nM (Albro, 2013)), allowing for a

sustained slow release from the ECM upon activation cessation. Further, the high rate of cell-mediated aTGF-β internalization ensures that aTGF-β is internalized upon release from binding sites, thus rapidly clearing it from the extracellular space. The physiologic implications of this interplay are intriguing, suggesting that relatively short periods of mechanical loading may provide synovial joint cells with a sustained, long-term exposure to moderated TGF-β activity levels. In this context, the ECM appears to function as a biochemical 'storage capacitor,' allowing for binding site 'charging' during periods of joint activity, followed by 'dissipation' during periods of resting. Ultimately, this mechanism may provide an important additional layer of TGF-β regulation in addition to that achieved by latency activation dynamics.

A second important insight advanced by computational models is the pronounced impact of chemical reactions on modulating the distribution of aTGF-β in synovial joint tissues. Due to the high density of ECM-aTGF-β binding sites in cartilage and the high cell-mediated aTGF-β internalization rate, the diffusion of free aTGF-β through the cartilage matrix is highly limited. In effect, this behavior creates a spatially compartmentalized regulation of the different LTGF-β pools in the synovial joint, whereby LTGF-β activated in SF acts predominantly on SF-peripheral tissues–-i.e., cartilage SZ and synovium—and LTGF-β activated within cartilage acts locally on chondrocytes in proximity to the activation site. These results support a dual physiologic role synovial joint LTGF-β, whereby SF LTGF-β regulates the secretion of biolubricants by SZ chondrocytes/synoviocytes and cartilage LTGF-β regulates the secretion of structural ECM constituents (GAG/collagen) by chondrocytes through the depth of cartilage (Fig.8B).

The performance of a direct *in vivo* validation of the accuracy of model predictions constitutes a significant challenge. However, our pilot demonstration that reaction-diffusion models can accurately predict the uptake of aTGF-β in cartilage explants (Fig.2) lends strong support to the quantitative accuracy of ECM binding and cell internalization chemical reactions in the framework. LTGF-β activation rate parameters are prescribed based on the best currently available estimates. SF LTGF-β activation rates of are adopted from *ex vivo* measures of activation in response to estimated physiologic shear rates (Albro, 2012). Cartilage LTGF-β activation rates of are adopted from bioassay measures in explants exposed to dynamic compressive strain (Kim et al., 2020). However, it is worth noting as a limitation, these estimates do not account for the complex mechanical deformation profiles (i.e., fluid shear rates and matrix strains) that exist in the synovial joint. The future development of more sophisticated strain-dependent activation rate measures may allow for improved quantitative accuracy of model predictions. As a further limitation, prescribed chemical reaction rate constants were adopted from characterizations on different TGF-β isoforms and models do not account for variability in parameter

estimates. In future work, it will be interesting to develop TGF-β isoform-specific models and explore the influence of specimen variability on model predictions.

The accompanying experimental *ex vivo* live tissue models advance our understanding of the impact of the distinct pools of endogenous TGF-β on the functional activity of synovial joint tissues. While the influence of TGF-β on chondrocyte biosynthesis has been explored extensively, these models provide novel characterizations of tissue response within the context of physiologic TGF-β activity regimens. In our initial *ex vivo* model, we examined the influence of SF LTGF-β on regulating SZ cartilage and synovium secretion of PRG4. Prior work has shown that continuous supraphysiologic TGF-β dose exposure can substantially enhance PRG4 secretion (Lee, 2008; Schmidt, 2008; Blewis et al., 2010). Here, we show that physiologic exposure regimens associated with shear-activated TGF-β (0.1 to 1ng/mL at 1 to 4 hours daily) are sufficient to significantly enhance PRG4 secretion in bovine SZ cartilage and synovium. For physiologic doses, PRG4 enhancements exhibited only modest further increases in response to TGF-β administered continuously, thus suggesting that short-term, physiologic daily regimens of LTGF-β shear-activation may be sufficient to maximize boundary lubricant replenishment into SF.

In our second *ex vivo* model, the influence of the cartilage LTGF-β on regulating tissue properties was examined. While the influence of TGF-β activity on cartilage health has been examined previously via *in vivo* genetic knockout studies (Serra, 1997; Chen, 2012), this model directly examines the impact of endogenous TGF-β activity in cartilage on maintaining tissue composition and mechanical properties. We observe that one month of endogenous TGF-β activity inhibition leads to a significant loss of GAG and substantial reduction of E_Y of bovine cartilage, supporting the critical role of TGF-β signaling in cartilage in regulating the functional mechanical performance of the tissue. A notable limitation of this work is that tissue property changes are monitored in the absence of dynamic loading, under which LTGF-β activation is expected to increase markedly (Madej, 2014; Madej, 2016). The observed property reductions in the absence of loading indicate that: 1) chondrocytes are capable of activating LTGF-β in the ECM to a degree via load-independent mechanisms—potentially from integrin-mediated cell traction (Wipff et al., 2007) or cell-secreted enzymes (Jenkins, 2008), and 2) these basal activity levels play a role in maintaining tissue properties. In future work, we will examine the impact of TGF-β inhibition on cartilage properties in response to loading, which is anticipated to yield greater property reductions, as well as explore the mechanistic details of aggrecan loss in response to TGF-β inhibition.

Human cartilage explant experiments further emphasize the important functional role of TGF-β regulation in maintaining synovial joint biosynthetic activities. Interestingly, prior studies on animal tissues have shown a decreased sensitivity of chondrocytes to TGF-β with of aging, attributed to receptor expression changes (Blaney Davidson et al., 2005; van Caam et al., 2016). Here, we show that cartilage from advanced age human donors (age range 51 to 74 years) responds to endogenous TGF-β activity in a manner that is strikingly similar to that observed for immature bovine cartilage. Similar PRG4 secretion enhancements in response to aTGF-β are observed in all four examined human donors. Similar reductions of modulus and GAG resulting from LTGF-β activity inhibition of in cartilage are observed in four of five human donors. These results suggest that TGF-β's significant role in regulating synovial joint functional activities may be important even at advanced ages.

The implemented reaction-diffusion framework and *ex vivo* tissue models may improve our understanding of TGF-β regulatory changes during aging and pathology. Aberrant TGF-β activity can contribute to pathologic features of osteoarthritis (e.g., osteophytes, synovial fibrosis (Blaney Davidson et al., 2007)) and steady-state levels of aTGF-β can reach as high as 3.8ng/mL in SF of osteoarthritis patients (Fava, 1989)—two orders of magnitude above levels during physiologic activation in healthy SF. It seems reasonable to hypothesize that aberrant aTGF-β levels may result, in part, from alterations in TGF-β chemical reaction rates following pathologic osteoarthritic tissue changes; altered LTGF-β activation may result from elevated enzyme activity (Zielinski et al., 2000) or joint biomechanics (Thomas et al., 2017), altered aTGF-β ECM binding may result from matrix degradation (Temple-Wong et al., 2009), and altered aTGF-β cell-internalization rates may result from receptor expression changes (van Caam, 2016). Further, LTGF-β pools may be differentially impacted by these changes; SF LTGF-β activation may be more susceptible to OA-associated SF enzymes, while cartilage LTGF-β activation rates may be more sensitive to chondrocyte integrin expression changes. As such, reaction-diffusion frameworks and *ex vivo* culture models provide a unique opportunity to study changes in spatiotemporal activity patterns of TGF-β during osteoarthritis and its effect on modulating synovial joint tissue behavior.

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6. Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

Figures

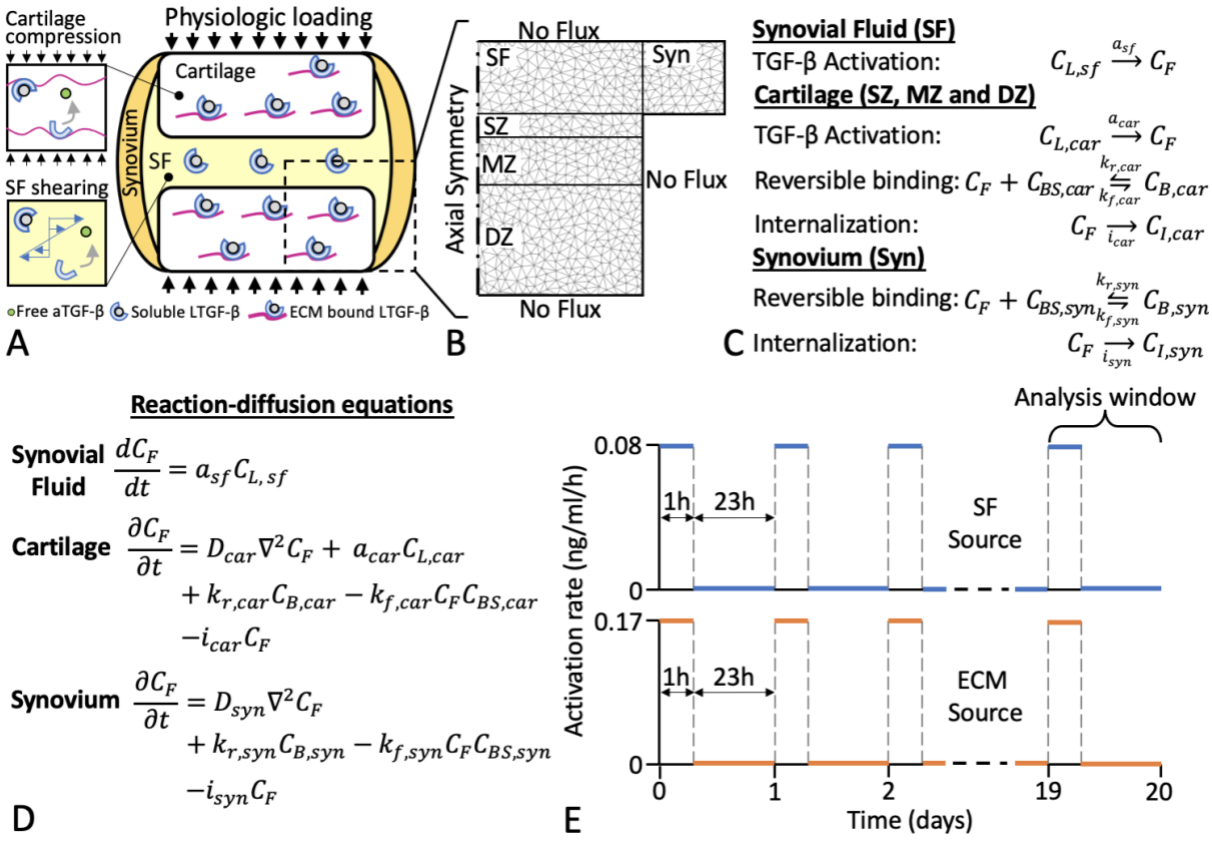

FIG. 1. Computational reaction-diffusion model of the spatiotemporal distribution of activated TGF-β in the synovial joint. (A) Schematic of mechanobiological activation of soluble LTGF-β in SF and ECM-bound LTGF-β in cartilage in response to physiologic mechanical joint activity. (B) Computational model mesh structure and boundary conditions in synovial joint tissue domains—synovial fluid (*sf*), articular cartilage (*car*) zones (SZ, MZ and DZ), and synovium (*syn*). (C) Chemical reactions modulate levels of LTGF-β (C_L), free aTGF-β (C_F), bound aTGF-β (C_B), and cell internalized aTGF-β (C_I) within each tissue domain. Governing parameters include: N_T (concentration of total aTGF-β–ECM binding sites), k_f and k_r (respective forward and reverse aTGF-β–ECM binding rate constants), i_{car} and i_{syn} (respective cartilage and synovium aTGF-β internalization rate constants), a_{sf} and a_{car} (respective activation rate constants of soluble LTGF-β in SF and ECM-bound LTGF-β in cartilage), and D (diffusivity). (D) Reaction-diffusion equations that describe (C_F), in each tissue domain. (E) Prescribed 1-hour daily load-induced activation rate profiles for each LTGF-β source (SF LTGF-β and cartilage LTGF-β) implemented in computational simulations and depiction of 24-hour analysis window.

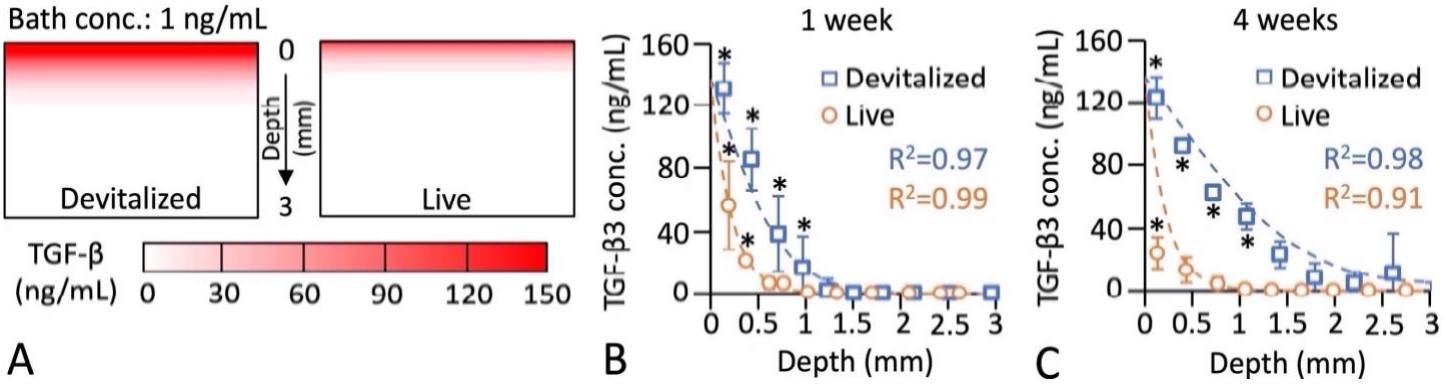

FIG. 2. Validation of reaction-diffusion modeling framework. (A) Model-predicted spatial distribution of exogenous aTGF-β (free + bound) through depth of live and devitalized articular cartilage explants after 1 week exposure to 1ng/mL TGF-β3 bath. Depth-dependent TGF-β uptake profiles from experimental measures (symbols) and computational model predictions (dashed lines) after (B) 1 week and (C) 4 week exposure durations. *$p<0.05$ represents statistical increase above corresponding 3mm cartilage depth value.

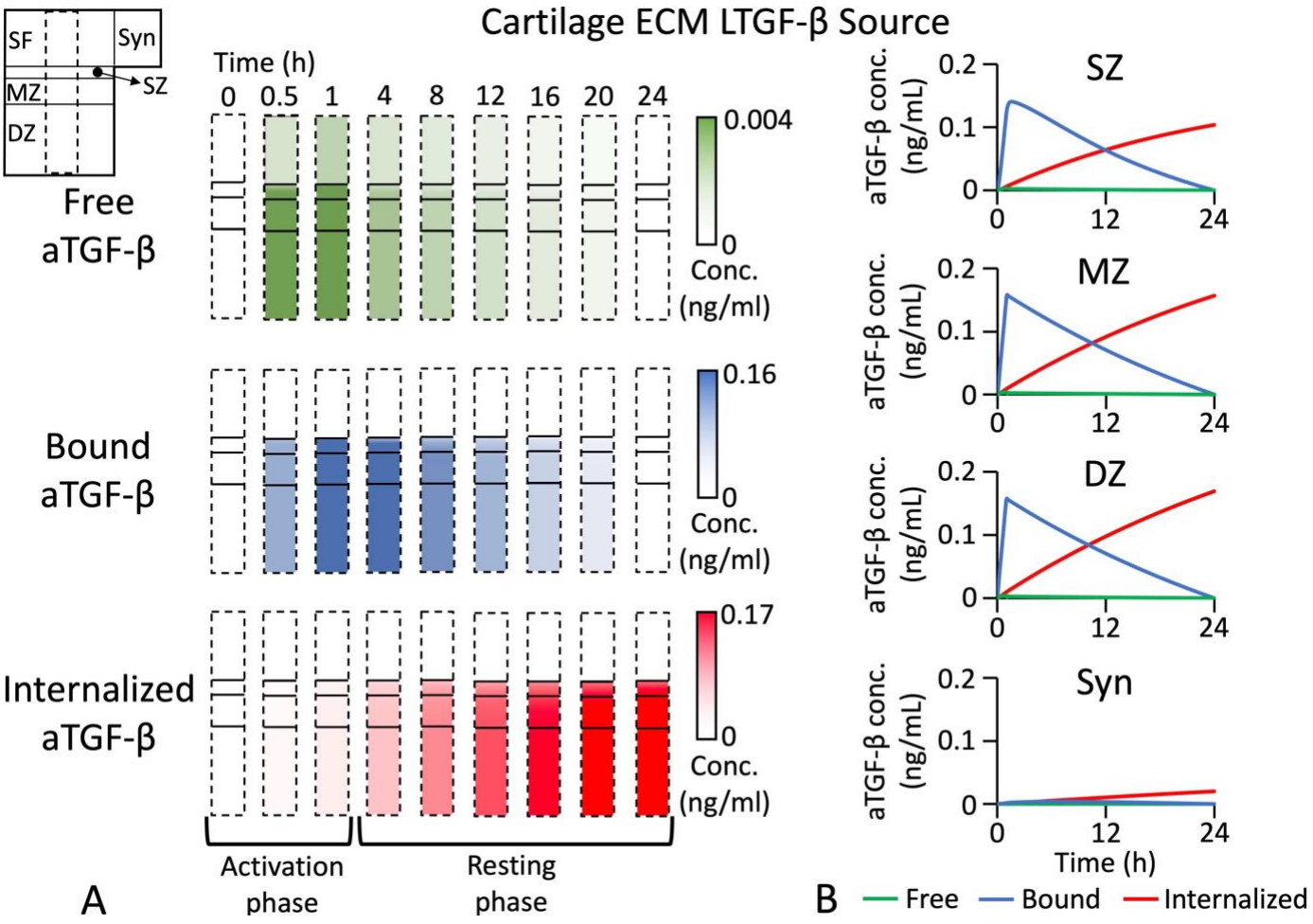

FIG. 3. Model-predicted spatiotemporal distribution of mechanochemical activated TGF-β in synovial joint tissues originating from cartilage ECM LTGF-β source. (A) Spatial distribution of activated free, ECM-bound, and cell-internalized TGF-β at varying time points, incorporating initial 1 hour activation phase, followed by a subsequent 23 hour resting phase. Spatial distribution in Syn is not depicted. Note different scale bars for each aTGF-β profile. (B) Transient free, ECM-bound, and cell-internalized aTGF-β concentrations averaged over entire region of SZ, MZ, DZ, and Syn.

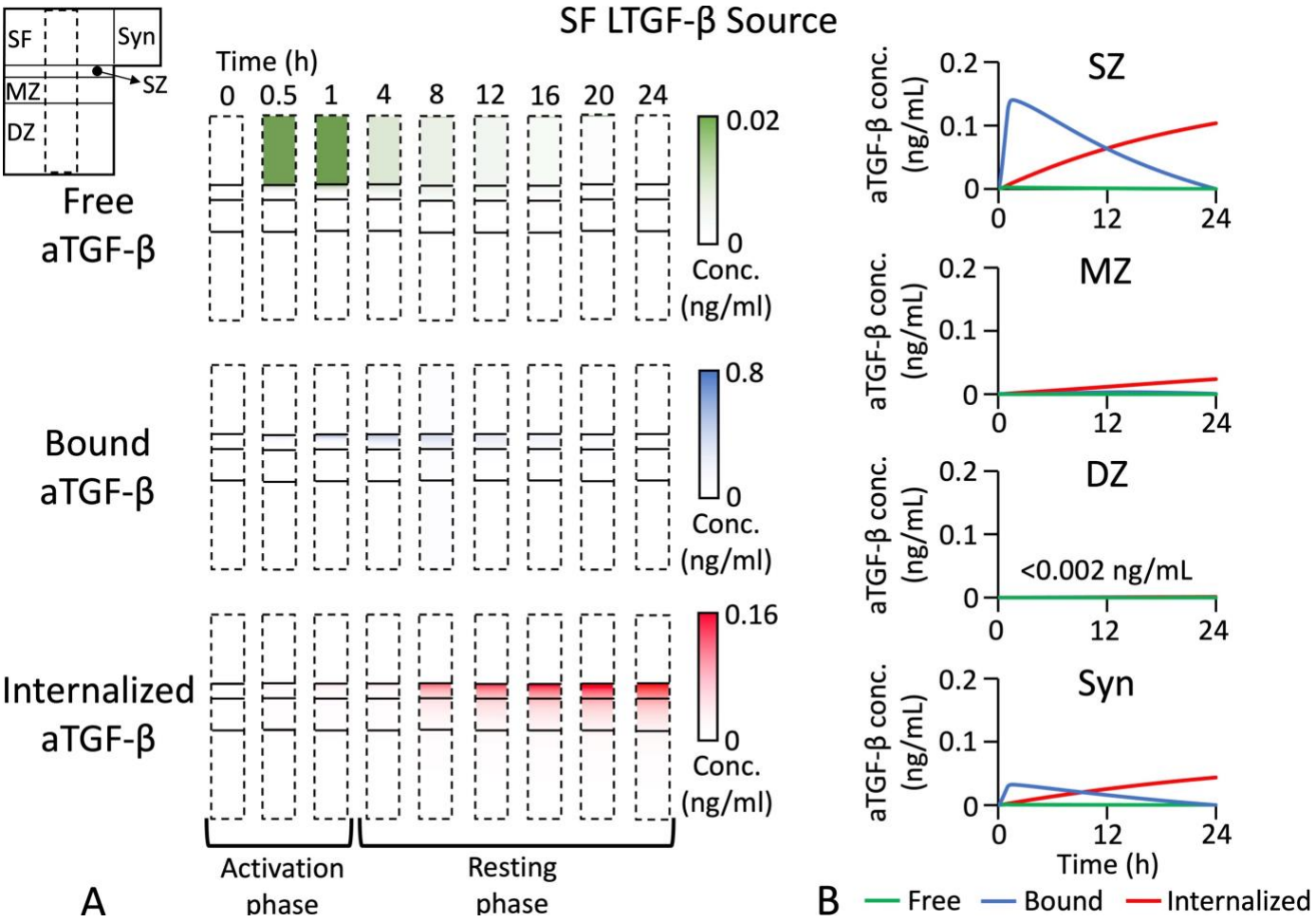

FIG. 4. Model-predicted spatiotemporal distribution of mechanochemical activated TGF-β in synovial joint tissues originating from SF LTGF-β source. (A) Spatial distribution of activated free, ECM-bound, and cell-internalized TGF-β at varying time points, incorporating initial 1 hour activation phase, followed by subsequent 23 hours resting phase. Spatial distribution in Syn is not depicted. Note different scale bars for each aTGF-β profile. (B) Transient free, ECM-bound and cell-internalized aTGF-β concentrations averaged over entire region of SZ, MZ, DZ and Syn.

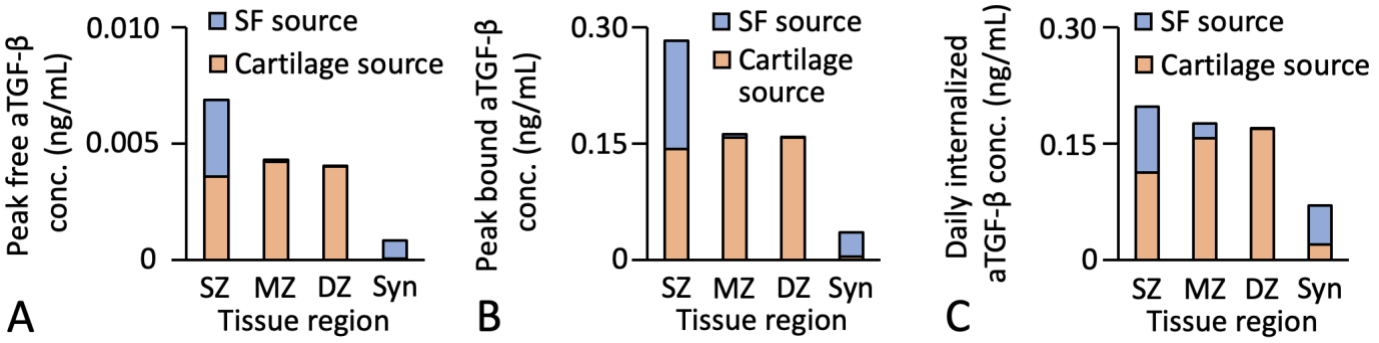

FIG. 5. Daily contribution of each LTGF-β source to different states of aTGF-β in synovial joint tissues. Transient (A) peak free, (B) peak ECM-bound and (C) daily cell-internalized aTGF-β concentrations in cartilage SZ, MZ, DZ, and Syn as a result of 1 hour daily activation of cartilage LTGF-β and SF LTGF-β pools.

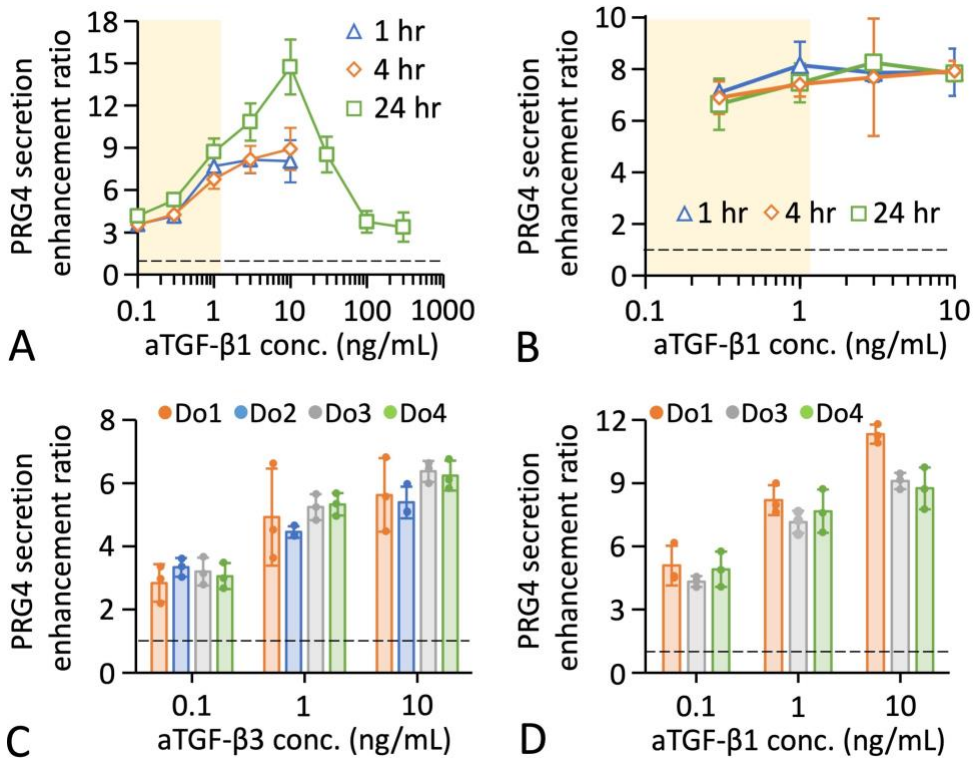

FIG. 6. Functional role of soluble LTGF-β in SF on regulation of synovial joint biolubricant secretion. Measured PRG4 secretion enhancement from (A) SZ bovine cartilage explants and (B) bovine synovium monolayers for varying aTGF-β exposure regimens, which encompass physiologic exposure doses (0.1 to 1ng/mL range; depicted by shaded background) and exposure durations (1-4 hour daily exposure). PRG4 secretion enhancements of SZ cartilage explants from four human donors in response to varying (C) aTGF-β3 or (D) aTGF-β1 exposure doses.

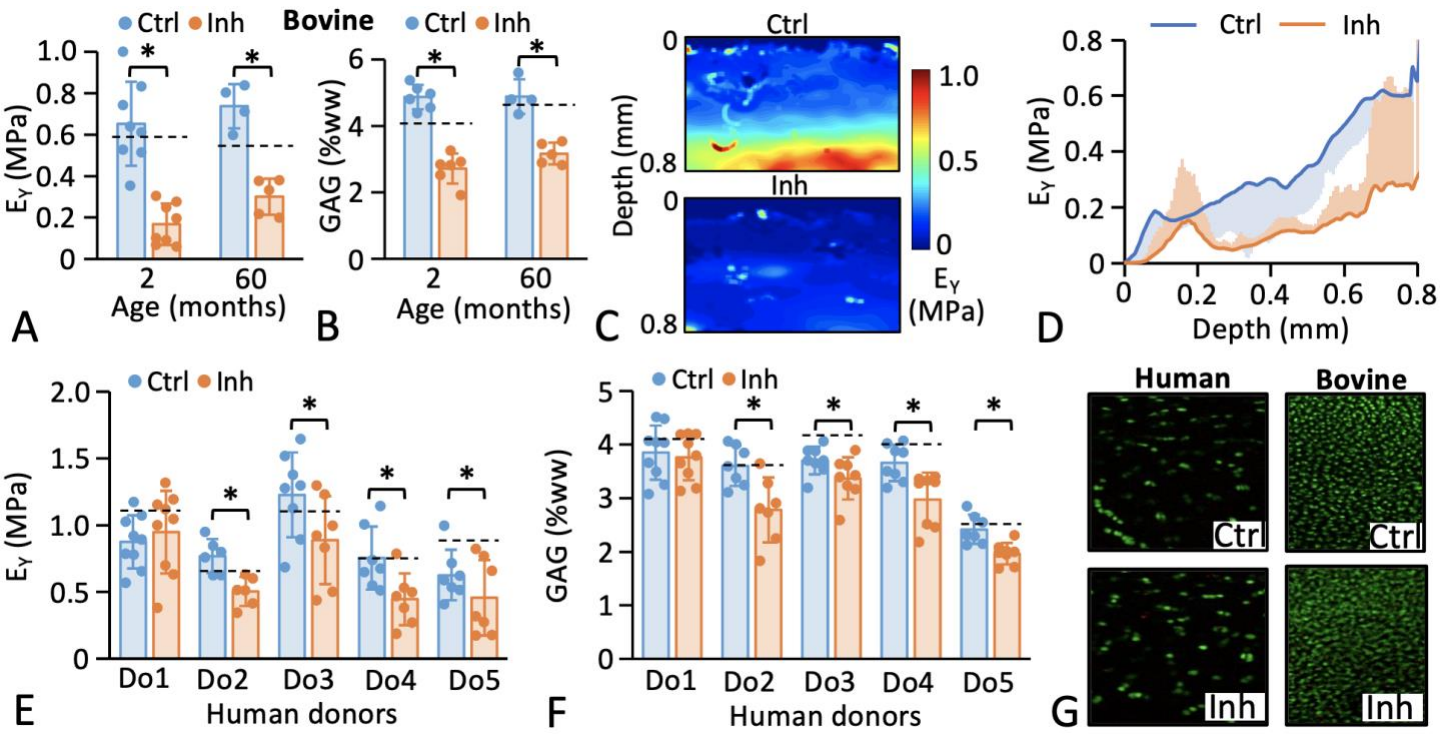

FIG. 7. Functional role of cartilage ECM LTGF-β on regulation of articular cartilage properties. (A) Young's modulus (E_Y) and (B) GAG content of bovine cartilage explants +/- TGF-β inhibition. (C) Representative E_Y distribution and (D) depth-dependent E_Y (mean+/-std dev) in bovine cartilage explants +/- TGF-β inhibition. (E) E_Y and (F) GAG content of cartilage explants for human donors +/- TGF-β inhibition. (G) Viability of human and bovine cartilage explants +/- TGF-β inhibition. Dashed lines represent day-0 measurement values. *$p<0.05$: denotes significant decrease below respective Ctrl group.

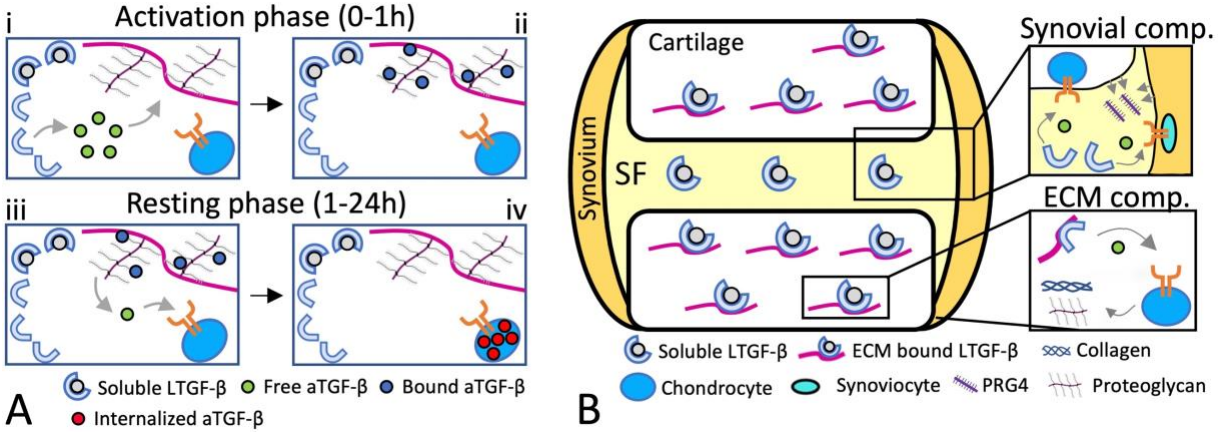

FIG. 8. Spatiotemporal regulation of mechanochemically activated TGF-β in the synovial joint. (A) During the load-induced activation phase, (i) LTGF-β is activated via mechanochemical stimuli and (ii) free aTGF-β is rapidly re-sequestered in the ECM due to binding site interactions. During the subsequent load-free resting phase, (iii) aTGF-β undergoes a slow, sustained release from binding sites, making it available for (iv) internalization by nearby chondrocytes or synoviocytes. (B) Due to chemical reactions, activated TGF-β acts locally on nearby tissue regions, creating a compartmentalized functional role in the synovial joint whereby: 1) activated LTGF-β in SF acts predominantly on SZ chondrocytes/synoviocytes to regulate secretion of biolubricants, and 2) activated LTGF-β in cartilage acts predominantly on nearby chondrocytes to regulate secretion of structural ECM constituents.

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