Supplementary Data

TGF-β Degradation Assessment

The potential for exogenous active TGF-β to undergo degradation upon transporting into tissue constructs through the action of chondrocyte-secreted proteases was analyzed by measuring the stability of TGF-β1 in the presence of construct-conditioned media. In order to mimic construct culture conditions, tissue constructs (∅ 4 × 2.3 mm at 60 × 10^6 cells per mL, n=5) were individually maintained in 0.5 mL media supplemented with 10 ng/mL of active TGF-β3 for 48 hours. At the end of this period, constructs were removed and discarded, and each conditioned media sample was supplemented with 10 ng/mL of TGF-β1. TGF-β1 levels in these conditioned medium samples were measured initially and after an additional 48 hours of incubation.

No statistical decrease was observed in TGF-β1 levels over the incubation period (Fig S1; p=0.63), indicating that exogenous active TGF-β is not degraded by chondrocyte-secreted proteases under tissue culture conditions.

Biosynthetic Effects of Different TGF-β Isoforms on Tissue Constructs

A preliminary study was performed to assess potential differential effects of exogenous active TGF-β1 and TGF-β3 on tissue construct biosynthesis. Here, tissue constructs (∅4 × 2.3 mm; 30 × 10^6 cells per mL) were cultured for 45 days in chondrogenic media supplemented with 10 ng/mL of TGF-β1 or TGF-β3, continuously or transiently (first 2 weeks only), or maintained TGF-β free (n=5 per group). Upon completion of culture, all constructs were analyzed for their GAG and collagen contents.

As demonstrated previously [1], TGF-β supplementation led to a significant increase in collagen content in tissue constructs whether applied continuously or transiently (Fig S2; p=0.002). A significant rise in GAG content was observed for transient TGF-β application (p<0.001). However, for both biochemical constituents and supplementation protocols (transient or continuous), the use of active TGF-β1 or TGF-β3 was not statistically different (p=0.62 for collagen; p=0.51 for GAG).

Computational Mechanical Modeling

The depth dependent and whole construct mechanical properties (equilibrium compressive Young's modulus) were assessed computationally for each of the samples in the active TGF-ß, latent TGF-ß, and control groups (n=8 for each group). Models were run in FEBio (http://www.febio.org, version 2.4.0), based to our recently submitted article: Nims, RJ et al., Continuum theory of fibrous tissue damage mechanics using bond kinetics: application to cartilage tissue engineering, which provides a theoretical and computational framework to model the mechanical growth (tissue swelling and compressive modulus development) of engineered cartilage based on the biochemical content (GAG and collagen content).

Models geometry consisted, initially, of ∅ 6 × 3.2 mm construct with 24 radial elements and four thickness elements. Radial symmetry was enforced to reduce the computational power required for simulations (3-degres slice). The engineered tissue was modeled as a mixture of a non-ideal Donnan equilibrium swelling model (describing the behavior of negatively charged proteoglycans, activity factor = 0.5) and a isotropic fiber distribution (describing the behavior of the collagens). The fiber distributions were applied multigenerationally to replicate successive generations of a deposited collagen network. Additionally, these fiber generations could become damaged due to excessive strain (due to the high deposition of GAGs), which our prior work has show is necessary to account for the growth patterns and mechanical behaviors of engineered cartilage constructs.

For each sample, the Donnan swelling term and fiber modulus within each slice was determined from the sample- and slice-specific biochemical content. The Donnan model parameter of fixed-charge density

was taken directly from the GAG content (assuming 513 g/mol for chondroitin sulfate). Solid volume fraction (for the FCD estimation) was approximated using a collagen and proteoglycan true density of 1.4 g/mL and an agarose content of 2% [1]. To calculate the GAG normalized to the initial construct wet weight, a measure of the GAG content in the reference configuration, the assumed tissue swelling of each slice was interpolated from the relation between swelling and FCD in the reference configuration from our submitted study. Relating material fiber modulus to collagen content was assumed as directly proportional to the collagen content and fiber modulus of our previous validation study (~1.5% collagen and 0.028 MPa fiber stiffness, respectively). As presented in that work, the collagen damage was based on Simo damage criterion according to a cumulative log-normal distribution (threshold = 0.08 $(mJ/mm^3)^{0.5}$ and shape parameter $\sigma_c = 1.5$) [2].

After simulating the "growth" period over 56 days by increasing the fixed-charge density (as governed by the experimental GAG content) and adding successive collagen generation deposition (governed by the experimental collagen content), the samples were "cored" to analyze the one-dimensional mechanical properties by reducing the modulus and swelling of the annuli to leave only the inner ∅ 3mm section for simulating the mechanical testing. Samples were first tared (5% displacement) before applying a 10% compression step, consistent with our mechanical testing protocols. The whole-construct Young's modulus was calculated as the difference between pre- and post- compression normalized to the strain of the tissue. Spatial modulus for each construct slice was calculated by normalizing the axial stress to prescribed axial strain (Green-Lagrange strain, referential set to configuration after the "growth" prior to the tare told).

Supplementary Data Citations

[1] Khoshgoftar, M, Wilson, W, Ito, K, Van Donkelaar, CC. The Effects of Matrix Inhomogeneities on the Cellular Mechanical Environment in Tissue-Engineered Cartilage: An In Silico Investigation. Tissue Eng C: 2013.

[2] Simo J. On a fully three-dimensional finite-strain viscoelastic damage model: formulation and computational aspects. Comput Methods Appl Mech Eng. 1987.

Supplementary Figure Captions

Figure S1: Stability of exogenous active TGF-β1 during incubation in conditioned medium of tissue constructs.

Figure S2: (A) GAG and (B) collagen content of tissue constructs supplemented with 45 day cultured continuous or transient active TGF-β1 or TGF-β3.