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RESEARCH ARTICLE

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Mechanical performance of collagen gels is dependent on purity, α1/α2 ratio, and telopeptides

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Abstract

This article describes the compositional, mechanical, and structural differences between collagen gels fabricated from different sources and processing methods. Despite extensive use of collagen in the manufacturing of biomaterials and implants, there is little information as to the variation in properties based on collagen source or processing methods. As such, differences in purity and composition may affect gel structure and mechanical performance. Using mass spectrometry, we assessed protein composition of collagen from seven different sources. The mechanics and gelation kinetics of each gel were assessed through oscillatory shear rheology. Scanning electron microscopy enabled visualization of distinct differences in fiber morphology. Mechanics and gelation kinetics differed with source and processing method and were found to correlate with differences in composition. Gels fabricated from telopeptide-containing collagens had higher storage modulus (144 vs. 54 Pa) and faster gelation (251 vs. 734 s) compared to atelocollagens, despite having lower purity (93.4 vs. 99.8%). For telopeptide-containing collagens, as collagen purity increased, storage modulus increased and fiber diameter decreased. As α1/α2 chain ratio increased, fiber diameter increased and gelation slowed. As such, this study provides an examination of the effects of collagen processing on key quality attributes for use of collagen gels in biomedical contexts.

KEYWORDS
atelocollagen, collagen, mass spectrometry, rheology, SEM

1 | INTRODUCTION

Type I collagen constitutes up to 30% of total protein mass in mammals and is the main structural component of mammalian extracellular matrices. Thus, due to its biocompatibility and capacity for remodeling, it is vitally important for model systems investigating cell migration, vascularization, and cell differentiation in the context of cancer, wound healing, and development.[1-4] Moreover, collagen has been widely used in the development of scaffolds for tissue engineering, where anatomically accurate geometries can be tailored to individuals through mold-based fabrication and additive manufacturing.[5] Important applications also include use of collagen as an injectable filler for cosmetic applications.[6-10] In these applications, collagen gels can be engineered with specified stiffness, pore size, and diffusivity through various fabrication and crosslinking techniques.[11] Through the development of these techniques, the gelation, viscosity, mechanics, and nanostructure of collagen gels have been investigated extensively.[11,12]

There is a wide variety in the sources of type I collagen, including anatomic source (skin and tendon), animal source (murine, bovine, porcine, and human), and production method (purification vs. recombinant synthesis).[11-14] Additionally, after collagenous materials are obtained, an additional processing steps may or may not include salt extraction, centrifugation, and enzymatic digestion.[11] As a result of these variations, materials may have significant differences in mechanics and properties such as purity and the ratio of α1 to α2

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chains, despite the fact that they are all derived from type I collagen. Another consequence of this variation is that approaches to fabrication and use of type I collagen are highly empirical. As such, specific protocols can produce reliable results for a given method and source of collagen, but there is a lack of greater understanding as to what attributes are most important for achieving desired mechanical performance.

Little is known about the effect of collagen source (e.g., species and tissue) and processing method on mechanical performance of gels fabricated from these materials. A common processing outcome is the removal of nonhelical regions at the end of collagen molecules, called telopeptides, through pepsin treatment. While collagen maintains its triple helical structure after pepsin treatment, the collagen produced (atelocollagen) has lower viscosity,[15] a decreased rate of fibrillogenesis,[16,17] and decreased denaturation energy.[18] On the microfibrillar scale, collagen's overlap structure necessitates interaction between telopeptide regions with the triple helical region of collagen molecules.[19] As such, telopeptides play a key role in the self-association of tropocollagen into microfibrils.[15] Furthermore, telopeptides account for the majority of crosslinking sites between adjacent collagen molecules, accounting for two thirds of crosslinks found in collagen dimers.[20] Collectively, these data point to an important role for telopeptide-based interactions in determining the mechanics and gelation kinetics of collagen-based gels.

An advantage of processes that remove telopeptides is that they produce very pure collagen gels with potentially lower immunogenicity.[21] However, it is unclear how the purity of collagen gels is related to gel mechanics. Thus, the purpose of this study was to assess critical features of composition in commercial and in-house preparations of collagen gels and determine how these compositional features relate to the mechanical performance. Specifically, we used mass spectrometry to measure collagen purity, identify major impurities, and determine the α_1/α_2 ratio of collagen preparations containing telopeptides (hereby referred to as "Telo") and without telopeptides (aTelo) from multiple manufacturers. We then related these compositional features to storage modulus, loss modulus, dynamic modulus, and gelation kinetics obtained from rheometry, as well as fiber structure obtained from scanning electron microscopy (SEM).

2 | EXPERIMENTAL

2.1 | Materials and methods

2.1.1 | Preparation of stock collagen solutions

Collagen prepared in house and by collaborators at Fesarius Therapeutics was extracted from rat tail tendons as previously described.[22] Briefly, rat tail tendons (BioIVT, Westbury, NY) were solubilized in 0.1% (vol/vol) acetic acid (Sigma, St. Louis, MO) at a concentration of 6.67 g/L at 4°C for a minimum of 48 hr. Solutions were centrifuged at 9,000 rpm (relative centrifugal force of 5,625) for 90 min at 4°C, with lyophilization of the supernatant to yield a collagen sponge. The lyophilized collagen was then reconstituted in 0.1% acetic acid at a concentration of 15 mg/ml.

Collagen extracted from neonatal bovine (Gold Medal Packing, Rome, NY) flexor tendons was prepared following the methods adapted from Zeugolis et al.[23] Briefly, minced tendons were rinsed with 1× phosphate-buffered saline (PBS) (Corning Cellgro, Manassas, VA) before solubilization in 0.5 M acetic acid for 5 days. Insoluble material was removed by filtration through 0.8 μm syringe filters, with subsequent salt precipitation (0.9 M NaCl), centrifugation at 9,000 rpm (relative centrifugal force of 5,625) for 45 min at 4°C, and resuspension in 0.5 M acetic acid for 24 hr. Following three stages of salt precipitation, the collagen solution was dialyzed (8,000 molecular weight cut-off) against 0.01 M acetic acid. The dialyzed solution was lyophilized and reconstituted in 0.1% acetic acid at 8 mg/ml.

Commercial collagen sources with concentration ranging from 5 to 10 mg/ml were obtained directly from Advanced Biomatrix (Carlsbad, CA), Collagen Solutions (Eden Prairie, MN), Corning (Corning, NY), and Koken (Tokyo, Japan). Both pepsin-treated (aTelo) and -untreated (Telo) collagen sources were purchased from each commercial source, where possible. Commercial sources are referred to by their source species and tissue as follows: rat tail tendon (RTC), bovine flexor tendon (BTC), or bovine dermal collagen (BDC).

2.1.2 | Scanning electron microscopy

Hydrogels were prepared from each collagen source by neutralization using a solution of 1 M sodium hydroxide (Avantor, Center Valley, PA), 10X PBS (Corning Cellgro), and deionized (DI) water to a final collagen concentration of 3 mg/ml and osmolarity of 300 mOsm/kg H_2O, as previously described.[22] All solutions were kept on ice before mixing and neutralized to a pH of 7.5 immediately prior to testing.

Gels were deposited in the wells of 24-well plates (Corning, Durham, NC) and incubated at 37°C for 2 hr. Following incubation, gels were fixed in 4% formalin in 1X PBS (Fisher Scientific, Fairlawn, NJ) at room temperature for 1 hr. Subsequently, gels were rinsed three times with 1X PBS (Corning Cellgro) for 10 min and then rinsed twice with DI water for 10 min. Gels were then fixed in 1% osmium tetroxide (OsO_4) (Alfa Aesar, Ward Hill, MA) in DI water for 1 hr. Samples were then dried with a series dilution of water into ethanol (30, 50, 70, 90, and then 100% [×2] ethanol in water) for 10 min each, and ethanol into hexamethyldisilane (HMDS) (Electron Microscopy Sciences, Hatfield, PA) (33, 50, 66, and 100% HMDS in ethanol) for 15 min each.[24] Samples were then dried for 8–12 hr on aluminum foil.

Directly prior to imaging, samples were fixed to 18 mm aluminum specimen mounts with double-sided copper tape and sputter coated with gold/palladium alloy for 20 s at a target current of 20 mA. Samples were then imaged on a TESCAN Mira3 scanning electron microscope at an accelerating voltage of 5 kV and 5 mm working distance. Samples from each collagen source were prepared and imaged in triplicate, with a minimum of three ×5,000 (41.5 μm view field) and ×30,000 (6.92 μm view field) images obtained per sample. Fiber diameter was then calculated manually from representative ×5,000 images

using ImageJ. Three grid lines were placed randomly over the image, and fibers crossing those lines were measured using a straight line across the width of the fiber with the measure function in ImageJ.[24]

2.1.3 | Compositional analysis

Collagen solutions were analyzed for purity and α1/α2 ratio by liquid chromatography–tandem mass spectrometry. Polyacrylamide gels electrophoresis with colloidal Coomassie staining was performed for initial compositional confirmation, with consequent full lane trypsin digestion. Liquid chromatography–tandem mass spectrometry was performed on an Orbitrap Fusion through the Cornell University Institute of Biotechnology, with database search performed using PD2.3 (Thermo Fisher Scientific, Bremen, Germany) with Sequest HT. Database search was performed against the NCBI protein database. The external calibration for all three mass analyzers (fourier transform, ion trap, and quadrupole mass analyzers) was performed using Pierce™ FlexMix™ Calibration solution (Thermo Fisher Scientific). A summary of the full data output has been provided in the Supporting Information. Purity was calculated as the ratio of type I collagen to other protein components on a molar abundance basis.

2.1.4 | Rheological characterization

Rheology was performed on a TA Instruments DHR3 rheometer using a 25-mm parallel plate geometry. Gels were prepared as described above, with neutralization to a pH of 7.5 directly prior to deposition on rheometer geometry. All tests were performed with a geometry gap of 1 mm with sinusoidal oscillation at 0.1 Hz and 0.5% strain for 5 min at 4°C followed by 30 min at 37°C. No-slip boundary conditions were ensured through use of glass cover slips (Neu-Vitro, Vancouver, WA) treated with 1% polyethyleneimine (Sigma) in water for 10 min, followed by 0.1% glutaraldehyde (Sigma) in water for 30 min.[25]

Storage (G') and loss (G'') moduli were recorded every 10 s throughout testing, with initial (G'_0, G''_0) and final (G'_∞, G''_∞) moduli values calculated as averages as the first 100 and last 300 s of testing, respectively. As some samples exhibited higher storage than loss moduli in both their sol and gel states, gelation time (t_g) was calculated as the point of maximum change in storage modulus over time. A custom MATLAB code was used for all rheological data analysis.

2.2 | Statistical analysis

Differences between grouped Telo and aTelo gels were determined by hypothesis testing under a Student's t distribution using Satterthwaite's approximation for degrees of freedom in MATLAB. Correlations were performed using a least squares linear regression with p-values calculated from Snedecor's F distribution with n-k-2 degrees of freedom, where k is the number of groups. Multiple linear regression was performed likewise, with inclusion of additional variables only where analysis of variance between the models favored the more complex model. Trend lines are plotted for significant correlations only, unless otherwise noted. Correlations between aTelo gels only were not plotted. Correlation and linear regression analysis were performed in R Studio.

3 | RESULTS

The majority of collagen sources exhibited similar nanoscale fiber morphology, with randomly aligned fibers forming a network (Figure 1a,b,e–g,i). The surfaces of collagen fibers appeared to be

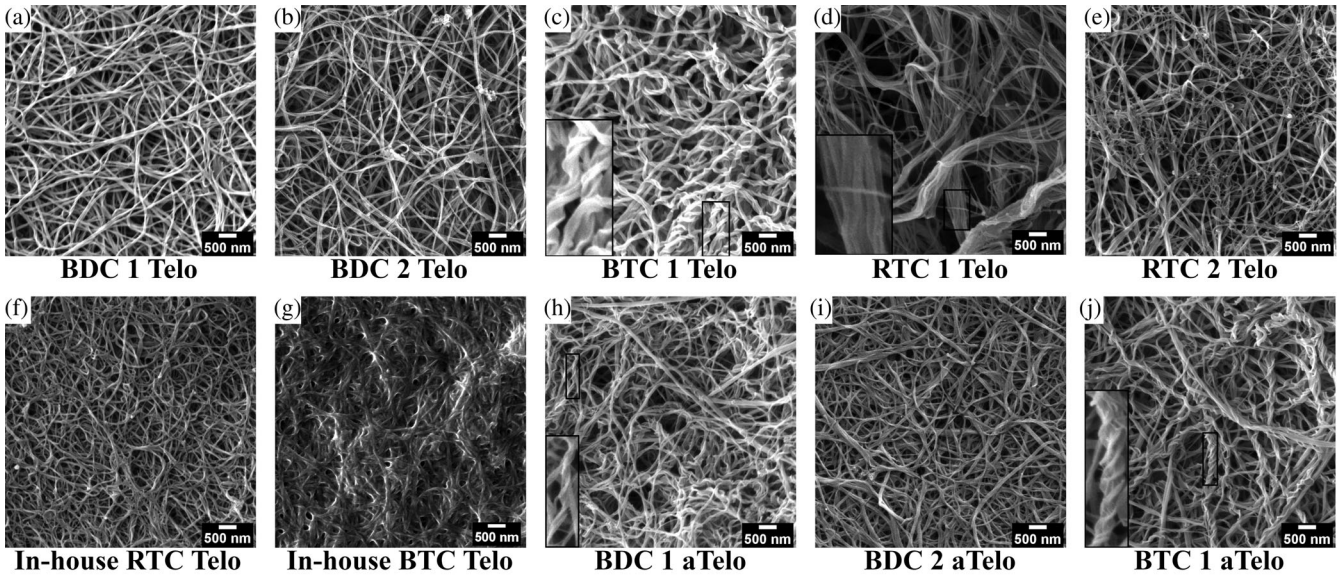

FIGURE 1 (a–j) Representative scanning electron micrographs of each collagen source. Scale bars are 500 nm. (c, h, j) Inset identifies characteristic "wound fiber" morphology. (d) Inset identifies large aligned fiber aggregates

smooth, although this may be due to the gold/palladium sputter coating on samples, which has a thickness of approximately 2–8 nm. The mean fiber diameter was found to be between 40 and 80 nm. While one source, BTC 1 telocollagen (Figure 1c), had a mean fiber diameter of 123 nm, the vast majority of fibers contained two or more smaller constitutive fibers (Figure 1c).

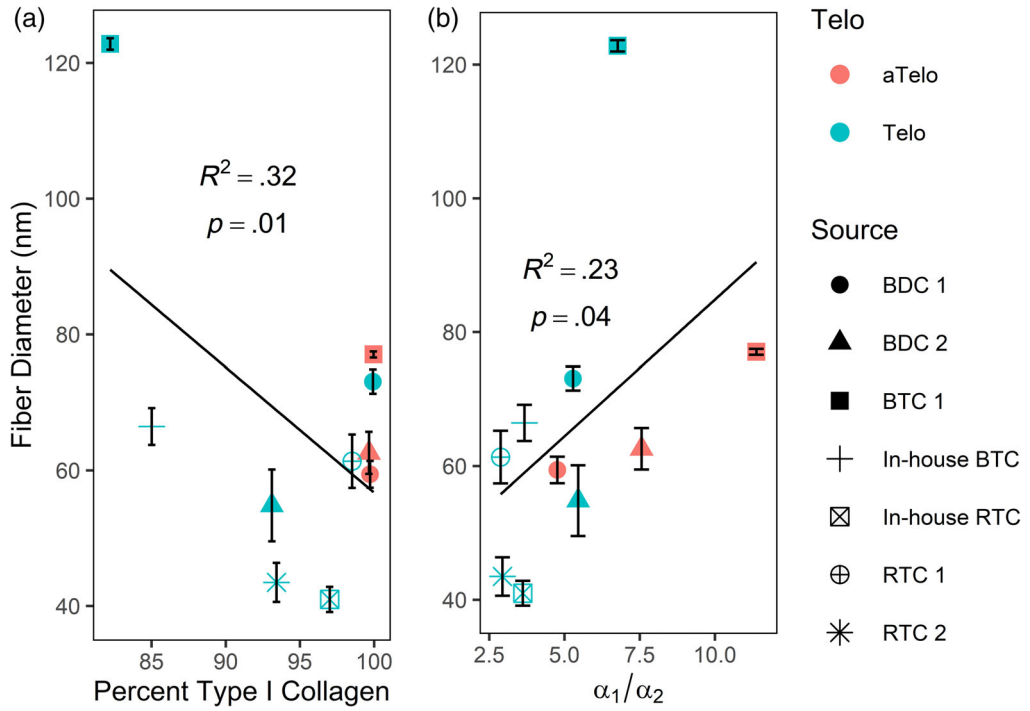

FIGURE 2 Fiber diameter as a function of (a) percent type I collagen and (b) α_1 to α_2 ratio. Shapes correspond to collagen source and color corresponds to the presence of telopeptides. Error bars represent *SD*. Black lines correspond to correlations on both Telo and aTelo sources ($n = 4$ for RTC 1, $n = 2$ for BTC 1 aTelo and in-house BTC, $n = 3$ otherwise)

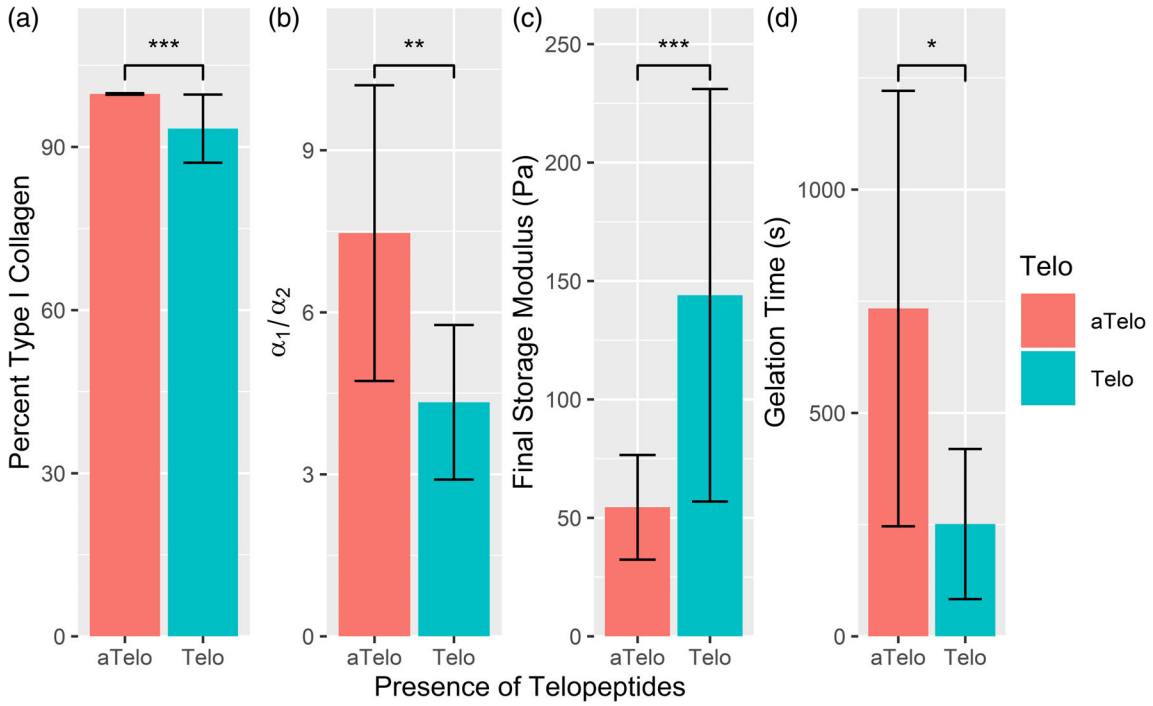

FIGURE 3 Comparison between grouped Telo and aTelo collagen sources based on: (a) percent type I collagen, (b) α_1 to α_2 ratio, (c) final storage modulus, and (d) time of maximum increase in storage modulus with time (dG'/dt). Error bars represent *SD*. *Significant difference with $p < .05$. **Significant difference with $p < .01$. ***Significant difference with $p < .001$ ($n = 21$ Telo, $n = 8$ for aTelo)

TABLE 1 Summary of compositional and rheological analyses

Source information			Compositional analysis				Rheological analysis				
Collagen type	Source tissue	Telo/aTelo	Purity (%)	α_1/α_2	α_1 (%)	α_2 (%)	G'_0 (Pa)	G''_0 (Pa)	G'_∞ (Pa)	G''_∞ (Pa)	t_g (s)
In-house RTC	Rat tail	Telo	97.0	3.6	76.0	21.0	0.77	0.52	160.4	25.4	262
In-house BTC	Bovine tendon	Telo	85.0	3.7	66.8	18.2	0.44	0.38	130.5	12.4	44
RTC 1	Rat tail	Telo	93.5	2.7	68.1	25.4	6.03	2.19	149.8	29.4	86
RTC 2	Rat tail	Telo	93.4	2.9	69.7	23.7	0.83	0.46	137.2	16.6	161
BDC 1	Bovine dermis	Telo	99.9	5.3	84.0	15.9	1.29	0.93	313.8	63.3	275
BDC 2	Bovine dermis	Telo	93.1	5.4	78.7	14.4	0.85	0.47	61.2	14.5	449
BTC 1	Bovine tendon	Telo	82.2	6.8	71.6	10.6	1.99	1.74	48.7	12.3	470
BDC 1	Bovine dermis	aTelo	99.7	4.8	82.4	17.3	0.76	0.50	62.5	14.7	1,178
BDC 2	Bovine dermis	aTelo	99.6	7.6	88.0	11.6	1.16	0.62	68.2	15.5	205
BTC 1	Bovine tendon	aTelo	99.9	11.4	91.9	8.1	1.02	0.52	21.9	5.1	861

Notably, some sources not only had a number of fibers of similar diameter and morphology, but also had a number of smaller fibers winding around those larger fibers. This "wound fiber" morphology was observed in both Telo and aTelo samples, including BTC 1 Telo, BDC 1 aTelo, and BTC 1 aTelo (Figure 1c,h,j). RTC 1 Telo collagen had unique fiber morphology compared to either Telo or aTelo sources. While having single fiber diameter similar to that of other sources, RTC 1 collagen also had large, aligned fiber aggregates (Figure 1d).

Fiber diameter was not different between Telo and aTelo gels but showed a weak negative correlation with purity ($R^2 = .32$, $p = .01$) (Figure 2a) and weak positive correlation with $\alpha1/\alpha2$ ratio ($R^2 = .23$, $p = .04$) (Figure 2b). Collectively, a multiple linear model showed a moderate correlation between diameter, purity, and $\alpha1/\alpha2$ ratio ($R^2_{adj.} = .56$, $p < .001$).

Telocollagen sources were less pure (93–99%) compared to those without telopeptides (>99.5%, $p < .001$) (Figure 3a) and had lower α_1/α_2 ratio (2.4–5.3 vs. 4.8–11.4, $p = .007$) (Figure 3b). Telo gels had higher final storage modulus compared to aTelo gels ($p < .001$) (Figure 3c), despite having lower purity and $\alpha1/\alpha2$ ratio. Likewise, Telo gels had higher initial loss modulus (0.3–5.7 Pa) than aTelo gels (0.31–0.69, $p = .044$) (Table 1, G'_0). Telo gels also gelled more quickly (60–583 s) than aTelo gels (40–1,318 s, $p = .0133$) (Figure 3d).

Protein impurities in each source were also determined in our compositional analysis. Impurities including albumin, annexin, histone, and decorin were found in multiple collagen sources (Table 2). However, each collagen source had unique overall composition of non-collagen protein components, with Telo sources having a greater number and fraction of impurities than aTelo sources overall. No relationship was found between storage or loss modulus and the presence of collagen-bound peptides, such as decorin, or structural proteins, such as keratin or any other impurities.

Storage modulus in Telo gels was found to increase with purity ($R^2 = .42$, $p = .012$), while aTelo gels did not exhibit increased final storage modulus with increasing purity, as aTelo gels were invariably quite pure (99.75 ± 0.13% collagen) (Figure 4a). While the correlation was not strong ($R^2 = .20$, $p = .056$), Telo and aTelo gels appeared to decrease in final storage modulus with increasing $\alpha1/\alpha2$ ratio (Figure 4b). Multiple linear regression analysis of final storage modulus as a function of purity, $\alpha1/\alpha2$ ratio, and the presence of telopeptides yielded a reasonably good fit ($R^2_{adj.} = .493$, $p = .003$), but found no evidence for inclusion of $\alpha1/\alpha2$ ratio in the model ($p = .968$) ($R^2_{adj.} = .512$, $p < .001$ for $G'_{(\infty)} \sim$ purity + Telo). In short, the improvement in model fit provided by inclusion of the $\alpha1/\alpha2$ term was not found to be statistically justifiable, suggesting that purity and the presence of telopeptides are sufficient to describe changes in storage modulus (Table 3).

Purity did not appear to have any effect on the gelation speed of Telo ($p = .26$) or aTelo ($p = .49$) gels (Figure 5a). Gelation time was however found to have a positive correlation with $\alpha1/\alpha2$ ratio ($R^2 = .66$, $p < .001$) in Telo gels, while no such relation was found for aTelo gels ($R^2 = .087$, $p = .63$) (Figure 5b).

4 | DISCUSSION

Sampling a variety of collagen sources revealed large compositional differences, with a high range of purity (93–99%) and $\alpha1/\alpha2$ ratio (2.7–11.4). These differences revealed key predictors of structure and mechanical performance, with purity correlating with fiber diameter and final storage modulus, and $\alpha1/\alpha2$ ratio correlating with fiber diameter and gelation time in Telo gels. The removal of telopeptides was found to significantly compromise performance, with a more than threefold decrease in average storage modulus, and a nearly threefold increase in average gelation time. For Telo gels, purity was found to be a good predictor of storage modulus ($R^2 = .42$), while $\alpha1/\alpha2$ ratio was a better predictor of gelation time ($R^2 = .66$). For Telo and aTelo gels, fiber diameter was found to be weakly correlated with both purity ($R^2 = .32$) and $\alpha1/\alpha2$ ratio ($R^2 = .23$).

This study found a significant increase in purity going from Telo to aTelo collagen sources, with aTelo sources having a minimum purity of 99.5% (compared to 82% for Telo sources). This increase in purity is likely due to the additional processing aTelo sources undergo

TABLE 2 Rank list of identified contaminants. Bolded contaminants constituted greater than 1% (mol/mol) of the mixture. Orange font color denotes blood associated proteins, while blue denotes collagen-bound proteins

Collagen type	In-house RTC	In-house BTC	RTC 1	RTC 2	BDC 1	BDC 2	BTC 1	BDC 1	BDC 2	BTC 1
Telo/ aTelo	Telo	Telo	Telo	Telo	Telo	Telo	Telo	aTelo	aTelo	aTelo
Ranked list 1	**Albumin**	**Vimentin**	**Albumin**	**Decorin**	Annexin	**Histone**	**Annexin**	Keratin 6A	Lysozyme	Junction plakoglobin
2	Collagen VI, α3	**Fibromodulin**	**Hemoglobin**	**Fibromodulin**	Lysozyme	**Vimentin**	**Histone**	Junction plakoglobin	Keratin 6A	
3	Decorin	**Annexin**	Decorin	Keratin 1	GAPDH	Decorin	**Allergen Bos d 2 precursor**		Junction plakoglobin	
4	Serotransferrin	Keratin	Creatine kinase	Keratin 6A	Actin	Allergen Bos d 2	**IGLL1**		Annexin	
5	Hemoglobin	Keratin II	Fibromodulin	POTEF	Procollagen C-endopeptidase	Mimecan	Decorin		GAPDH	
6	PEDF	Actin	Serotransferrin	PEDF	Junction plakoglobin	Keratin 6A	Mimecan		Peroxiredoxin	
7	Collagen VI, α1	Biglycan	Myosin	Keratin 10	Vimentin	Annexin	Vimentin			
8	GAPDH	Decorin	Tetranectin	Albumin		SRPX	MBL1			
9	Creatine kinase	Serpin F1	Pre- anionic trypsin-1	Keratin 19		Odorant-binding protein	SRPX			
10	Collagen XII, α1	Fibulin-5	Parvalbumin alpha	Keratin 42		MBL1	Keratin 6A			

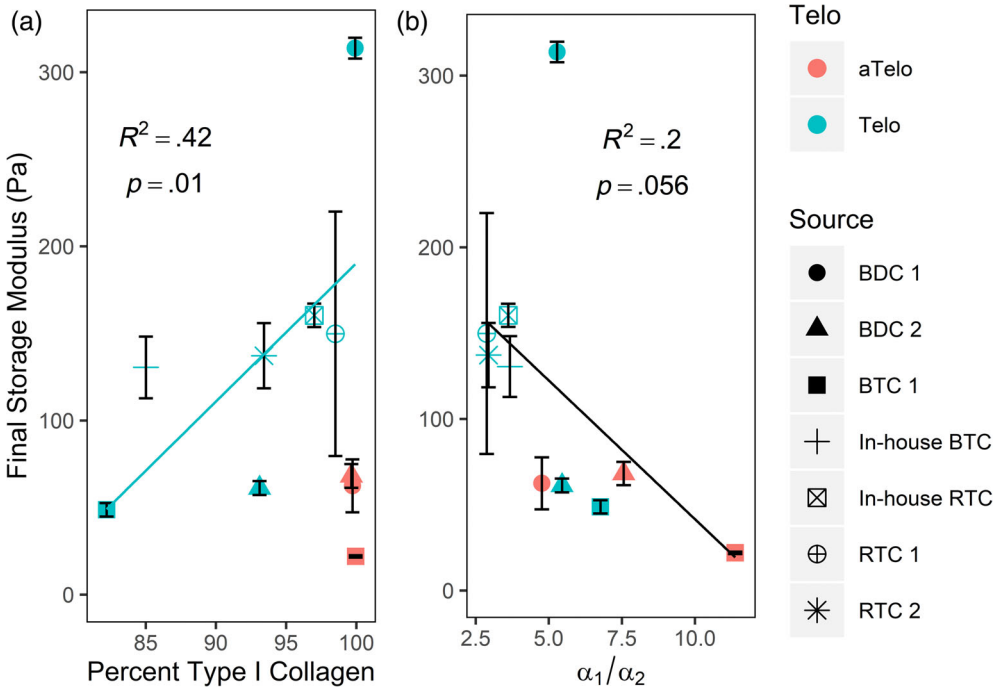

FIGURE 4 Final storage modulus as a function of (a) percent type I collagen and (b) α_1 to α_2 ratio. Shape corresponds to collagen source and color corresponds to the presence of telopeptides. Error bars represent *SD*. Blue line corresponds to correlation on Telo sources only. Black line corresponds to (nonsignificant) correlation on both Telo and aTelo sources ($n = 4$ for RTC 1, $n = 2$ for BTC 1 aTelo and in-house BTC, $n = 3$ otherwise)

to remove their telopeptides. The removal of telopeptides through pepsin digestion is generally accompanied by at least one additional stage of purification, which is generally not matched in the production of telocollagen. Indeed, many of the contaminants seen in Telo collagen, such as annexin, histone, and hemoglobin, are of much smaller molecular weight than collagen α1 and α2 helices, and as such would be removed to a greater degree with centrifugation. Other proteins, including albumin, vimentin, and fibromodulin, would be separated in acid–salt precipitation stages due to their acidic isoelectric points. Pepsin digestion and additional purification stages may also affect collagen α1 and α2 helices differently, contributing to significantly increased α1/α2 ratio in aTelo collagen sources.

It has been shown by multiple studies that Telo collagen sources are more immunogenic than aTelo sources, but most of these studies do not control for the increased purification of atelocollagen.[26] In this study, we show that the increased processing associated with the removal of telopeptides also removes several protein impurities. Many of these impurities are potential sources of immunogenicity, and likely contribute to the difference in immune response between Telo and aTelo gels.[27,28]

TABLE 3 Correlation R^2 values. Correlations for all sources (top-right, grey) and for Telo sources only (bottom-left, blue) shown. Significant correlations ($p < .05$) are bolded

All / Telo	G'_0	G''_0	G'_∞	G''_∞	t_g	Purity	α1/ α2	α1	α2	Diameter
	Rheological analysis					Compositional analysis				SEM
G'_0	-	**.866**	.000	.002	.034	.006	.028	.017	.068	.003
G''_0	**.867**	-	.002	.010	.023	.006	.013	.034	.025	.082
G'_∞	.003	.005	-	**.895**	.183	.070	.198	.010	.179	.045
G''_∞	.000	.001	**.890**	-	.080	.130	.081	.010	.068	.005
t_g	.039	.000	.104	.012	-	.034	.183	**.218**	.180	.026
Purity	.028	.001	**.423**	**.438**	.105	-	.005	**.460**	.033	**.324**
α1/ α2	.031	.000	.042	.000	**.665**	.254	-	**.542**	**.828**	**.228**
α1	.003	.003	**.297**	**.524**	.146	**.360**	.136	-	**.359**	.000
α2	.065	.006	.055	.005	**.597**	**.357**	**.949**	.080	-	**.359**
Diameter	.003	.087	.056	.003	.200	**.422**	**.583**	.005	**.501**	-

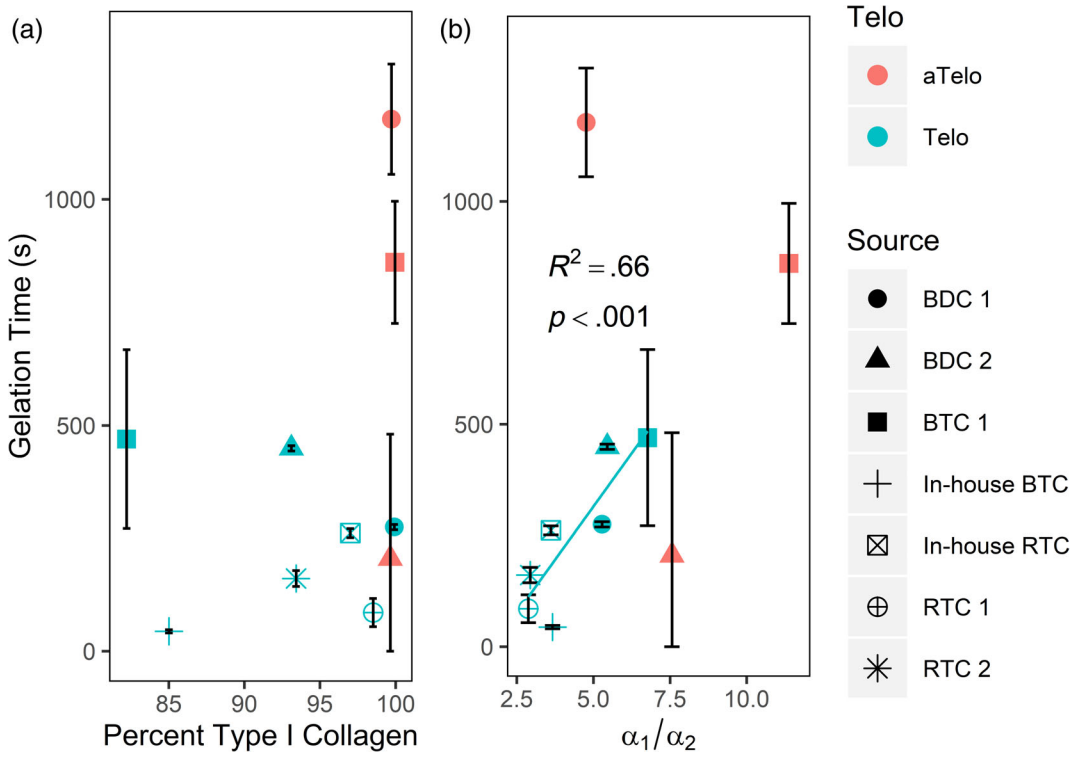

FIGURE 5 Gelation time as a function of (a) percent type I collagen and (b) α_1 to α_2 ratio. Shape corresponds to collagen source and color corresponds to the presence of telopeptides. Error bars represent *SD*. Blue line corresponds to correlations on Telo sources only ($n = 4$ for RTC 1, $n = 2$ for BTC 1 aTelo and in-house BTC, $n = 3$ otherwise)

Despite their increased purity, aTelo gels were found to gel more slowly than Telo gels. This finding is largely in line with previous literature. Studies going back four decades have found slower fiber assembly in pepsin-treated collagen gel with multiple studies confirming this phenomenon more recently.[15,16] The proposed mechanism for this decrease in gelation speed with the removal of telopeptide domains is linked to a decrease in fiber nucleation rate. It has been shown that the transient association of collagen molecules due to increased affinity between telopeptide-containing collagen molecules may well account for this increase in nucleation rate.[15]

Pepsin-treated gels were also found to have lower storage moduli than telocollagen gels, as well as higher loss modulus prior to gelation. Similar in rationale to the increase in gelation time with removal of telopeptides, the effect of telopeptides on gel mechanics may be due to the increased affinity between telopeptide-containing collagen molecules.[23] This increased affinity may lead to the formation of longer-range intermolecular structures that contribute to the observed increase in pre- and post-gelation storage and loss moduli in our study as well as others.[15,29]

The formation of longer-range intermolecular structure in telopeptide-containing gels potentially contributes to the difference in fiber nanostructure observed in this study, specifically, the higher incidence of "wound fiber" morphology in aTelo gels.[15] The different fiber structure between Telo and aTelo gels also likely stems from their difference in fibrillogenesis kinetics. This difference has been demonstrated through optical density measurement during gelation.[15-17,29] Additionally, it has been demonstrated that differences in collagen fiber structure as a result of various conditions can be monitored using real-time confocal reflectance and second harmonic generation imaging.[30-33] However, due to the extensive preparation required for high-resolution SEM images, it is not feasible to monitor differences in fiber structure in the early stages of gelation.

While purity had a significant effect on the storage modulus of Telo collagen sources, we found no such relation in aTelo collagens. This lack of correlation is likely due to the overall high purity of aTelo collagen sources, which were all higher than 99.5% collagen by protein mass. Such invariably high concentration did not, however, lead to stiffer gels. Despite the high purity of these collagens, their final storage modulus was only a fraction of even the least pure Telo collagen sources. Instead of purity, $\alpha 1/\alpha 2$ ratio was a much better predictor of gel mechanics for both Telo and aTelo sources. With $\alpha 1/\alpha 2$ ratios increasing past the native ratio of 2:1, we found storage modulus decreased significantly compared to sources with ratios closer to that of native collagen. This correlation is in part driven by the high $\alpha 1/\alpha 2$ ratios seen in aTelo collagens compared to Telo sources, and their significantly lower storage moduli. There is evidence to suggest that $\alpha 1$ homotrimers have a propensity to form poorly defined nonfibrillar aggregates during the gelation process,[34] but this propensity has not been shown in telopeptide-containing collagen gels.

A major limitation of this study is the selection of sources for analysis. As there were a limited number of manufacturers whose

collagen was available in solubilized form, and at a high enough concentration to make 3 mg/ml gels, the number of sources tested remains low (10 sources from six manufacturers). Furthermore, as few manufacturers source collagen at sufficiently high concentrations to form moldable or printable constructs, the chosen concentration cut-off of 3 mg/ml (final concentration) may not be representative of the mechanics of high concentration collagen gels. Even though collagen sources were ultimately diluted to the same concentration and pH, storage medium differed between manufacturers, with commercial bovine sources solubilized in dilute hydrochloric acid, and rat tail sources solubilized in dilute acetic acid. Additionally, certain collagen sources such as recombinant human, porcine, and equine dermal collagen were not available at the time of testing. In terms of analysis, a major limitation of this study is that while mass spectrometry provides useful quantitative comparison between sources with similar overall composition, the calculated ratios between protein components are not absolute. As such, the calculated $\alpha1/\alpha2$ ratios are only accurate as relative comparisons between the analyzed sources.

5 | CONCLUSION

The objective of this study was to determine whether distinct compositional and mechanical differences could be found between gels fabricated using a variety of collagen sources and processing methods. As hypothesized, differences in purity and relative abundance of $\alpha1$ and $\alpha2$ chains were found to relate with differences in gelation kinetics, mechanics, and fiber structure. Telo gels with higher purity were found to have higher storage modulus and lower fiber diameter, while those with higher $\alpha1/\alpha2$ ratio were found to have higher fiber diameter and slower gelation. Overall, Telo gels had lower purity and $\alpha1/\alpha2$ ratio than aTelo gels but had higher storage modulus and faster gelation. Collectively, these results show the relevance of tuning purity and $\alpha1/\alpha2$ ratio to affect the mechanics of collagen gels.

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DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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