



# In Vitro Collagenase Degradation of Grafts Used Clinically for Anterior Cruciate Ligament Reconstruction: Human Tendon Data

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## Abstract

Increasing anterior cruciate ligament (ACL) rupture rates are driving the need for new graft materials which undergo testing to characterize material properties and function. The in vitro collagenase assay is routinely used to determine the degradation rate of collagenous materials. At times, it is used to screen new biomaterials on the basis that results reflect breakdown rates in vivo. However, its predictive potential is inconclusive with no guidelines for acceptable in vitro rates of degradation. Reference data from target tissue/s or existing clinical materials are needed to determine appropriate thresholds. From a summary of reported protocols, the most common bench conditions (bacterial collagenase; unloaded samples) were used to evaluate the in vitro degradation of human tendons used as ACL allografts: patellar, semitendinosus, gracilis, Achilles, tibialis anterior and posterior. Tendons were sectioned in equal volumes and exposed to 100 U collagenase for 1, 2, 4 or 8 h. The change in dry weight was analysed using mixed linear regression. All tendon samples demonstrated a significant reduction in mass over time but the patellar tendon degraded significantly faster than all other tendons ( $P \leq 0.004$ ). As all tendons used in this study are clinically accepted, this study provides a range of human tendon reference data for comparative assessment of new tendon and ligament biomaterials. However, the more rapid degradation of the patellar tendon, one of the most successful ACL graft materials, also highlights the limitations of common collagenase assay conditions for predicting in vivo performance, particularly in the absence of suitable comparative controls.

**Keywords** Collagen · Graft · ACL · Tissue engineering · Biomaterials · Bench test

## Introduction

Novel biomaterials for tendon and ligament applications range from natural and synthetic polymers, decellularised extracellular matrices and patient-derived constructs [1–3]. A large proportion of biomaterials are predominantly composed of collagen to match the major compositional element of tendons and ligaments (65–80% of tissue dry mass) [4, 5]. Collagen is susceptible to degradation in vivo by a number of different enzymes including matrix metalloproteinases designated as collagenases [6]. These enzymes participate in the natural turnover of tendons, ligaments and other tissues [7], and play an active role in the integration of graft materials which often include periods of inflammation, cellular proliferation, revascularization and remodelling and maturation [8]. In vivo resorption and remodelling affect the mechanical strength of graft materials which is a critical factor for load-bearing applications. As such, graft materials need to maintain sufficient structural integrity while

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the processes of cellular penetration, vascularization, and neogenesis occur. Ideally, the rate of material resorption matches the rate of tissue repair/integration. Thus, degradation characteristics and breakdown rates are key considerations in the development of tendon and ligament biomaterials, particularly after processes such as decellularization, crosslinking and sterilization which can alter the overall structure, mechanical strength and degradation susceptibility of materials [9].

High-throughput evaluation of materials *in vivo* is costly, time consuming, and ethically against safety practices and aims to replace, reduce, and refine the use of animals. *In vitro* tests are, therefore, critical tools for early-stage screening of new materials. The collagenase assay is commonly used as an *in vitro* test of the enzymatic susceptibility of materials. It is listed as an outcome measure in several standards of the American Society for Testing and Materials (ASTM International):

- Collagenase susceptibility, ASTM F3354 Standard Guide for Evaluating Extracellular Matrix Decellularization Processes [10];
- Collagenase resistance, ASTM F2212 Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs) [11];
- Collagenase degradability, ASTM F3089 Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions [12]

While the collagenase assay may be a useful method for characterizing material behaviour, the standards do not establish guidelines for use of the collagenase assay as an *in vitro* screening tool that is predictive of *in vivo* performance. As such, there are significant variations in reported protocols across the literature (Table 1) including the specific collagenase used (origin), concentration, starting material size, and duration of exposure (digestion time), and appropriate controls or acceptance criteria have not been defined. The existing evidence for the predictive potential of the collagenase test is limited and conflicting [13–18]. In the absence of a direct correlative study of *in vitro* and *in vivo* results, comparative reference data may be determined by evaluating the *in vitro* degradation of the target tissue or materials in current clinical use with known performance outcomes *in vivo*. Additionally, reference datasets are of particular use where access to comparative materials may be limited and/or cost prohibitive.

For tendon and ligament applications, human tendons used as autografts or allografts with well-established clinical outcomes are an ideal starting point. Reconstructions of the anterior cruciate ligament (ACL) routinely use various

human autograft and allograft tendons and is an area of growing demand with increasing numbers of reconstructions being performed globally. For example, the annual incidence of reconstructions increased 54.8% in Italy between 2001 and 2015 [38], 43% in Australia between 2000 and 2015 [39], and 22% in the US between 2002 and 2014 [40]. Tendon autografts are the current gold standard in which the most common are a bone-patellar tendon-bone (BPTB) graft or hamstring tendons (semitendinosus and gracilis) [41]. Allograft tendons also include the tibialis anterior/posterior tendons and the Achilles tendon [42]. The insufficiencies and limitations of autografts and allografts, and the increasing rates of both primary ACL ruptures and re-ruptures are driving research into the development of suitable alternatives. For novel biomaterials designed to replace the ACL, an acceptable range of *in vitro* degradation rates may be determined by assessment of human tendons used as autografts and allografts. Therefore, the aim of this study was to evaluate and compare the *in vitro* collagenase degradation rates of human patellar, semitendinosus, gracilis, Achilles, and tibialis anterior and posterior tendons, using some of the most frequently reported test conditions (bacterial collagenase and unloaded, stress-deprived samples) to establish a comparative reference dataset.

## Materials and Methods

### Tissue Collection

All protocols in this study were approved by the Northern Sydney Local Health District Human Research Ethics Committee. Informed consent was obtained from all donors or next of kin. Tendons were retrieved from 6 fresh-frozen human cadavers (3 male, 3 female; 49–62 years old). Donors had no documented history of musculoskeletal injury or disease, and no surgery performed on the lower limbs. Experimental samples were obtained opportunistically alongside samples used for other research studies. For this experiment, a small portion of tissue was collected from the mid-tendon of the patellar, semitendinosus, gracilis, Achilles, tibialis anterior, and tibialis posterior tendons which represent tendons used as graft materials for ACL reconstruction [41, 42]. Quadriceps tendons were not evaluated in this study despite their increasing use for ACL reconstruction.

### Collagenase Digestion

The collected tissue segment was sectioned into 10 mm × 2 mm × 2 mm volumes. Samples from the same donor tendon were collected and allocated to 1 of 4 digestion timepoints: 1, 2, 4, or 8 h ( $n = 4\text{--}6$  samples/tendon/time point). There were no samples for the gracilis tendon

**Table 1** Summary of varying collagenase assays used to assess collagen-based materials

Test Sample	Control Sample	Collagenase Origin	Collagenase Concentration	Sample Size	Digestion Time (h)	Ref
Human acellular dermal matrix	Native human acellular dermal matrix	<i>Clostridium histolyticum</i> (Type I)	100 U/mL	~ 100 mg	0–16	[13]
Commercial scaffolds	Porcine dermis and bovine pericardium matrix	<i>Clostridium histolyticum</i> (Type I)	100 U/mL	60–80 mg	0–16	[14]
Cross-linked decellularized bovine tendon-derived scaffolds	Non-cross-linked decellularized bovine tendon-derived scaffolds	<i>Clostridium histolyticum</i>	1 mg/mL (10–12 CDU/mg)	10–12 mg (~ 20 mm x 5 mm)	0–96	[15] <sup>a</sup>
Sterilized reconstituted scaffold	Non-sterilized reconstituted scaffold	Not specified	250 µg/mL	8 mm diameter punch, 7 mm thickness	0–504	[16]
Cross-linked decellularized bovine tendon-derived scaffolds	–	<i>Clostridium histolyticum</i>	3.5 U/cc (0.008 mg/cc)	0.032 mm × 1.3 mm tape, 3 cm long	–	[17] <sup>c</sup>
Irradiated/cross-linked kangaroo tail tendon	Non-cross-linked or fresh-frozen kangaroo tendon	<i>Clostridium histolyticum</i>	0.18 U/mL	20 mm long (~ 3 mm <sup>2</sup> diameter)	0–240	[18, 19] <sup>d</sup>
Various biological scaffolds	Porcine small intestine sub-mucosa	<i>Clostridium histolyticum</i> (Type I)	20 U/mL	“Dog bone” 1.0 cm × 6 cm (mid: ~ 0.4 cm × 1.5 cm)	0–30	[20]
Decellularized bovine pericardium/dermis	–	<i>Clostridium histolyticum</i>	2.5 mg/mL	1 cm × 1 cm	0–120	[21]
Decellularized bovine pericardium	Sterilized bovine pericardium	<i>Clostridium histolyticum</i>	0.1 mg/mL	1 cm × 1 cm	0–200	[22]
Cross-linked dermis tissue grafts	Non-chemically cross-linked collagen sponge	<i>Clostridium histolyticum</i> (Type I, Type II)	50, 100, 200 U/mL	~ 0.05 mg	0–24	[23] <sup>b</sup>
Devitalized rabbit tendon (loaded under tension)	Devitalized rabbit tendon (unloaded)	<i>Clostridium histolyticum</i> (Type VII)	60 U/mL	Entire specimen (~ 12 mm <sup>2</sup> area, 22 mm length)	20	[24] <sup>c</sup>
Cross-linked dermal sheep collagen	Non-cross-linked dermal sheep collagen	<i>Clostridium histolyticum</i> (Type I)	100 U/mL (10 U/mg)	10 mg	0–25	[25]
Cross-linked bovine ventral pericardium	Untreated bovine pericardium	<i>Clostridium histolyticum</i> (Type I)	5 mg/mL	20–30 mg	0–18	[26]
Decellularized porcine tendon	Untreated porcine tendon	<i>Clostridium histolyticum</i>	100 U/mL	5 mg	0–24	[27]
Commercial scaffolds	–	<i>Clostridium histolyticum</i>	100 U/mL	5 mg	0–24	[28]
Irradiated AlloDerm	AlloDerm	<i>Clostridium histolyticum</i> (Type I)	~ 0.4 mg/mL	20–30 mg	0–7	[29] <sup>a</sup>
Disinfected skin biopsy	Cross-linked skin	<i>Clostridium histolyticum</i> (Collagenase A)	200 U/mL	5 mm diameter biopsy	0–4	[30] <sup>a</sup>
Cross-linked collagen gels	Glutaraldehyde-cross-linked collagen	<i>Clostridium histolyticum</i> (Type I)	200 U	5 mg	0–24	[31]
Cross-linked porous collagen scaffold	Uncross-linked scaffold	Collagenase (Type I)	278 U/mg	Not specified	0–12	[32]
Mammalian Collagen Type I	–	<i>Clostridium histolyticum</i> (Type II)	50 U/mL	~ 5 mg	0–24	[33] <sup>a</sup>

**Table 1** (continued)

Test Sample	Control Sample	Collagenase Origin	Collagenase Concentration	Sample Size	Digestion Time (h)	Ref
Sterilized and cross-linked bovine tendon-derived scaffolds	Non-cross-linked bovine tendon-derived scaffold	<i>Clostridium histolyticum</i> (Type IV)	10 U/mL	Not specified	0–24	[34]
Commercial porcine collagen matrices	–	<i>Clostridium histolyticum</i> (Type V)	2 IU/mL	10 mm x 10 mm	0–1200	[35] <sup>d</sup>
Decellularized murine annulus fibrosus	–	Recombinant human MMP-1	100 ng/mL	Spinal motion segment	0–24	[36] <sup>e</sup>
Modified murine-derived collagen fibrils/fibres	Native murine-derived collagen fibrils/fibres	Recombinant MMP-1, MMP-8, MMP-13	400 nM, 1 µM	0.6 mg/mL, 1 mg	2, 4 or overnight	[37]

Digestion with agitation<sup>a</sup>, static<sup>b</sup>, samples under load<sup>c</sup>, or with collagenase replenished over time<sup>d</sup>. MMP = matrix metalloproteinase

at 1 h and only  $n=5$  at 2 and 8 h. Only  $n=4$ , samples were obtained for the semitendinosus tendon at 1 h.

Samples were dehydrated using a vacuum centrifuge for 2 h. Dry tissue mass was recorded, and samples were rehydrated in 1 mL buffer solution containing 0.1 M Tris buffer and 0.05 M calcium chloride (pH 7.4). Samples were incubated at 37 °C for 1 h. 100 U of collagenase (*Clostridium histolyticum*, Type I-A, Sigma Aldrich, #C2674) was added to each sample with a further 1 mL buffer solution (final concentration: 50 U/mL; 0.4 mg/mL). Samples were returned to 37 °C on a 3D-printed rotator [43] allowing for constant agitation. After incubation, 200 µL of 0.25 M EDTA was added and samples placed on ice for 10 min to halt the reaction. Samples were centrifuged at 3000 g for 20 min and the supernatant removed. The final dry weight was recorded after another 2 h of dehydration.

### Statistical Analysis

The change in dry weight after collagenase digestion was evaluated using mixed model linear regression (Stata/SE 15.1, StataCorp LP, College Station, TX), accounting for tendons obtained from the same donor, and digestion time, sex, and initial dry weight as covariates. Donor age was not included in the final model due to the narrow age range and absence of a significant effect. The Benjamini–Hochberg procedure was used to adjust for multiple comparisons between tendons with a 5% false discovery rate.

### Results

All tendons demonstrated a reduction in tissue mass over time (Fig. 1). At 1 h, the average loss in mass was greatest in the patellar tendon, losing over 25% of its initial mass compared to

all other tendons which retained more than 75% of their initial mass. After 4 h, the patellar tendon continued to degrade more rapidly, losing 77.2% of its mass compared to less than 46.89% for all other tendons.

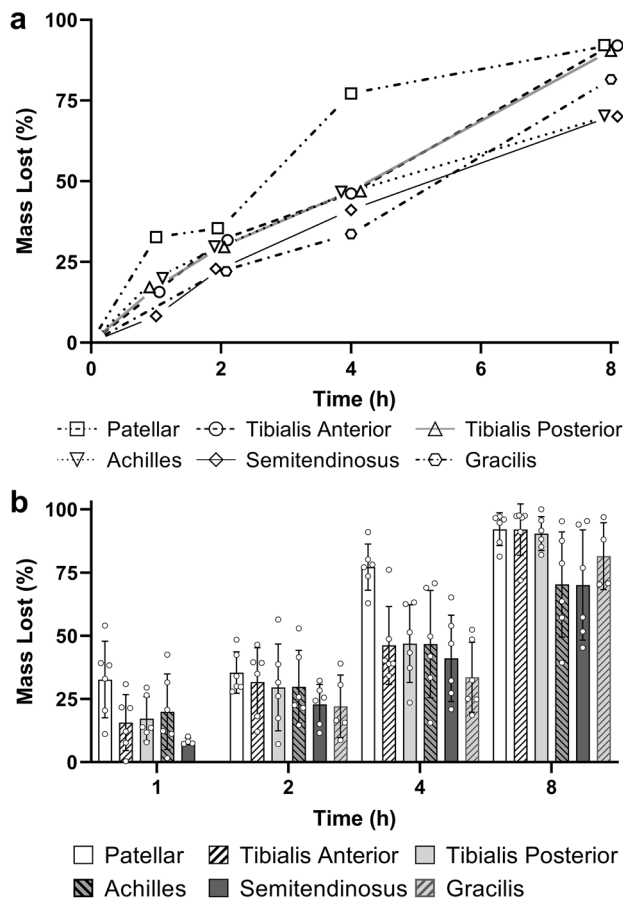
By 8 h, the average percentage loss was greater than 90.45% for patellar, tibialis anterior, and tibialis posterior tendons. Gracilis, Achilles, and semitendinosus tendons were reduced by 81.53%, 70.31%, and 70.08%, respectively. Overall, change in mass was significantly greater in the patellar tendon compared to all other tendons ( $P \leq 0.004$ , Table 2). Tissue loss was also significantly higher in tibialis posterior compared with semitendinosus ( $P \leq 0.0128$ , Table 2).

### Discussion

Under the conditions of this study, all tendons tested were found to be susceptible to collagenase-mediated digestion. On average, the tendons most commonly used for auto- and allograft ACL reconstruction represented both the most (patellar) and the least (hamstrings: semitendinosus and gracilis) degraded tendons. As both BPTB and hamstring tendons remain the most clinically accepted and used graft materials, the results of this study may provide a useful benchmark and acceptable range for the assessment of alternative ACL graft materials under similar conditions. However, interpretation and use of these results requires careful consideration as collagenase degradation alone may not provide a clear indication of graft success in vivo.

### Using the Collagenase Assay as an Indicator of Performance

The collagenase assay is used in vitro to simulate and characterize a key process involved in graft remodelling in vivo,



**Fig. 1** Tissue mass lost during the collagenase assay for individual tendon types. Mass lost is expressed as the percentage change between the initial and final dry weights. **a** Mean tissue mass lost for each tendon type: □, square=patellar; ○, circle=tibialis anterior; △, up pointing triangle=tibialis posterior; ▽, down-pointing triangle=Achilles; ◇, diamond=semitendinosus; ⬡, hexagon=gracilis. **b** mean ± standard deviation of tissue mass lost for each tendon type. White bar=patellar; black and white diagonal lines=tibialis anterior; light grey=tibialis posterior; black and grey diagonal lines=Achilles; dark grey=semitendinosus; light and dark grey diagonal lines=gracilis

specifically enzymatic material breakdown. Collagenase degradation is often associated with a loss of mechanical strength [20] and as such, a more slowly degrading material is typically considered to be more advantageous/mechanically stable which is an important factor for load-bearing applications. In isolation, the results indicate that the patellar tendon is most susceptible to enzymatic degradation, and it may be subsequently inferred that the patellar tendon would lose mechanical strength more rapidly compared with hamstring tendons in vivo. However, clinical studies report lower re-injury rates following BPTB ACL reconstructions compared with hamstring tendon grafts [44–48]. These findings were confirmed in a meta-analysis by Samuelsen et al. [49], although the meta-analysis by Gabler et al. reported

no significant differences in ACL graft failure rates between the two donor tendons [50]. In this context, a faster in vitro degradation rate is not necessarily predictive of risk of ACL graft failure. Additionally, there are known differences in the baseline mechanical properties of different tendons [51], and a limitation of the present study is an absence of correlated changes in mechanical properties with collagenase digestion. It may be that the expected initial loss of mechanical strength in vivo [52] could be sufficiently overcome through the selection of a material with either a higher starting strength or a lower loss of mechanical properties with collagenase digestion.

Remodelling of the graft is also an important part of the ligamentization process, facilitating integration and/or replacement with host tissue. Inadequate integration and maturation of the implanted material can affect fixation of the graft and may delay restoration of mechanical properties [52]. Again, comparing the tendons with the fastest (patellar) and slowest (hamstring) collagenase degradation results, it has been reported that BPTB grafts have a slightly faster maturation rate compared with hamstring tendons [53, 54] which may indicate a faster restoration of mechanical properties. In this context, the greater susceptibility of the patellar tendon to enzymatic degradation may be more beneficial.

Suitable control or reference materials are essential for the interpretation of in vitro results, particularly in the absence of established acceptance criteria. This is the first study to evaluate the collagenase susceptibility of gold-standard ACL graft materials (human tendons) and demonstrates a range of acceptable in vitro degradation profiles for comparisons with new materials for ACL reconstruction. As noted previously, however, the rates of degradation and remodelling need to be carefully aligned in order to prevent early failure. The use of an in vitro collagenase assay to predict in vivo performance is a difficult undertaking given the exclusion of other catabolic and anabolic processes. Furthermore, the in vivo maturation process of an ACL graft can be active for up to 3 years post-surgery [8], potentially remains incomplete for up to 9–10 years [55–57], and occurs more rapidly in animal models versus humans [53]. There have been several studies which have evaluated both in vitro and in vivo breakdown/resorption characteristics of biomaterials in different settings, but the outcomes are not always consistent. A good association between collagenase digestion and in vivo resorption is typically observed when comparing cross-linked versus non-cross-linked materials [15, 17, 18] and has also been demonstrated when comparing diverse materials such as porcine pericardium versus dermis [21]. In contrast, other studies evaluating sterilization effects have shown somewhat opposite outcomes with the fastest degrading in vitro materials having slightly better resistance to in vivo resorption [16] or retention of mechanical strength [13], although these were not always significant.



**Table 2** Statistical analysis using mixed model linear regression

Variable	$\beta$	95% CI	P
<i>Covariates</i>			
Starting Dry Weight	0.49	[0.41, 0.58]	<0.0001 <sup>a</sup>
Sex (Male vs Female)	1.39	[− 1.25, 4.03]	0.3006
Digestion Time (h)	2.29	[2.02, 2.56]	<0.0001 <sup>a</sup>
<i>Pairwise Comparisons</i>			
Semitendinosus vs Patellar	− 6.62	[− 9.10, − 4.15]	<0.0001 <sup>b</sup>
Gracilis vs Patellar	− 5.80	[− 8.58, − 3.02]	<0.0001 <sup>b</sup>
Achilles vs Patellar	− 4.36	[− 6.85, − 1.87]	0.0006 <sup>b</sup>
Tibialis Anterior vs Patellar	− 3.48	[− 5.85, − 1.11]	0.0040 <sup>b</sup>
Tibialis Posterior vs Patellar	− 4.21	[− 6.64, − 1.78]	0.0007 <sup>b</sup>
Gracilis vs Semitendinosus	0.82	[− 1.99, 3.63]	0.5663
Achilles vs Semitendinosus	2.26	[− 0.42, 4.95]	0.0989
Tibialis Anterior vs Semitendinosus	3.15	[0.67, 5.62]	0.0128 <sup>b</sup>
Tibialis Posterior vs Semitendinosus	2.41	[− 0.06, 4.88]	0.0557
Achilles vs Gracilis	1.44	[− 1.60, 4.48]	0.3531
Tibialis Anterior vs Gracilis	2.32	[− 0.45, 5.09]	0.1006
Tibialis Posterior vs Gracilis	1.59	[− 1.13, 4.31]	0.2527
Tibialis Anterior vs Achilles	0.88	[− 1.61, 3.38]	0.4878
Tibialis Posterior vs Achilles	0.15	[− 2.55, 2.85]	0.9137
Tibialis Posterior vs Tibialis Anterior	− 0.73	[− 3.15, 1.68]	0.5519

$\beta$  = regression coefficient; CI = confidence interval

<sup>a</sup>Significant effect ( $P < 0.05$ ); <sup>b</sup>Significant effect after Benjamini–Hochberg adjustment for multiple comparisons (5% FDR)

The limitations of using the in vitro collagenase assay to predict the in vivo response is clearly demonstrated in the study by Sun et al. [14] Two of the commercial products compared (XenMatrix and Stratrice) were both non-cross-linked materials derived from porcine dermis and processed using proprietary methods. In vitro XenMatrix had a significantly greater susceptibility to collagenase digestion compared with Stratrice. However, the opposite occurred in vivo with XenMatrix demonstrating slow degradation, poor integration and a significant and sustained inflammatory response. Poor host integration in vivo can also occur in materials which have demonstrated strong resistance to in vitro degradation through the use of chemically induced cross-links [22, 58]. Thus, while the collagenase assay may be a useful method for determining the breakdown characteristics of different materials in vitro, in isolation, it cannot provide a definitive indication of a material's performance in vivo. The assay should be conducted alongside other biocompatibility tests to better reflect the complexity of the in vivo environment and host response.

### Assay Considerations

While this study was performed under commonly reported test conditions, interpreting the results of the collagenase assay needs careful consideration of the parameters of the

test (e.g. Table 1) and the intended application of the material. A key factor often highlighted in in vitro studies is the use of bacterial collagenase which targets different cleavage sites compared with endogenous enzymes, may not represent all of the proteases present, and is used in higher concentrations than what would be present in vivo [16, 23, 59]. A further limitation of the present and published studies is the incubation of tendon samples in a stress-deprived state despite ACL reconstructions being a load-bearing application. Studies have shown that collagen under tension [24] is more resistant to enzymatic breakdown due to configuration changes altering the exposure of cleavage sites [59]. This would suggest that under tension, the human tendons would have degraded at a slower rate. Conversely, processed and manufactured materials may have specific characteristics that respond differently to load and strain. For example, Bourne et al. found that glycosylated tendon fibres were instead more susceptible to degradation when loaded versus stress deprived [60]. This highlights the importance of testing samples in conditions that are relevant to the intended application. The current study, in contrast to many others, was also conducted under moderate agitation which simulates a dynamic environment and contributes to mechanical disruption of the tissue as it is exposed to collagenase. Lastly, differences in sample dimensions/size may affect comparisons between material degradation rates. This study attempted

to mitigate any indirect effects by trimming all samples to similar dimensions; however, the starting dry mass remained variable (Online Resource Fig. S1) and was found to have a significant effect on the final percentage change in mass (Table 2). The data were reviewed to determine if the initial wet tissue mass could be used to assist sample standardization and a significant positive correlation was found when evaluating all tendons ( $r=0.83$ ,  $P<0.0001$ ; Fig. S1a). Closer inspection of individual tendon types using predictive margins calculated from a mixed linear regression model demonstrated different relationships for each individual tendon, although ~50 mg wet tissue weight may produce the most consistent dry weight across the tendons tested in this study (Fig. S1b). However, this particular relationship between sample wet weight and dry weight is likely to be highly specific to the current study using unprocessed tendons from mid-aged donors.

In addition to experimental factors, other tendon- or material-specific variables should be taken into consideration to assist the interpretation of results. For example, within the present study, collagenase susceptibility may be influenced by differences in tendon ultrastructure (e.g. collagen fibril diameters and packing densities) or native tissue cross-links which can vary with tissue origin and age [61–64]. The greater susceptibility of the patellar tendons to enzymatic degradation may be due to a lower number of collagen fibrils and greater spacing between fibrils (reported as a lower fibril:interstitium ratio) when compared with other tendons such as the more slowly degrading hamstring tendons [56, 57, 61]. Another study also appears to demonstrate that tibialis anterior tendons, which were 92% degraded at 8 h, have greater spacing between collagen fibrils when compared with Achilles tendons which were only 70% degraded at 8 h [65]. This spacing (and cross-links) between fibrils and fibres likely affects fluid flow throughout the sample and the overall area exposed to collagenase, which when combined with the dynamic motion of the experimental setup, results in a more rapid structural disintegration (i.e. as the tissue separates, there is greater exposure to the enzyme and thus more rapid digestion). Additional comparative studies of variations in tendon ultrastructure and composition are needed to provide further insight.

## Conclusions

The collagenase assay is widely used for characterizing a variety of biomaterials in vitro, and at times, the results are used to select materials for further development. As summarized in Table 1, there exists notable variations in published protocols as well as the samples used as comparisons or controls. In the absence of established acceptance criteria, existing materials in clinical use provide a useful comparison

for development of new biomaterials. This study used commonly reported testing conditions to determine the range of in vitro degradation rates of human tendons used for ACL reconstruction and discusses some important considerations for the use and interpretation of the collagenase assay.

## Supporting Information

The correlation of starting wet and dry tissue mass and the predictive margins for different tendons is available as a supplementary figure (Online Resource Fig. S1).

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s44174-022-00046-9>.

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## Declarations

**Conflict of interest** Funding bodies had no role in the design of the study; or in the collection, analyses, or interpretation of data. The authors BV, DMA, PH, SAH, and CLB have no competing interests to declare. Authors ECC, NH, and CBL declare the following potential conflicts of interest: Authors receive research support as principal investigators from Bone Ligament Tendon Pty Ltd (ECC and CBL); Allegra Orthopaedics Ltd (ECC); Ceva Animal Health, Concentric Analgesics Inc, Cynata Therapeutics and Regeneus Ltd (CBL). Authors serve on the boards of the Lincoln Centre for Research into Bone and Joint Diseases (ECC); Bone Ligament Tendon Pty Ltd (NH); and Allegra Orthopaedics Ltd (NH). Authors own stock or stock options in Bone Ligament Tendon Pty Ltd (NH) and Allegra Orthopaedics Ltd (NH).

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