Basic Research

Can Genipin-coated Sutures Deliver a Collagen Crosslinking Agent to Improve Suture Pullout in Degenerated Tendon? An Ex Vivo Animal Study

Roland S. Camenzind MD, Timo O. Tondelli PhD, Tobias Götschi, Claude Holenstein, Jess G. Snedeker PhD

Abstract

Background The suture-tendon interface is often the weakest link in tendon-to-tendon or tendon-to-bone repair. Genipin is an exogenous collagen crosslink agent derived from the gardenia fruit that can enhance suture force to failure of the tendon-suture interface. Viable methods for intraoperative clinical delivery of genipin could be of clinical utility, but to our knowledge have not yet been extensively studied.

R. S. Camenzind, T. O. Tondelli, T. Götschi, C. Holenstein, J. G. Snedeker, Department of Orthopedics, Balgrist University Hospital, Zurich, Switzerland

T. Götschi, C. Holenstein, J. G. Snedeker, Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

R. S. Camenzind (✉), Department of Orthopedics Balgrist University Hospital Forchstrasse 340 8008 Zurich, Switzerland e-mail: roland.camenzind@bluewin.ch

Questions/purposes The purposes of this study were (1) to evaluate whether sutures precoated with genipin can augment the suture-tendon interface to improve force to failure, stiffness, and work to failure in healthy and degenerated tendons; and (2) to determine the effect of genipin on the extent and distribution of crosslinking.
Methods Single-stitch suture pullout tests were performed ex vivo on 25 bovine superficial digital flexor tendons. To assess effects on native tissue, one group of 12 tendons was cut in proximal and distal halves and randomized to treatment (n = 12) and control groups (n = 12) in a matched-pair design. One simple stitch with a loop with either a normal suture or genipin-coated suture was applied to tendons in both groups. To simulate a degenerative tendon condition, a second group of 13 tendons was cut in proximal and distal halves, injected with 0.2 mL of collagenase D (8 mg/mL) and incubated for 24 hours before suturing with either a genipin-coated suture (n = 13) or their matched controls (n = 13). Sutures from all groups then were loaded to failure on a universal materials testing machine 24 hours after suturing. Suture pullout force, stiffness, and work to failure were calculated from force-displacement data and compared between the groups. Additionally, fluorescence was measured to determine the degree of crosslinking quantitatively and a qualitative analysis of the distribution pattern was performed by microscopy.
Results In healthy tendon pairs, the median maximum pullout force was greater with genipin-coated sutures than with control sutures (median, 42 N [range, 24–73 N] versus 29 N [range, 13–48 N]; difference of medians, 13 N; p = 0.003) with corresponding increases in the required work to failure (median, 275 mJ [range, 48–369 mJ] versus 148 mJ [range, 83–369 mJ]; difference of medians, 127 mJ; p = 0.025) but not stiffness (median, 4.1 N/mm [range, 2.3–8.1 N/mm] versus 3.3 N/mm [range, 1.1–9.6 N/mm]; difference

of medians, 0.8 N/mm; p = 0.052). In degenerated tendons, median maximum pullout force was greater with genipin-coated sutures than with control sutures (median, 16 N [range, 9-36 N] versus 13 N [range, 5-28 N]; difference of medians, 3 N; p = 0.034) with no differences in work to failure (median, 75 mJ [range, 11–249 mJ] versus 53 mJ [range, 14–143 mJ]; difference of medians, 22 mJ; p = 0.636) or stiffness (median, 1.9 N/mm [range, 0.7–13.4 N/mm] versus 1.6 N/mm [range, 0.5–5.6 N/mm]; difference of medians, 0.3 N/mm; p = 0.285). Fluorescence was higher in tendons treated with genipin-coated sutures compared with the control group, whereas higher fluorescence was observed in the treated healthy compared with the degenerated tendons (difference of means -3.16; standard error 1.08; 95% confidence interval [CI], 0.97–5.34; p = 0.006/healthy genipin: mean 13.04; standard error 0.78; 95% CI, 11.47-14.62; p < 0.001/degenerated genipin: mean 9.88; SD 0.75; 95% CI, 8.34-11.40; p < 0.001).

Conclusions Genipin-coated sutures improved force to failure of a simple stitch at the tendon-suture interface in healthy and degenerated tendons in an ex vivo animal model. Fluorescence was higher in tendons treated with genipin-coated sutures compared with the control group.

Clinical Relevance A genipin-coated suture represents a potential delivery vehicle for exogenous crosslink agents to augment suture retention properties. In vivo animal studies are the next logical step to assess safety and efficacy of the approach.

Introduction

Tendon injuries are common and often result in surgical repair using open or arthroscopic means, but these repairs do not always heal. Followup studies suggest that between 13% and 68% of patients who undergo rotator cuff repair have retears [19, 21–23, 26, 31, 41] with suture cut-through constituting the most common failure mode [3, 11]. The causes of retears are multifactorial and more frequently occur in the older patient [25]. Age-related degenerative changes of the rotator cuff [40, 42, 46] likely play a role. Such degeneration histologically presents with disorientation and thinning of collagen fibers and reduced collagen content [24, 34].

To overcome the limitations imposed by weakened tissue structures that undergo repair, approaches to improve the mechanical stability of the initial surgical repair have been proposed. These include improved stitch configuration [9, 21], tendon grasping techniques [2, 29], and tissue augmentation by incorporating reinforcing materials such as human dermal allograft [5]. Another possible tissue augmentation technique is application of exogenous crosslinking agents with the aim to strengthen the tissue surrounding the suture and thereby increase resistance to suture cut-through. Crosslinking agents have been reported to increase the tensile strength of tendon tissue up to 110% [17]. Genipin is a naturally occurring crosslinking agent derived from Gardenia jasminoides (an evergreen flowering plant) with relatively low cytotoxicity [15-17]. Genipin has emerged as a promising candidate for preclinical investigation with results of an earlier study showing potential to increase suture-tendon force to failure in an ex vivo sheep model [10]. In that study, sheep infraspinatus tendons were incubated in a 20-mmol/L genipin solution for 24 hours; this process increased the maximum suture pullout force of a single suture stitch by 30%. Preliminary work has shown that embedding the crosslinking agent in the suture may enhance tissue delivery [38], although the utility of this approach for arthroscopic repair of torn tendons with underlying tissue degeneration remains uninvestigated, to the best of our knowledge [38]. The aim of the current investigation was to assess the potential of a genipin-releasing suture to augment suture retention strength using a simple stitch configuration in healthy and degenerative tissues, whereby tendon degeneration was modeled through collagenase injection to disrupt the collagen matrix [7, 14, 27, 43].

Specifically, we sought (1) to evaluate whether sutures precoated with genipin can augment the suture-tendon interface to improve suture force to failure, stiffness, and work to failure in healthy and degenerated tendons; and (2) to determine the effect of genipin on the extent and distribution of crosslinking.

Materials and Methods

A total of 25 superficial digital flexor tendons from approximately 2-year-old cattle were obtained from a local abattoir. These freshly harvested tendons were grossly examined for pathologies, wrapped in gauze, moistened with phosphate-buffered saline (PBS) solution, and stored at -20° C until testing.

Preparation of Suture Coating

Suture coating was prepared according to the description of Sundararaj et al. [38]. The formula consists of a genipin-cosolvent solution with dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA) and acetone (50:50 v/v). Genipin (500 mg/mL) (Challenge Bioproducts Co, Ltd, Taiwan, Republic of China) was added to the cosolvent solution. Acid end-capped poly(lactic-co-glycolic acid) (Akina, Inc, West Lafayette, IN, USA) with a lactic acid:glycolic acid ratio of 50:50 and polyethylene glycol (M_n 400) was dissolved in the genipin-cosolvent solution at 1% and 35%, respectively, to

form the coating formulation. High-strength braided polyethylene suture (ORTHOCORD® USP #2; DePuy Synthes, New Brunswick, NJ, USA) was submerged in the coating solution for 10 minutes. In modification of the protocol proposed by Sundararaj et al. [38], soaked sutures were not air-dried but vacuum-dried for 2 hours to ensure complete evaporation of the solvent.

Tendon Preparation

Before testing, tendon explants were thawed, cut in proximal and distal equally sized halves, and randomly allocated to the specific test group in a matched-pair design. The samples of the degenerated testing group were injected with 0.2 mL collagenase D (Roche Diagnostics GmbH, München, Germany) in PBS (8 mg/mL) at the site of subsequent repair 24 hours before suturing. This tendon degeneration protocol was established in a preliminary experiment and has been used with similar dosage in previous in vivo [33, 43, 44] and ex vivo [7, 14] work. All tendons were wrapped in gauze moistened with PBS and stored at room temperature for 24 hours. Either a 14-cm long untreated (Fig. 1A) or genipin-coated suture (Fig. 1B) was inserted with a simple stitch into the tendon at a distance of 10 mm from the tendon end and tied in a single-loop configuration with two sliding half hitches followed by alternating half hitches for a total of seven throws. After suturing, all tendons were wrapped in gauze moistened with PBS and stored at room temperature for 24 hours until mechanical testing.

Mechanical Testing of Suture Pullout

The tendon ends were wrapped in PBS-soaked pieces of cloth and fixed in clamps [14]. Cyanoacrylate adhesive was used to reduce slippage. The suture loop then was connected to a 1-kN load cell (GTM Gassmann Theiss Messtechnik GmbH, Bickenbach, Germany) of a universal materials testing machine (Zwick 010; Zwick GmbH, Ulm, Germany) (Fig. 1A-B). Specimens were preloaded at 5 N for 1 minute and then loaded to failure with a force increment of 1 N per second [10]. Force (N) and displacement (mm) were recorded with dedicated software (testExpert® 10; Zwick-Roell, Ulm, Germany). The samples were sprayed intermittently with PBS during measurement to prevent drying.

Data Analysis of Mechanical Testing

For data analysis, a corrected force-displacement signal was used. In a separate mechanical test, the force-displacement behavior of only the suture loop was recorded. This force-displacement behavior was then subtracted from the force-displacement data of the current experiments to yield a signal irrespective of suture strain (Fig. 1C).

Stiffness and work to failure calculations were based on the transformed data. Force to failure was defined as the maximum force achieved. Stiffness (N/mm) was calculated as the slope of the linear curve from 5 N to maximum force. Work to failure (mJ) was computed from the end of preload (5 N) to maximum load.

Optical Spectral Properties

Normally opaque collagen tissue turns blue (ie, absorbs light at 590 nm) after exposure to genipin and these crosslinks emit fluorescence at 645 nm when excited at 590 nm [1, 30, 37]. These specific optical properties were used to determine the amount and distribution pattern of genipin-induced crosslinks. For this purpose, after mechanical testing, a total of seven tissue extracts of approximately 60 mg were taken at increasing distances from the location of the suture entrance in 3-mm increments (Fig. 2). The tissue samples were lyophilized for at least 6 hours (Alpha 2-4 LSCplus; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and dry weight was recorded. Subsequently, the tissue samples were digested for 72 hours in 0.6 mg/mL papain in 0.9 mL of an EDTA disodium salt solution (100 mM sodium phosphate buffer/10 mM Na_2EDTA) at 65° C [35]. After digestion, samples were centrifuged at 8000 relative centrifugal force for 1 hour. Three aliquots per sample were pipetted on a 96-cell culture well-plate (Thermo Fisher Scientific, Waltham, MA, USA). A fluorescence assay was performed at 590 nm excitation and 645 nm emission wavelength (SpectraMax® GeminiXS; Molecular Devices, LLC, Sunnyvale, CA, USA). Absorption was recorded with a spectrophotometer (Epoch; BioTek Instruments GmbH, Luzern, Switzerland) for wavelengths from 380 to 700 nm at 10-nm increments. To yield a measure for genipin-specific optical properties, fluorescence and absorption readouts were standardized by dry weight and readouts of the healthy untreated tendons at the suture insertion site. Hence, fluorescence and absorption outcome measures are relative to untreated tendons and have no unit of measurement.

Microscopy

To qualitatively assess the spatial fluorescence emission of genipin-induced crosslinks, confocal image acquisition was performed using an inverted spinning disc confocal
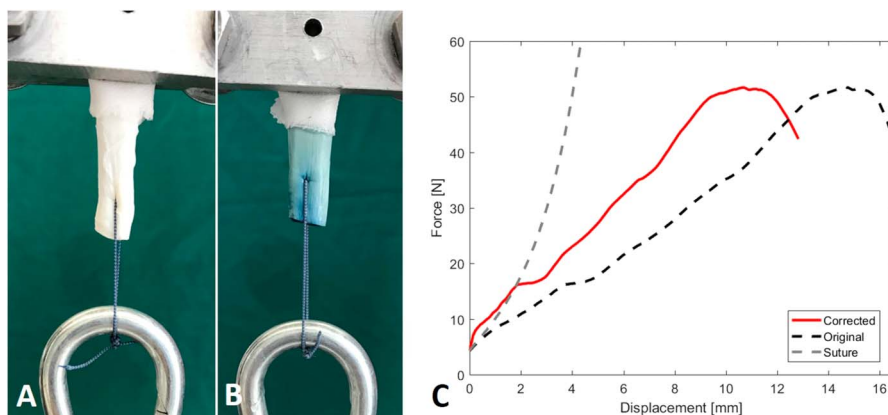
Fig. 1 A-C The experimental setup used to measure the tendon-suture pullout force is shown. (**A**) A single-loop stitch with an untreated suture was performed in a bovine superficial digital tendon. (**B**) The tendon was blue after 24 hours of exposure to a genipin-coated suture. (**C**) Single-loop curves for measurement of maximum pullout force (F_{max}) are shown. Suture deformation as a function of force-displacement (gray dashed line) then was subtracted from the raw signal (black dashed line) so that the computed signal solely reflects force displacement of the suture in the tissue (red solid line).

microscope (iMic; FEI Munich GmbH, Munich, Germany) using a 4x (0.16 NA) objective (Olympus™ U Plan S-Apo; Thermo Fisher Scientific) and a Hamamatsu Orca®-flash 4.0 V2 Digital CMOS camera C11440-22CU (Hamamatsu Photonics KK, Hamamatsu City, Japan). Genipin crosslinked tendon samples were mounted in a 30-mm petri dish and submerged in 1x PBS during imaging. A laser excitation wavelength of 405 nm was used to observe the autofluorescence of collagen type I [20] and the genipin-induced crosslinking was imaged with an excitation wavelength of 560 nm. Single z-stacks were acquired covering the whole length of the sample and subsequently tiled using the embedded function of Live acquisition Software, Offline Analysis application (FEI Munich GmbH, Munich, Germany). Final image processing (maximum intensity projection and composite) was done using ImageJ (https://imagej.nih.gov/ij/).

Statistical Analyses

As a result of the matched-pair design, mechanical data of uncoated and genipin-coated sutures were analyzed using ratio t-tests. According to D'Agostino's K-squared test [12], the ratios of treated to control for all mechanical variables were normally distributed. The significance level was set at 0.05 and the results are reported as medians and range if not stated otherwise. All significance tests were two-sided. One-way analysis of variance and t-tests were used to analyze differences in the level of absorption and fluorescence across groups. The longitudinal distribution pattern of fluorescence and absorption was analyzed by

linear regressions using fluorescence as the dependent variable and distance from the suture as well as group as a dummy-coded independent variable. The regression coefficients reflect the difference in percent along the tendon and across groups. The statistical analyses and graphs were computed using MATLAB® (MATLAB® and Statistics Toolbox™ Release 2016b; MathWorks, Inc, Natick, MA, USA), GraphPad Prism® 7.02 for Windows (GraphPad Software, La Jolla CA, USA), or Stata® 14.0 for Windows (StataCorp LP, College Station, TX, USA).

Results

Genipin-loaded Sutures Showed Improved Pullout Characteristics

Median force to failure in a healthy tendon with a genipin-coated suture was 42 N (range, 24–73 N) compared with 29 N (range, 13–48 N) in the control group (difference of medians, 13; $p = 0.003$) (Fig. 3). In the tendons pretreated
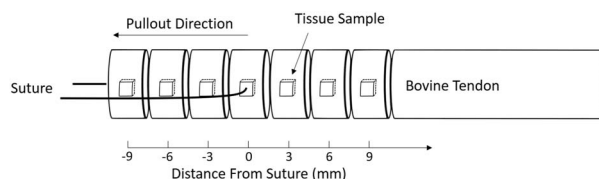
Fig. 2 Tissue samples of approximately 60 mg were taken at the suture canal and longitudinal to the tendon in both directions in 3-mm increments, yielding a total of seven tissue extracts per tendon sample for fluorescence assay.
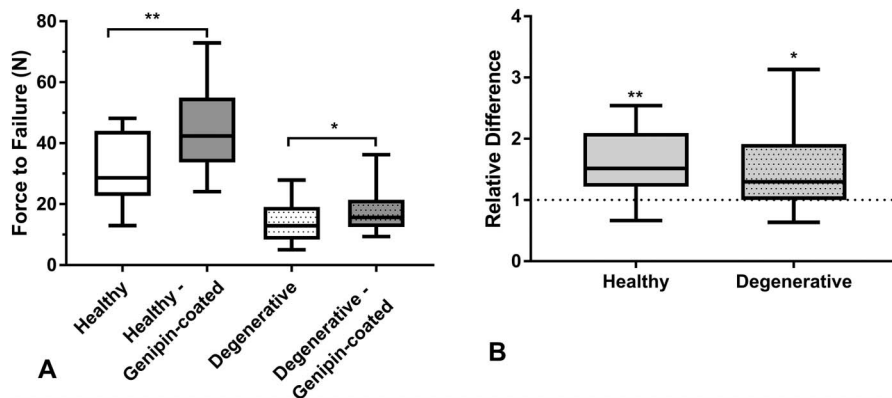
Fig. 3 A-B (**A**) Suture pullout properties of the healthy and degenerated tendons and (**B**) relative differences between genipin-treated and control tendons are shown. The white bars show the values of tendons in the control group and the gray bars show the values of the genipin-pretreated tendons. The whiskers represent minimum to maximum values. In healthy tendons treated with genipin-coated sutures, a 60% improvement of pullout force was observed (p = 0.0033). In degenerated tendons treated with genipin-coated sutures, a 59% improvement of pullout force was observed (p = 0.0344).

with collagenase, median force to failure with a genipin-coated suture was 16 N (range, 9-36 N) compared with 13 N (range, 5-28 N) in the control group (difference of medians, 3; p = 0.034). No difference in stiffness with the numbers available was detected in the healthy tendons (4.1 N/mm [range, 2.3-8.1 N/mm] versus 3.3 N/mm [range, 1.1-9.6 N/mm]; differences of medians, 0.8; p = 0.052) nor in the degenerated tendons (1.9 N/mm [range, 0.7-13.4 N/mm] versus 1.6 N/mm [range, 0.5-5.6 N/mm]; difference of medians, 0.3; p = 0.285) (Fig. 4A-B). Genipin coating increased the work to failure in the healthy tendons (275 mJ [range, 48-369 mJ] versus 148 mJ [range, 83-369 mJ]; difference of medians, 127; p = 0.025), but with the numbers available, we observed no difference in work to failure in the degenerated tendons (75 mJ [range, 11-249 mJ] versus 53 mJ [range, 14-143 mJ]; difference of medians, 22; p = 0.636) (Fig. 4C-D). The failure mode in all tests was suture cutting through the tendon in all cases. Comparing the two control groups (healthy tendons with uncoated sutures versus degenerated tendons with uncoated sutures), decreases in force to failure (p = 0.0002), stiffness (p = 0.0383), and work to failure (p = 0.0022) of the degenerated tendons with uncoated sutures were observed (Table 1).

Genipin-coated Sutures Increased Localized Tissue Crosslinking

The level of fluorescence across groups (Fig. 5A) was found to be different (p < 0.001). Mean fluorescence, that indicates genipin-induced crosslinking (emission 590 nm, excitation 645 nm), of the central part was higher in the healthy (difference of means +12.26; standard error [SE], 1.23; 95% confidence interval [CI], 9.62-14.60; p < 0.001/ healthy genipin: mean 13.04; SD 0.78; 95% CI, 11.47-14.62; p < 0.001) and degenerated (difference of means +9.10; SE 1.21; 95% CI, 6.65-11.56; p < 0.001; degenerated genipin: mean 9.88; SD 0.75; 95% CI, 8.34-11.40; p < 0.001) genipin-coated groups compared with the healthy (mean 0.78; SD 0.95; 95% CI, -1.15 to 2.71; p = 0.44) and degenerated (mean 0.93; SD 0.75; 95% CI, -1.00 to 2.86; p = 0.36) control. A lower magnitude of fluorescence was observed in the degenerated genipin-coated group compared with the healthy genipin-coated group (difference of means. -3.16; SE 1.08; 95% CI, -5.34 to -0.97; p = 0.006). No difference was observed between the healthy control group and the collagenase-pretreated control group (difference of means -0.16; SE 1.36; 95% CI, -2.57 to 2.88; p = 0.909).

As described in detail in the Methods, the longitudinal distribution of fluorescence was analyzed by linear regression. Accordingly, the regression coefficients are reported that express fluorescence differences in percentage between the groups along the tendon. The relative distribution of fluorescence along the tendon did not vary between the healthy and degenerated genipin tendons (in pullout direction: coefficient -0.2%, SE 7.0%; 95% CI, -19.5% to 19.0%; p = 0.983; opposite direction: coefficient -8.8%, SE 9.6% 95% CI, -10.4% to 28.0%, p = 0.364). Fluorescence was unchanged in the pullout direction for the healthy and degenerated genipin groups (coefficient +10.5%; SE 7.1%; 95% CI, -3.4% to 24.4%; p = 0.135). However, fluorescence decreased in the opposite direction by 22.5% (SE 6.9%; 95% CI, 8.7%-36.4%; p = 0.002) for healthy and degenerated genipin tendons, respectively.
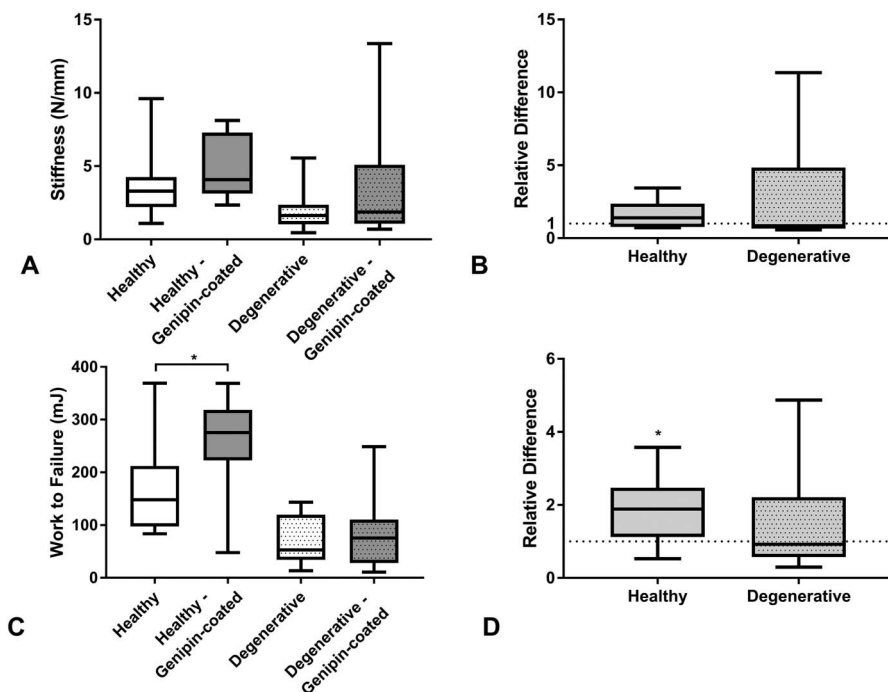
Fig. 4 A-D (**A**) Stiffness values of the healthy and degenerated tendons and (**B**) relative differences are shown. The white bars show the values of tendons in the control group and the gray bars show the values of the genipin-pretreated tendons. The whiskers represent minimum to maximum values. No difference in stiffness of either the healthy or degenerated tendons was detected. (**C**) Work to failure values of the healthy and degenerated tendons and (**D**) relative differences are shown. For healthy tendons, work to failure increased (p = 0.015) but was not different in degenerated tendons.

Absorption peaked at a wavelength of 590 nm for both genipin groups (Fig. 5B). Therefore, the following absorption analyses were focused on a wavelength of 590 nm. Both genipin groups absorbed more at 590 nm than the control and degenerated tendons (Fig. 5C). In contrast to the fluorescence results, the mean absorption of the healthy genipin group (mean 16.65; SD 1.59; 95% CI, 13.44-19.85; p > 0.001) was lower compared with the degenerated genipin group (difference of means -6.18; SE 2.19; 95% CI, -10.62 to -1.73; p = 0.008/degenerated genipin: mean 22.82; SD 1.52; 95% CI, 19.74-25.90; p > 0.001). However, there was no difference in the relative

distribution of absorption between these groups along the tendon. As described in the Methods in detail, the absorption distribution was analyzed by regression. For both groups, absorption increased by 21.3% (SE 8.6%; 95% CI, 4.2%-38.4%; p = 0.015) in direction of suture pullout, whereas there was a -53.7% (SE 8.1%; 95% CI, 70.8% to -36.6%; p = 0.179) decrease toward the opposite end.

The fluorescent emission of the genipin-induced crosslinking was also observed by fluorescence microscopy (Fig. 6A-B), allowing qualitative assessment of the optical spectral properties. The fluorescent signal intensity emitted by the genipin-induced crosslinking was strongest in the

Table 1. Mechanical properties of healthy and degenerated tendons treated with genipin-coated or untreated sutures

Mechanical property	Healthy tendons				Degenerated tendons			
	Control	GP-coated	Difference of medians	p value*	Control	GP-coated	Difference of medians	p value*
Force to failure (N)	29 (13-48)	42 (24-73)	13	0.003	13 (5-28)	16 (9-36)	3	0.035
Stiffness (N/mm)	3.3 (1.1-9.6)	4.1 (2.3-8.1)	0.8	0.052	1.6 (0.5-5.6)	1.9 (0.7-13.4)	0.3	0.285
Work to failure (mJ)	148 (83-369)	275 (48-369)	127	0.025	53 (14-143)	75 (11-249)	22	0.636

All values are presented as medians (range).
*ratio paired t-test; GP = genipin.
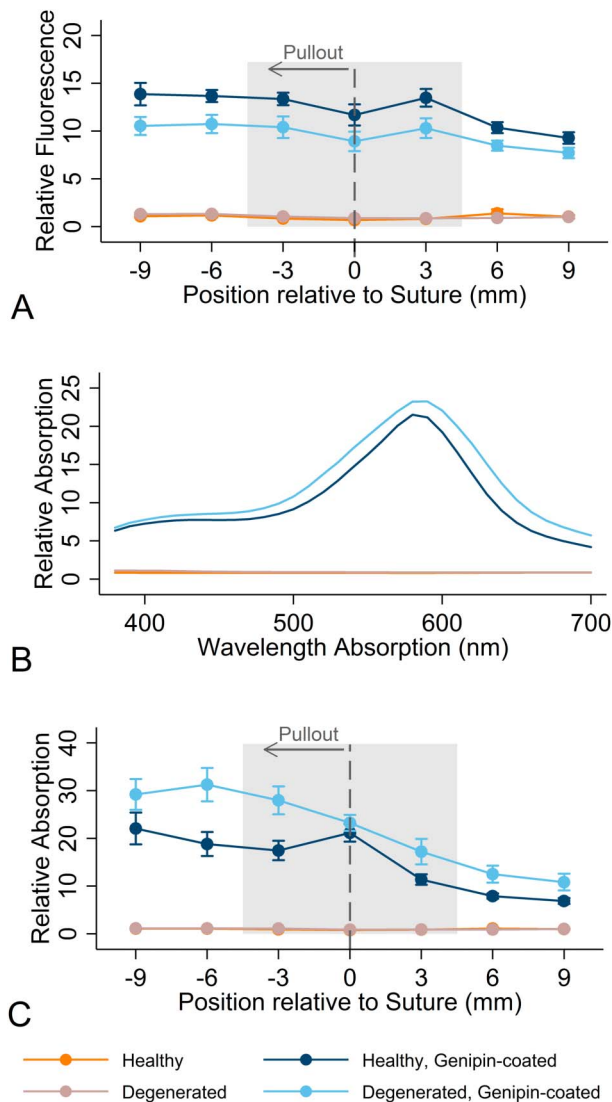
Fig. 5 A-C Results of optical spectral properties. Fluorescence and absorption are measured relative to the control group and therefore have no unit. (**A**) Levels of genipin-induced crosslinking fluorescence (emission 590 nm, excitation 645 nm) across the four groups are shown parallel to suture pullout direction. The vertical axis represents the distance in millimeters from the initial suture position before pullout. The highest signal was recorded in the healthy genipin group followed by the degenerated genipin group. (**B**) The absorption of the tendon at the suture position across different wavelengths (vertical axis) is shown. Peak absorption is measured at 590 nm for both genipin groups shown. (**C**) The level of genipin-induced blue coloration at 590 nm is shown parallel to suture pullout direction. The vertical axis represents the distance in millimeters from the initial suture position before pullout. The healthy genipin group had a lower blue signal compared with the degenerated genipin group.

tissue area surrounding the genipin-coated suture (red box) and slightly decreasing toward the ends of the tendon sample.

Discussion

Suture cut-through constitutes the most common reason for retear of arthroscopically performed rotator cuff tendon repairs. Age-related alterations of the tendon tissue can additionally lead to a reduction in suture retention strength. Therefore, strategies to improve the structural resistance to a suture complex are of interest. Genipin has shown potential to augment resistance at the suture-tendon interface for a simple stitch pattern [10]. A clinically feasible method for localized and concentrated genipin delivery is missing for possible in vivo preclinical evaluation. We therefore investigated the potential of suture-based delivery of genipin to improve arthroscopic suture retention in an in vitro model where tissue degeneration was induced by collagenase before testing. In our study setting, genipin-coated sutures increased force to failure in healthy and in degenerated tendons.

This study had several limitations. First, ex vivo injection of collagenase to induce collagen breakdown and matrix disorganization was used to simulate degenerative changes found in vivo [4, 7, 14, 18, 28]. Other biologic changes found in tendinopathic specimens such as hypercellularity and increased vascularity, however, are not taken into account [45]. This enzymatic weakening of the tendon was intended to mimic solely the structural features found in degenerated human tendons and address the need to consider age- and tissue quality-associated factors in patients with tendon tears [13, 32, 36, 39, 46]. As such, the collagenase pretreated control (uncoated suture) tendons showed a lower maximum pullout force of -123%, lower stiffness of -106%, and work to failure of -179% compared with the untreated control group. Second, the study was performed in an ex vivo bovine animal model. As a result of potential interspecies differences in tendon properties, caution is warranted when extrapolating these findings to humans. In this ex vivo setting, the biologic response to genipin treatment as well as long-term effects cannot be studied. Third, the mechanical testing protocol does not reflect the regime of high cyclic loading during daily activities. However, during early rehabilitation after rotator cuff repair, one involuntary movement of the arm can overload the suture-tendon interface and lead to suture cut-through [2]. Application of genipin is therefore aimed to increase primary stability of the construct in the context of a single precipitating event leading to a potential retear rather than long-term tissue fatigue.

During arthroscopic surgery, a genipin-coated suture would be exposed to flowing saline. A potential washout of genipin during this period was not investigated and should be the subject of future work. Once the suture is in place, however, fluid flow during arthroscopy at the suture segment of interest would be minimal. We further tested a single loop stitch, which is more practical in an arthroscopic setting than
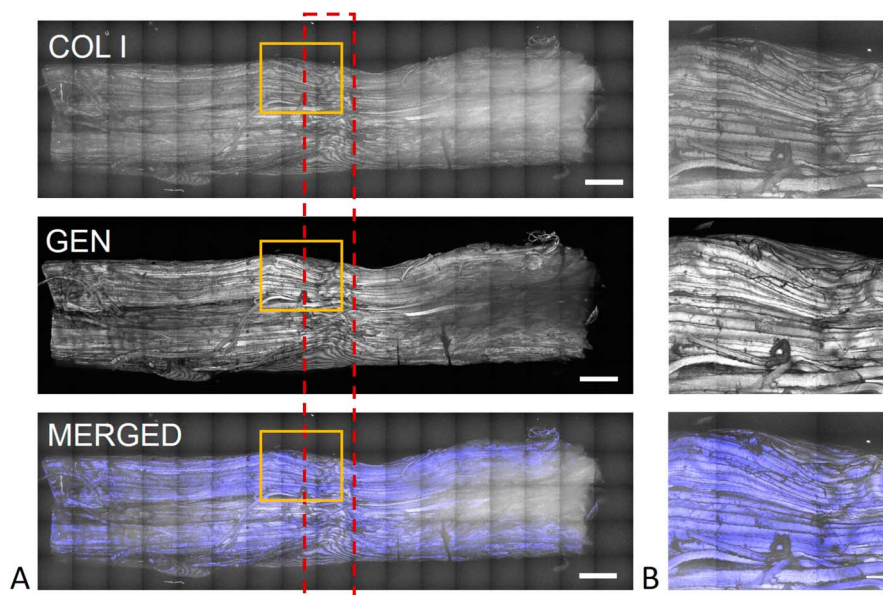
Fig. 6 A-B Confocal imaging of the genipin-induced crosslinking is shown. (**A**) Overall scans of the tendon sample imaged with a spinning-disc confocal microscope are shown. Collagen type I autofluorescence was imaged with a 405-nm laser excitation wavelength (COL I) and genipin-induced crosslinking was imaged with a 560-nm excitation wavelength (GEN). The composite image (MERGED) shows the area with stronger crosslinks in the tissue around the suture (red box). Blue indicates the genipin-induced crosslink signal was arbitrarily chosen. Scale bar = 1.5 mm. (**B**) Enlargements of the area indicated by the yellow box in A are shown. Scale bar = 0.25 mm.

a more complex stitch configuration such as the modified Mason-Allen, which had no relevant additional pullout retention properties in combination with genipin [10].

A recent study showed that exogenous genipin collagen crosslinking could increase median pullout force of a single-loop stitch by > 30% in a highly simplified sheep model of rotator cuff tendon repair [10]. This increase was attained when the healthy ovine infraspinatus tendon was incubated for 24 hours in a relatively high concentration solution of genipin (20 mmol/L)—an approach that results in substantial unintended crosslinking of the surrounding tissue. Using the suture as a delivery vehicle is easily applicable and has been shown to be compatible with genipin use [38]. We refined this approach, demonstrating that it can be potentially relevant for a clinical application to improve mechanical suture performance while minimizing unintended tissue crosslinking.

A major aim of our study was to confirm effective local delivery of genipin crosslinks to the tissue surrounding the suture with a degree of crosslinking thought to be proportional to the effective tensile strength of the tissue [8]. Quantification of the amount of genipin-induced collagen crosslinking was performed using fluorescence measurements at appropriate wavelengths [30, 35, 37]. Genipin-related fluorescence decreased by 23% at a distance of

9 mm from the suture, suggesting reasonable localization of genipin crosslinking. Degenerated tendon tissue showed a lower level of genipin-specific fluorescence compared with healthy tendon with no apparent qualitative difference in distribution characteristics. The altered arrangement and disorganized structure of fibrillary collagen by collagenase treatment may reduce genipin-induced crosslinking. In contrast, genipin-specific absorption generally was increased in the collagenase-treated tissue, possibly attributable to the facilitated polymerization of genipin molecules in higher molecular weight chains with the increased presence of free amino acid ends [30, 35]. Using a confocal microscope for a visual investigation of the tendon, the fluorescent signal emitted by the genipin-induced crosslinking was found to be high with the maximum intensity in the tissue surrounding the suture channel.

The tissue-augmenting effect of genipin in living animals has not yet been fully investigated. However, a recent study showed collagen crosslinking to be highly persistent 1 year after intradermal genipin injection in horses [6]. That study investigated the toxicologic effect of genipin with good tolerability and no adverse effects.

Using an ex vivo animal model, we showed that suitable genipin coating can increase suture retention strength in healthy and degenerated bovine tendon. Fluorescence-

based analysis confirmed localization of genipin-induced crosslinking at the margins of the delivery suture. This study thus provides initial support that application of genipin as a crosslinking agent to sutures may have clinical utility in the arthroscopic repair of degenerated tendons. Future studies might investigate the influence of genipin-augmented sutures in vivo to study the tissue reaction to a genipin-coated suture and to evaluate the effect over time. Followup studies, especially in human tissue with a repair stitch configuration, are needed for evaluation of a genipin-coated suture toward a clinical application.

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