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

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1 **Accepted version**

2

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

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6

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8

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14

15

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18

19 **Abstract**

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

41

42 **Introduction**

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

57

58 Simulations based on computational models can aid the understanding of mechanobiological
59 processes during tissue healing by predicting spatio-temporal tissue composition and
60 organization. Two studies have investigated the *temporal* effect of reduced loading on
61 collagen orientation, anisotropy and synthesis/content during tendon healing (Chen et al.,
62 2018; Richardson et al., 2018). However, these computational studies did not investigate
63 mechanoregulated *spatial* variations of tendon composition and organization that occurs
64 during tendon healing. We have previously developed a mechanoregulatory framework that
65 addresses this gap (Notermans et al., 2021). The framework is able to describe the spatio-

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

68

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

78

79 During tendon healing, intrinsic repair is performed by tendon cells originating from the intact
80 tendon, whereas extrinsic healing involves the recruitment of external cells (e.g. matrix-
81 producing fibroblasts) to the defect site from neighboring tissues or blood supply (here called
82 *the extrinsic compartment*) (Snedeker and Foolen, 2017; Nichols et al., 2019). Chen et al.
83 (2018) modeled cell migration and proliferation from the tendon stumps towards the callus
84 core during early healing. However, no modeling approach has incorporated extrinsic cell
85 infiltration (migration and proliferation) during tendon healing, which is believed to be key as
86 most cells enter the defect extrinsically since the tendon stumps have low cellularity
87 (Snedeker and Foolen, 2017; Nichols et al., 2019).

88

89 In this study, we aimed to determine if our previously developed mechanoregulatory
90 framework (Notermans et al., 2021) can be developed to investigate the load level-dependent
91 evolution of heterogeneous tissue distribution and the mechanical properties *in silico*,
92 including both elastic and viscoelastic properties of the tendon throughout healing.
93 Simulations were compared with data from rats undergoing tendon healing (Khayyeri et al.,
94 2020). We hypothesized that extrinsic cell infiltration may play a role in heterogeneous tissue
95 production. Therefore, we investigated the effect of cell infiltration on collagen distribution in
96 the callus by including cell density-dependent tissue production in our mechanobiological
97 framework.

98

99 **Methods**

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

109

110 *The Finite Element Model*

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

122

123 The full loading FE-model was subjected to 2.0 N tensile load, representing the maximum
124 force during gait in adult female Sprague-Dawley rats (Song et al., 2019). The reduced
125 loading FE-model was subjected to estimated reduced load levels. As the load level following
126 a botox injection is unknown, three levels of reduced loading were screened, specifically 0.25
127 N, 0.5 N, and 1.0 N, representing 12.5-50% of the physiological loading during gait.

128 Mechanical loading was modeled as a linear ramp, with a rate of 1.1 N/s.

129

130 *Adaptive mechanobiological model*

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

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

143

144 A new feature depicting cell migration and proliferation was added to the previous framework
145 of Notermans et al. (2021), where cells infiltrated from the extrinsic compartment of the
146 callus (this assumption was varied, as shown in Suppl. Fig 5). The process was modeled as
147 diffusion, where the diffusion constant in Fick's law describes the rate of cell infiltration. The
148 value of the diffusion constant was set such that the average cell density in the callus reached
149 95% after 2 weeks (Suppl. Fig. 3-4). This assumption was motivated by studies measuring
150 temporal evolution of cell density and proliferation in tendon defects that identified maximum
151 cell density and proliferation rate at 1-2 weeks post-rupture (Ackerman et al., 2019;
152 Chamberlain et al., 2013; Dymant et al., 2013; Dymant et al., 2014; Galatz et al., 2006). The
153 local tissue production (P_{total}) in the callus was implemented such that mechanoregulated
154 production ($P_{mechanical}$; following the strain-regulated production law depicted in Fig. 1)
155 depended on the local cell density (ρ_{cell} , ranging from 0-1), according:

$$156 \quad P_{total} = \rho_{cell} * P_{mechanical} \quad [1]$$

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

159 the local cell density is 100% ($\rho_{cell}= 1$). 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.

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

188

189 **Results**

190 *Load-dependent distribution of collagen content*

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

195

196 In general, the high strain magnitudes predicted limited collagen production in the tendon
197 core (see simulation results for 2N and 1N load level), compared to the more reduced load
198 levels (0.25N and 0.5N, see Figs. 4-5). Although simulations predicted higher collagen
199 content in the core at week 1 when the load levels were lower, the final collagen content in the
200 callus core at week 4 was higher in simulations of higher load levels (Fig. 4, 5). For the
201 scenarios without cell infiltration, simulations predicted higher tissue density in the periphery
202 of the callus only at week 1 (see simulation results with 2.0N and 1.0N) and week 2 (see
203 simulation results with 2.0N). Instead, the healing simulations that included cell infiltration
204 consistently predicted higher tissue production in the periphery compared to in the callus core
205 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

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

236

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

243

244 **Discussion**

245 In this study, we investigated how different levels of external mechanical load affected spatio-
246 temporal development of collagen content, collagen reorientation and temporal evolution of
247 mechanical properties (stiffness, Young's modulus, creep magnitude, creep ratio, tissue
248 strain) in healing tendons, using an existing 3D mechanoregulatory healing framework for
249 tendons (Notermans et al., 2021). The framework was further developed by adding features
250 that consider cell infiltration from the extrinsic compartment in to the healing tendon. The
251 numerical predictions were validated against the experimental data from Khayyeri et al.
252 (2020). We showed that accounting for cell infiltration and mechanoregulation can capture
253 heterogeneous tissue production and temporal evolution of mechanical properties as observed

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

257

258 We predicted heterogeneous strain distributions throughout the callus in all healing
259 simulations (Fig. 3). Like our previous work (Notermans et al., 2021), we found
260 supraphysiological strains ($>15\%$) in all simulations up to 2 weeks post-rupture. These results
261 highlight the local heterogeneous strain response from mechanical loading in the Achilles
262 tendon throughout early healing. The results further support that supraphysiological ($>15\%$)
263 strain levels could govern spatial variations in tissue production through a mechanism that
264 collagen production decreases for high strains ($>15\%$) as hypothesized in Notermans et al.
265 (2021).

266

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

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

286

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

303

304 A limitation in this study is that the FE geometries developed for the simulations do not adapt
305 throughout the healing process. Since the geometry is based on 1 week post-rupture
306 measurements, the Young's modulus (Fig. 8) is predicted correctly at week 1, but it is
307 overpredicted at week 2 and 4. In general, experimental studies have showed that length and
308 width of the callus increased considerably with increased level of loading (Andersson et al.,
309 2009; Eliasson et al., 2020; Hammerman et al., 2018; Khayyeri et al., 2020). In this study, we
310 mimicked this by using different sized FE models for the fully loaded and the reduced loaded
311 scenarios (Suppl. Fig. 1). Using two different FE-models, with different callus sizes, enabled
312 us to better compare our simulations to experimental findings. But the different geometries
313 stem for mechanobiological reasons, where loading seems to have an enlarging effect on the
314 callus diameter. The mechanobiological mechanisms behind this needs a mesh-adaptive
315 model in order to be explored. Future numerical studies should include the adaptation of
316 callus geometry during tendon healing.

317

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

329

330 Due to the lack of measurements of spatio-temporal evolution of tissue properties (e.g.
331 collagen content, orientation and structure) in healing tendon, we used quantitative SAXS
332 measurements (intrafibrillar order) as pseudo-measure for spatial distributions of collagen
333 content and quality. We mimicked callus content and quality by implementing a callus density
334 function (ρ) that relates the stress in the callus to the stress in intact tendon material:

335 $\sigma_{\text{callus}}^{\text{tissue}} = \rho * \sigma_{\text{intact}}^{\text{tissue}}$ as in Notermans et al. (2021). This scaling, just as the SAXS parameter,
336 may reflect changes in both quantity and quality of the collagen matrix.

337

338 There are many mechanobiological facets that future healing models may address, particularly
339 upon the arrival of more elaborate experimental validation work. Such features may entail
340 how loading affects the collagen reorientation rate, load-dependent adaptation of the callus
341 geometry, utilizing vivo measurements of tendon loading during different rehabilitation
342 scenarios, more thorough (viscoelastic, fatigue, heterogeneous) biomechanical validation, and
343 many more. Therefore, our model is a simplification of a very complex biological system.
344 However, this study presents an important first step, where we investigate spatial variations
345 underlying tendon healing using a mechanoregulated computational framework to simulate
346 different levels of external mechanical loading. Although many variables may affect the
347 healing process, we focused on exploring the cell infiltration and the effect of reduced
348 loading, with focus on direct comparison with our experimental available SAXS data. Further
349 development of the computational framework can improve the predictive capacity of the
350 healing framework and enable more elaborate in silico investigations of tendon healing.
351 Identifying the mechanisms underlying heterogeneous tendon healing may help designing
352 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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363 **Figure legends**

364 **Figure 1.** Schematic overview of the healing framework. Finite element simulations were
365 performed to predict the maximum principal strain distribution in the healing callus. Each
366 iteration (~1 day of healing), tissue production was guided by local strain magnitude and
367 collagen fibrils were reoriented towards the maximum principal strain direction. Daily tissue
368 production depended on the local cell density. For cell infiltration, three different diffusion
369 constants were used to allow the average cell density to reach 95% after 1, 2 or 3 weeks. For
370 clarity, only the results for cell infiltration over 2 weeks are shown in the main manuscript.
371 The tendon model was stimulated with various load levels to mimic full physiological load
372 (2.0N) and different levels of reduced loading (0.25N, 0.5N, and 1.0N).

373

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

382

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

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

389

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

395

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

399

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

406

407 **Figure 7.** Temporal evolution of mechanical properties. Stiffness, Young's modulus, callus
408 strain measured at 2N, creep magnitude and creep ratio is displayed for simulations with full
409 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 \pm standard deviation) from free cage activity loaded and botox unloaded tendons from
412 Khayyeri et al. (2020).

413

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