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Predicting the effect of reduced load level and cell infiltration on spatio-temporal Achilles tendon healing

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Abstract

Mechanobiology plays an important role in tendon healing. However, the relationship between mechanical loading and spatial and temporal evolution of tendon properties during healing is not well understood. This study builds on a recently presented mechanoregulatory computational framework that couples mechanobiological tendon healing to tissue production and collagen orientation. In this study, we investigated how different magnitudes of mechanical stimulation (principal strain) affect the spatio-temporal evolution of tissue production and the temporal evolution of elastic and viscoelastic mechanical parameters. Specifically, we examined the effect of cell infiltration (mimicking migration and proliferation) in the callus on the resulting tissue production by modeling production to depend on local cell density. The model predictions were carefully compared with experimental data from Achilles tendons in rats, at 1, 2 and 4 weeks of healing. In the experiments, the rat tendons had been subjected to free cage activity or reduced load levels through intramuscular botox injections. The simulations that included cell infiltration and strain-regulated collagen production predicted spatio-temporal tissue distributions and mechanical properties similarly to that observed experimentally. In addition, lack of matrix-producing cells in the tendon core during early healing may result in reduced collagen content, regardless of the daily load level. This framework is the first to computationally investigate mechanobiological mechanisms underlying spatial and temporal variations during tendon healing for various magnitudes of loading. This framework will allow further characterization of biomechanical, biological, or mechanobiological processes underlying tendon healing.

Introduction

Mechanobiology plays a key role in the adaptation of tendon properties to external mechanical loading (Snedeker and Foolen, 2017). Many experimental studies have investigated the effect of (reduced) loading on the temporal evolution of overall tendon properties (Andersson et al., 2009, 2011; Eliasson et al. 2009; Eliasson et al., 2011; Eliasson et al., 2012, Hammerman et al., 2018; Freedman et al., 2016; Freedman et al., 2017a; Freedman et al., 2017b; Huegel et al., 2019; Hillin et al., 2019). Yet, very sparse data is available on the effect of load levels on both *spatial* and *temporal* evolution of tissue constituents (e.g. production of collagen I and III, different cell populations, elastin, proteoglycans) and the resulting mechanical properties of healing tendons. Recent studies have begun to characterize this, where e.g. Khayyeri et al. (2020) used small angle x-ray scattering to visualize the spatial variation in collagen properties during Achilles tendon healing in Sprague-Dawley rats. Understanding the spatial and temporal characteristics of healing soft tissue, can help reveal underlying biological mechanisms that are critical for tendon recovery and strength.

Simulations based on computational models can aid the understanding of mechanobiological processes during tissue healing by predicting spatio-temporal tissue composition and organization. Two studies have investigated the *temporal* effect of reduced loading on collagen orientation, anisotropy and synthesis/content during tendon healing (Chen et al., 2018; Richardson et al., 2018). However, these computational studies did not investigate mechanoregulated *spatial* variations of tendon composition and organization that occurs during tendon healing. We have previously developed a mechanoregulatory framework that addresses this gap (Notermans et al., 2021). The framework is able to describe the spatio-

temporal evolution of tendon properties, i.e. collagen content and alignment, in rats subjected to *full* physiological daily loading (free cage activity) during early tendon healing.

In addition, several experimental studies have investigated how reduced loading affects the temporal evolution of geometrical and mechanical properties throughout the first weeks of healing in fully transected nonrepaired Achilles tendons in rats (Eliasson et al., 2009; Andersson et al., 2012; Hammerman et al., 2018; Khayyeri et al., 2020). We recently characterized viscoelastic properties for fully loaded (free cage activity) and unloaded (through botulinum toxin A (botox) injection in calf muscle) tendons over time (Khayyeri et al., 2020). Differences in cross-sectional area, gap distance, stiffness, creep magnitude, creep ratio and peak force were found in the early time points, but the effect of unloading diminished by 4 weeks of healing.

During tendon healing, intrinsic repair is performed by tendon cells originating from the intact tendon, whereas extrinsic healing involves the recruitment of external cells (e.g. matrix-producing fibroblasts) to the defect site from neighboring tissues or blood supply (here called *the extrinsic compartment*) (Snedeker and Foolen, 2017; Nichols et al., 2019). Chen et al. (2018) modeled cell migration and proliferation from the tendon stumps towards the callus core during early healing. However, no modeling approach has incorporated extrinsic cell infiltration (migration and proliferation) during tendon healing, which is believed to be key as most cells enter the defect extrinsically since the tendon stumps have low cellularity (Snedeker and Foolen, 2017; Nichols et al., 2019).

In this study, we aimed to determine if our previously developed mechanoregulatory framework (Notermans et al., 2021) can be developed to investigate the load level-dependent evolution of heterogeneous tissue distribution and the mechanical properties *in silico*, including both elastic and viscoelastic properties of the tendon throughout healing. Simulations were compared with data from rats undergoing tendon healing (Khayyeri et al., 2020). We hypothesized that extrinsic cell infiltration may play a role in heterogeneous tissue production. Therefore, we investigated the effect of cell infiltration on collagen distribution in the callus by including cell density-dependent tissue production in our mechanobiological framework.

Methods

An existing mechanobiological framework (Notermans et al., 2021) was further developed and implemented to predict tendon healing based on different magnitudes of external mechanical stimulation (Fig. 1). We used a 3D finite element framework of a healing tendon (Abaqus v2017, Dassault Systèmes Simulia Corp., Johnston, RI, USA) with a subroutine describing the fibre-reinforced hyper-visco-poro-elastic material (Khayyeri et al., 2016; Notermans et al., 2019). An iterative framework was implemented (Matlab R2019b) to predict daily collagen production and collagen reorientation in the healing callus (see Notermans et al. (2021) for detailed descriptions of the framework). This framework was expanded by adding a cellular component to account for the effect of cell infiltration on tissue production.

The Finite Element Model

The healing tendon consisted of two stumps with longitudinally aligned collagen fibres, and a bulging healing callus with 13 discrete fibres in every material point. The initial densities of collagen and ground matrix in the callus were set to 1% (w.r.t. the tendon stumps) and fibres were organized randomly. Two tendon finite element (FE) meshes were created based on the callus geometries from healing tendons reported by Khayyeri et al. (2020) at 1 week post-transection from groups that had been subjected to free cage activity (called *full loading*) and intramuscular botox treatment (called *reduced loading*). Based on the experimental data, the callus was assumed to cover 50% of the stumps in the two FE-models respectively (Suppl. Fig. 1). To reduce computational cost, the implemented finite element meshes were a quarter of a cylinder and symmetry conditions were implemented to mimic a complete 3D cylinder (see Suppl. Fig. 1-2 for details on geometry, mesh and boundary conditions).

The full loading FE-model was subjected to 2.0 N tensile load, representing the maximum force during gait in adult female Sprague-Dawley rats (Song et al., 2019). The reduced loading FE-model was subjected to estimated reduced load levels. As the load level following a botox injection is unknown, three levels of reduced loading were screened, specifically 0.25 N, 0.5 N, and 1.0 N, representing 12.5-50% of the physiological loading during gait. Mechanical loading was modeled as a linear ramp, with a rate of 1.1 N/s.

Adaptive mechanobiological model

The healing framework describing collagen production and reorientation laws and rates, were implemented as described in Notermans et al. (2021) with the novel addition of the cell density-dependent tissue production. Briefly described; a single mechanical load was applied to represent mechanical stimulation during 1 day of healing. Using a strain-regulated

production law, tissue (collagen and ground matrix) were produced based on the magnitude of the resulting maximum principal strain in each element (Fig. 1). The production law entails a higher tissue production rate for increasing strains up to 10%, followed by a plateau, and thereafter a reduced tissue production rate for supraphysiological (>15%) strains. The maximum tissue production rate was 2%/day. During the first 5 days of healing, the maximum mechanoregulated tissue production rate was 1%/day plus 1%/day as baseline tissue production rate, assumed to be driven by acute inflammation. After 5 days, the tissue production rate was solely mechanoregulated (Notermans et al., 2021).

A new feature depicting cell migration and proliferation was added to the previous framework of Notermans et al. (2021), where cells infiltrated from the extrinsic compartment of the callus (this assumption was varied, as shown in Suppl. Fig 5). The process was modeled as diffusion, where the diffusion constant in Fick's law describes the rate of cell infiltration. The value of the diffusion constant was set such that the average cell density in the callus reached 95% after 2 weeks (Suppl. Fig. 3-4). This assumption was motivated by studies measuring temporal evolution of cell density and proliferation in tendon defects that identified maximum cell density and proliferation rate at 1-2 weeks post-rupture (Ackerman et al., 2019; Chamberlain et al., 2013; Dyment et al., 2013; Dyment et al., 2014; Galatz et al., 2006). The local tissue production (P_{total}) in the callus was implemented such that mechanoregulated production ($P_{mechanical}$; following the strain-regulated production law depicted in Fig. 1) depended on the local cell density (ρ_{cell}, ranging from 0-1), according:

$$P_{total} = \rho_{cell} * P_{mechanical} \quad [1]$$

Hence, no tissue production ($P_{total} = 0$) occurs if there are no cells present (ρ_{cell}= 0), regardless of the mechanical cue, and tissue production is allowed fully (maximum 2%/day) if

159 the local cell density is 100% (ρ_{cell}= 1). In each iteration, the collagen fibrils (13/material
160 point with random initial orientation) in the callus were rotated in the direction of the
161 maximum principal strain (Notermans et al., 2021; Tanska et al., 2018; Wilson et al., 2006).
162 The fibril reorientation from random to longitudinal alignment was set to occur within 4
163 weeks (see Notermans et al., 2021).

164

165 *Validation of collagen content and mechanical properties*

166 To validate the model predictions, spatio-temporal evolution of collagen content was
167 compared to spatial data from small angle x-ray scattering (SAXS) measurements of fully
168 loaded and botox-treated rat tendons from Khayyeri et al. (2020). Additionally, we compared
169 the evolution of mechanical properties (stiffness, Young's modulus, creep magnitude, creep
170 ratio and strain levels in the callus) in our healing models to experimental data from Khayyeri
171 et al. (2020). The experimental creep test (load to 5N with loading rate 1.1N/s, followed by a
172 constant load held for 300s) was simulated at 1, 2 and 4 weeks post-rupture. For each
173 simulation, stiffness and Young's modulus were determined from the force-displacement and
174 stress-strain curve, at 4 - 4.5N. The strain level in the callus was determined at 2N tensile load
175 and assumed to represent a normal physiological load level. The creep magnitude was
176 measured as the final displacement of the whole tendon after maintaining 5N for 300 second,
177 as also measured in the experiments. Creep ratio was defined as the creep magnitude
178 normalized by gap distance.

179

180 To summarize, four load levels (0.25; 0.5; 1.0; 2.0 N) were simulated over time, with
181 (assuming 100% cell density was reached from day 1) or without cell infiltration (i.e. cell
182 infiltration over 2 weeks), and the output was reported at 1, 2, 3 and 4 weeks of healing (Fig.

2). All mechanobiological simulations ran without numerical complications. However, two creep simulations (out of 32) had numerical instabilities. In these two cases, the creep magnitude and ratio were estimated by instead measuring the creep displacement from a 4-parameter exponential function that was fitted to the converged part of the simulation using the *fminsearch* function in MATLAB.

Results

Load-dependent distribution of collagen content

All the simulations consistently predicted higher strain levels in the core of the tendon callus than in the periphery (outer area) (Fig. 3). The simulations with cell infiltration predicted higher strains throughout the callus, for at least 2 weeks, compared to the simulations with homogeneous high cell density.

In general, the high strain magnitudes predicted limited collagen production in the tendon core (see simulation results for 2N and 1N load level), compared to the more reduced load levels (0.25N and 0.5N, see Figs. 4-5). Although simulations predicted higher collagen content in the core at week 1 when the load levels were lower, the final collagen content in the callus core at week 4 was higher in simulations of higher load levels (Fig. 4, 5). For the scenarios without cell infiltration, simulations predicted higher tissue density in the periphery of the callus only at week 1 (see simulation results with 2.0N and 1.0N) and week 2 (see simulation results with 2.0N). Instead, the healing simulations that included cell infiltration consistently predicted higher tissue production in the periphery compared to in the callus core with all levels of loading (Fig. 4-6).

206

207 *Comparison with data from SAXS measurements*

208 The predicted spatial maps of the callus content from the healing simulations with infiltrating
209 cells (Fig. 4) resembled the experimental SAXS data (Khayyeri et al., 2020) showing highest
210 tissue content in the callus periphery. The results from the simulations show that higher load
211 levels during healing affects the spatial distribution of tissue production between week 2 and
212 4 by shifting the main production from the periphery to the callus core and callus-stump
213 interface. Comparing the healing simulations including cell infiltration quantitatively to
214 SAXS line profiles through the callus (Fig. 6) revealed that the simulations showed increased
215 peripheral production throughout healing, as seen experimentally. For simulations with
216 reduced load levels, the 0.5N load scenario was similar to the 2.0N load scenario in terms of
217 collagen content profile at week 1. This similarity between the two load scenarios was also
218 found experimentally. However, all simulations underpredicted the production of collagen in
219 the periphery, observed in the experimental data between week 2 and 4. Similar to the
220 experimental data, none of the reduced loading scenarios predicted a higher collagen content
221 at 2 or 4 weeks compared to the simulations where the tendon was fully loaded.

222

223 *Analysis of mechanical properties*

224 Healing simulations that included cell infiltration predicted a stiffness that was within the
225 range of experimental data for both full and reduced load scenarios, except at week 1 where
226 the simulations of reduced load levels underpredicted the experimental data (Fig. 7). When
227 comparing the simulation results from full and reduced loading scenarios, the model captured
228 a trend where reduced load levels resulted in stiffer tendons than tendons healing subjected to
229 full loading conditions. The predictions for Young's modulus fall mostly within the range of

the experimental data. However, the simulations with reduced load levels overpredicted the Young's modulus at week 2 and 4. The predictions for creep magnitude and creep ratio were in the range of experimental data at most time points (Fig 7). The magnitude of strain measured in the callus at 2N was high (>10%) (Fig. 7). The simulations predicted callus strains in the range of the experimental data for full (week 1, 2) and reduced load (week 2, 4) levels.

For the healing simulations with cell infiltration, the effect of the different levels of reduced loading (0.25; 0.5; 1.0N) on the temporal evolution of mechanical properties shifts over time (Fig 7; Suppl. Table 1). Comparing the simulations with and without cell infiltration revealed that adding cell infiltration slightly decreased the stiffness and Young's modulus for all load levels, had minor effects on creep magnitude and creep ratio, and increased strains at 2N (Suppl. Table 1).

Discussion

In this study, we investigated how different levels of external mechanical load affected spatio-temporal development of collagen content, collagen reorientation and temporal evolution of mechanical properties (stiffness, Young's modulus, creep magnitude, creep ratio, tissue strain) in healing tendons, using an existing 3D mechanoregulatory healing framework for tendons (Notermans et al., 2021). The framework was further developed by adding features that consider cell infiltration from the extrinsic compartment in to the healing tendon. The numerical predictions were validated against the experimental data from Khayyeri et al. (2020). We showed that accounting for cell infiltration and mechanoregulation can capture heterogeneous tissue production and temporal evolution of mechanical properties as observed

experimentally. Adding cell infiltration to the framework predicted limited early tissue production in the callus core for all load levels and showed minor effects on the temporal evolution of mechanical properties.

We predicted heterogeneous strain distributions throughout the callus in all healing simulations (Fig. 3). Like our previous work (Notermans et al., 2021), we found supraphysiological strains (>15%) in all simulations up to 2 weeks post-rupture. These results highlight the local heterogeneous strain response from mechanical loading in the Achilles tendon throughout early healing. The results further support that supraphysiological (>15%) strain levels could govern spatial variations in tissue production through a mechanism that collagen production decreases for high strains (>15%) as hypothesized in Notermans et al. (2021).

In general, our simulations with reduced load levels showed improved collagen production in the tendon core in the first week of healing (Fig. 4), identifying a possible therapeutic benefit for reduced load level during early healing. On the other hand, the absolute callus content decreased with reduced load level at 4 weeks of healing (Fig. 4), indicating a possible drawback of a prolonged reduced daily loading. Although the predicted collagen content in the simulation with 2N and 1N loading closely resembled the experimental data at week 1 and week 2 (Fig. 4 and 6), all healing simulations without cell infiltration underpredicted the collagen production in the periphery of the callus at week 4. Adding cell infiltration promoted collagen production in the periphery and reduced early production in the callus core such that simulation with all load levels roughly predicted collagen production that matched the spatial patterns observed experimentally (Fig. 4-6). We tested the sensitivity of the framework, by simulating different cell infiltration rates. However, the rate of cell infiltration did not affect

the spatial tissue production patterns but only affected the temporal evolution of tissue production (Suppl. Fig. 5). A decreased cell infiltration rate resulted in slower development of stiffness and Young's modulus. However, the evolution of creep properties was barely affected (Suppl. Table 1). Our framework predicted increased stiffness and Young's modulus and decreased creep properties at 1 week post-rupture when healing was simulated with reduced load levels (Fig. 7; Suppl. Table 1). This is similar to what we found in the animal experiments for tendons treated with botox (Khayyeri et al., 2020).

The implementation of cell infiltration, modeled as diffusion, was designed to resemble extrinsic cells invading the callus area (Suppl. Fig. 4) according to earlier work in bone healing (Isaksson et al., 2006). In our study, we did not consider that the rate of cell migration and proliferation may be mechano-regulated. Instead, our implementation of cell infiltration scales the mechano-regulated response, as the mechano-regulated tissue production is modeled here to depend on cell density. Also, we investigated the effect of allowing cell infiltration from the tendon stumps into the callus area (Suppl. Fig. 6), yet we observed no major differences in the predicted tissue production when cells were allowed to enter from the callus periphery and/or tendon stumps. Overall, spatio-temporal data on cells during tendon healing is lacking, and more elaborate experimental data on mechano-regulated cell behaviour during tendon healing will prove valuable in characterizing the role of cells in heterogeneous tendon healing. However, there may be other biological stimuli, e.g. oxygen or blood supply, that could stimulate early tissue production in the periphery in a similar manner. However, no computational framework of tendon healing has so far included spatio-temporal distributions of metabolic agents, e.g. oxygen, blood supply or lactate, although these could be modeled with diffusion-equations as in this study.

A limitation in this study is that the FE geometries developed for the simulations do not adapt throughout the healing process. Since the geometry is based on 1 week post-rupture measurements, the Young's modulus (Fig. 8) is predicted correctly at week 1, but it is overpredicted at week 2 and 4. In general, experimental studies have showed that length and width of the callus increased considerably with increased level of loading (Andersson et al., 2009; Eliasson et al., 2020; Hammerman et al., 2018; Khayyeri et al., 2020). In this study, we mimicked this by using different sized FE models for the fully loaded and the reduced loaded scenarios (Suppl. Fig. 1). Using two different FE-models, with different callus sizes, enabled us to better compare our simulations to experimental findings. But the different geometries stem for mechanobiological reasons, where loading seems to have an enlarging effect on the callus diameter. The mechanobiological mechanisms behind this needs a mesh-adaptive model in order to be explored. Future numerical studies should include the adaptation of callus geometry during tendon healing.

To induce reduced physiological load levels, experiments commonly use methods of botox injection (Eliasson et al., 2009), tail suspension (Andersson et al., 2009), boot/orthosis (Hammerman et al., 2018) or cast immobilization (Freedman et al., 2017a). However, there is no study that quantified the resulting loading on the Achilles tendon in vivo. Furthermore, the botox injection may affect the tendon healing process, for example, by affecting cell signaling, in addition to a mechanical effect, which has not been considered in the model. For now, our study assumed 12.5-50% of the maximum physiological load as approximations of the reduced load scenarios. The resulting range seems reasonable considering the literature, where e.g. Song et al. (2019) characterized ground reaction forces during maturation of Sprague-Dawley rats and found them to increase from ~1.4N to ~2.2N from 8 weeks to 20 weeks of age.

Due to the lack of measurements of spatio-temporal evolution of tissue properties (e.g. collagen content, orientation and structure) in healing tendon, we used quantitative SAXS measurements (intrafibrillar order) as pseudo-measure for spatial distributions of collagen content and quality. We mimicked callus content and quality by implementing a callus density function (ρ) that relates the stress in the callus to the stress in intact tendon material: $\sigma_{\text{callus}}^{\text{tissue}} = \rho * \sigma_{\text{intact}}^{\text{tissue}}$ as in Notermans et al. (2021). This scaling, just as the SAXS parameter, may reflect changes in both quantity and quality of the collagen matrix.

There are many mechanobiological facets that future healing models may address, particularly upon the arrival of more elaborate experimental validation work. Such features may entail how loading affects the collagen reorientation rate, load-dependent adaptation of the callus geometry, utilizing vivo measurements of tendon loading during different rehabilitation scenarios, more thorough (viscoelastic, fatigue, heterogeneous) biomechanical validation, and many more. Therefore, our model is a simplification of a very complex biological system. However, this study presents an important first step, where we investigate spatial variations underlying tendon healing using a mechanoregulated computational framework to simulate different levels of external mechanical loading. Although many variables may affect the healing process, we focused on exploring the cell infiltration and the effect of reduced loading, with focus on direct comparison with our experimental available SAXS data. Further development of the computational framework can improve the predictive capacity of the healing framework and enable more elaborate in silico investigations of tendon healing. Identifying the mechanisms underlying heterogeneous tendon healing may help designing better treatment following tendon rupture. Given that both experimental and numerical studies

353 investigating spatial variations underlying tendon healing are limited, this study establishes a
354 valuable and necessary computational tool for investigating different biomechanical and
355 (mechano) biological aspects involved in tendon healing.

356 Conflict of interest statement

357 T. Notermans, H. Khayyeri and H. Isaksson have no conflicts of interest to declare.

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Figure legends

Figure 1. Schematic overview of the healing framework. Finite element simulations were performed to predict the maximum principal strain distribution in the healing callus. Each iteration (~1 day of healing), tissue production was guided by local strain magnitude and collagen fibrils were reoriented towards the maximum principal strain direction. Daily tissue production depended on the local cell density. For cell infiltration, three different diffusion constants were used to allow the average cell density to reach 95% after 1, 2 or 3 weeks. For clarity, only the results for cell infiltration over 2 weeks are shown in the main manuscript. The tendon model was stimulated with various load levels to mimic full physiological load (2.0N) and different levels of reduced loading (0.25N, 0.5N, and 1.0N).

Figure 2. Overview of the finite element simulations. Simulations of full loading, 2N load, were based on the FE-geometry created from the average geometry of healing rat Achilles tendons exposed to free cage activity (FCA) (see Suppl. Fig. 1-2). Simulation of the reduced load levels (0.25; 0.5; 1.0N) were based on the FE-geometry created from the average healing geometry of the botox-treated group (see Suppl. Fig. 1-2). All load scenarios were simulated both with and without cell infiltration. In summary, eight mechanoregulated healing scenarios were simulated. For each healing scenario, a creep test was simulated at 1, 2, 3 and 4 weeks of healing, corresponding to 32 creep simulations.

Figure 3. Spatial and temporal evolution of maximum principal strain for all healing simulations, with homogeneous cell distribution and cell infiltration for 2 weeks. The initial strain profile in the whole callus is presented for both full (A) and reduced load level (C) geometry. The strain profiles along the midline (B) are presented for 0, 1, 2 and 4 weeks post-

rupture. The black dotted lines in the meshes (A, C) denote the callus midline highlighted in (B).

Figure 4. Spatio-temporal evolution of collagen content in the callus for full load (2N) and reduced load (1.0; 0.5; 0.25N) levels with cell infiltration over 2 weeks, at 1, 2 and 4 weeks post-rupture (B). Simulation output is compared to typical experimental data from free cage activity (A) or botox-treated (C) healing tendon per time point from Khayyeri et al. (2020), where intrafibrillar order reflects the collagen matrix.

Figure 5. Spatio-temporal evolution of collagen content along the midline of the callus for full loading (2N) and reduced load levels (0.25; 0.5; 1.0N), with homogeneous cell distribution and cell infiltration over 2 weeks, at 1, 2 and 4 weeks post-rupture.

Figure 6. Spatio-temporal evolution of collagen content along the midline of the callus for simulations with full load (2N) and reduced load (0.5, 1.0N) as indicated in the schematic to the right. All simulations include cell infiltration for 2 weeks, and results are compared to experimental data at 1, 2 and 4 weeks post-rupture. Specifically, simulation output is compared to experimental data from free cage activity loaded and botox-treated tendons from Khayyeri et al. (2020). All callus widths have been normalized.

Figure 7. Temporal evolution of mechanical properties. Stiffness, Young's modulus, callus strain measured at 2N, creep magnitude and creep ratio is displayed for simulations with full load (2N) and reduced load (0.25; 0.5; 1.0N) levels with cell infiltration over 2 weeks, at 1, 2,

410 3 and 4 weeks post-rupture. Healing simulation output is compared to experimental data
411 (mean ± standard deviation) from free cage activity loaded and botox unloaded tendons from
412 Khayyeri et al. (2020).

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