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CONSTITUTIVE MODELING OF COMPRESSIBLE TYPE-I COLLAGEN HYDROGELS

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Abstract

Collagen hydrogels have been used ubiquitously as engineering biomaterials with a biphasic network of fibrillar collagen and aqueous-filled voids that contribute to a complex, compressible, and nonlinear mechanical behavior - not well captured within the infinitesimal strain theory. In this study, type-I collagen, processed from a bovine corium, was fabricated into disks at 2, 3, and 4% (w/w) and exposed to 0, 10⁵, 10⁶, and 10⁷ microjoules of ultraviolet light or enzymatic degradation via matrix metalloproteinase-2. Fully hydrated gels were subjected to unconfined, aqueous, compression testing with experimental data modeled within a continuum mechanics framework by employing the uncommon Blatz-Ko material model for porous elastic materials and a nonlinear form of the Poisson's ratio. From the Generalized form, the Special Blatz-Ko, compressible Neo-Hookean, and incompressible Mooney-Rivlin models were derived and the best-fit material parameters reported for each. The average root-mean-squared (RMS) error for the General (RMS=0.13±0.07) and Special Blatz-Ko (RMS=0.13±0.07) were lower than the Neo-Hookean (RMS=0.23±0.10) and Mooney-Rivlin (RMS=0.18±0.08) models. We conclude that, with a single fitted-parameter, the Special Blatz-Ko sufficiently captured the salient features of collagen hydrogel compression over most examined formulations and treatments.

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KEY TERMS

Compressible hydrogels; Blatz-Ko material; Type-I Collagen; unconfined compression

1. INTRODUCTION

Collagen is a naturally occurring polymer that provides a low immunogenic and biologically compatible platform for cellular attachment [1,2]. Type-I collagen, the most abundant of the collagens, endows a diverse group of mammalian tissues with their mechanical properties [3,4]. This material can be processed from primary tissue sources into acidic solutions, molded into a variety of shapes, and reconstituted into gels using pH neutralization [5]. As such, its applications in biomedical engineering are vast. These applications include scaffolding for tissue engineering, biomimetics, and drug delivery [2,6–8]. Clinically, collagen hydrogels have been used as one of the major constituents of bioactive skin substitutes, corneal shields, and as drug-loaded wound dressings that enhance the healing and recovery processes [9,10]. Injectable collagen hydrogels are currently being investigated to stabilize and improve ventricle remodeling after myocardial infarction [11]. When physically loaded, (e.g., following implantation or when seeded with contractile cells) collagen gels undergo compression and compaction due to the collapse of aqueous voids within the material [12–15]. Accurate interpretation of the response of these materials to applied loads of these (e.g., using finite element analysis), requires the application of an appropriate constitutive model that captures the complex mechanical behavior.

Diverse testing techniques have been employed to measure the mechanical properties of collagen hydrogels (e.g., shear rheometry, tension/compression, indentation, dynamic mechanical analysis). The best experimental tool is selected based on the range of anticipated material behavior, conditions of interest, availability of equipment, and intended material application [5,16–20]. In simple unconfined axisymmetric compression tests, as performed here, two impermeable and rigid parallel plates compress a specimen submerged in an aqueous solution while permitting lateral deformation to occur [5,21]. With the direct measurement of deformed cross-sectional area and force, the Cauchy stress can be calculated at each experimental state and a relationship between the lateral extension and axial compression (Poisson's ratio) can easily be formulated [17,22,23].

Assuming the orientation of the collagen fibers are random (cf., Figure 1) and the time-scale of experimentation is such that viscoelastic and plastic effects are negligible, the material can be modeled within the finite strain theory as being nonlinearly-elastic and isotropic [5,13,24–26]. To encompass the salient features of these compressible collagen hydrogels within a mechanical formulation on the continuum scale, we employ an uncommon strain-energy function developed by Blatz and Ko in 1982 [27]. In its full form the model is called the General Blatz-Ko, aptly named after the pioneering work of these two investigators whose original experiments were used to explain a class of foamed polyurethane rubbers in uniaxial and biaxial tension [27]. Despite previous validation in both tension and compression by Beatty and others [27–29], this framework has been used sparingly to model biological tissues. An immediate advantage to this model is that, at most, only three material

parameters are needed; one of which is measured directly, another can be measured or prescribed, and the last is related to the fraction of voids within the material. From the General form of the Blatz-Ko, the popular compressible Neo-Hookean and incompressible Mooney-Rivlin models can be derived [30]. A further reduced form, called the Special Blatz-Ko, has only a single fitted parameter thereby greatly simplifying the required mechanical measurements and facilitating comparisons between groups of gels with different material characteristics.

In this work we prepared hydrogels using collagen processed from a bovine corium and modified the hydrogel's mechanical properties through manipulations of concentration, UV crosslinking, and enzymatic degradation. We then tested the hypothesis that the lesser-known Blatz-Ko material model could provide a better, and simpler, representation of a diverse group of collagen hydrogels under compressive loading compared to the popular Neo-Hookean or Mooney-Rivlin models. As a representative example of the model's utility, we applied these findings to the gel contraction assay using neonatal rat cardiac fibroblasts (NCFs) and calculated the acute energy required to deform this material in culture. In doing so, a constitutive model for hydrogel mechanics was developed and validated that can easily be used to interpret clinical or experimental results.

2. MATERIALS AND METHODS

2.1 Collagen Isolation and Preparation

Type-I collagen was isolated from a bovine corium through mechanical separation of the dermis and epidermis following the procedure outlined in Yost et al. 2004 [6]. Briefly, hair follicles and non-collagenous proteins were removed using a $\text{Ca}(\text{OH})_2$ and a solution of NaHS and subsequently treated with pepsin, grinded with ice, and mixed with acetic acid to create a gel solution. Collagen proteins were then salted out of the solution, collected via centrifugation, and then dialyzed against dH_2O to remove excess salts. A collagen type-I standard and a resultant hydrogel sample of extracted collagen were then pipetted onto a polyvinylidene difluoride (PVDF) membrane and incubated with a mouse primary type-I collagen antibody diluted (1:1000) using Tween Tris Buffered Saline (TTBS) for one hour. The membrane was washed and placed in a secondary goat anti-mouse antibody (1:3000) tagged with horseradish peroxidase (HRP) solution for one hour. After serial washings, the membrane was exposed to 3,3'-Diaminobenzidine (DAB) chromogen which produced a colorimetric change on the membrane after reduction by HRP. The presence of type-I collagen was confirmed using the dot-blot assay as indicated by a color change on the PVDF membrane upon reduction of the DAB substrate.

The pH of the collagen solution was adjusted to 5.5 and the desired concentrations (2, 3, and 4% w/w) were obtained via dilution and centrifugation. Non-polymerized collagen gels were then loaded into 10 mm diameter by 3.5 mm height molds and subsequently polymerized via pH neutralization using a 10x HEPES buffer (pH = 7.8). To create stiffer gels, a Stratalinker 2400 UV Crosslinker was employed at 10^5 , 10^6 , and 10^7 μJ . On the other hand, to reduce stiffness, proteolysis was induced via the addition of matrix metalloproteinase-2 (MMP2). Here a pro-MMP2 standard solution was diluted to a concentration of 8 nM with PBS. The MMP solution was subsequently activated with the addition of 40 mM p-aminophenyl

mercuric acetate (APMA) solution and incubated for 1 hour at 37°C. Collagen hydrogel disks were then submerged in a 1:1 mixture of activated MMP solution and reaction buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, 0.0025% Brij-35; pH = 7.5) for 2 hours at 37°C and then removed from the MMP2 solution and placed in PBS supplemented with Ethylenediaminetetraacetic acid (EDTA) to inhibit MMP activity.

2.2 Microscopy

A subset of samples were imaged using confocal reflectance microscopy that were first fixed in 4% paraformaldehyde (PFA) at 4°C overnight and then mounted. A Zeiss LSM 510 Meta (Carl Zeiss Microimaging, Inc.) with a 488 nm wavelength Argon laser and a photomultiplier tube was used with a 475 nm long-pass filter placed before the PMT to detect wavelength reflectance at 475 and 494 nm of collagen fibers in the sample. 63x was the maximum objective available so that confocal microscopy was used as a qualitative measure of random fiber dispersion within a sample (Figure 1).

2.3 Unconfined Compression Testing

Fully-hydrated disks were submerged in phosphate buffered saline (PBS) and compressed using a MARK-10 mechanical test rig (ESM301-L) with the M5-5 (25 N capacity, 0.005 N resolution) force transducer at rate of 10 mm/min. Synchronous measurements of radial and axial dimensions were taken using a Thorlabs CMOS Camera (DCC1645C) with 1280 × 1024 resolution and a maximum of 24.9 frames-per-second (FPS). At least 15 measurements were recorded for each sample at a rate of 1.0 FPS. At 13 mm in diameter, the upper platen was sufficiently large so as to provide a continuous surface during radial expansion but small enough to prevent camera interference. We assumed that inertial effects between the solid and liquid phases were negligible at low strain-rates and that the compression surfaces were frictionless so that “simple” compression could occur [31]. This assumption was validated by noting the lack of curvature at the sample edge during compression. Very little change in load or dimensions at maximum compression under static conditions were observed suggesting minimal relaxation. To best recreate the immediate physical response to applied compression, specimens were tested unidirectionally with data collected for a single loading cycle in the loading regime occurring prior to failure. Failure was indicated by discontinuity in the loading curve and a sudden, asymmetric, radial expansion of the sample. Any sample that failed prematurely (i.e., prior to 30% compression), occurring in less than 10% of the samples, was discarded and excluded from mechanical analysis. Experimental Cauchy stress was calculated by $\sigma^{\text{exp}} = f/a$, with $a = \pi r^2$ the current (or deformed) cross sectional area (r the radius of that deformed area) and f the force measured at the deformed state (Figure 2).

2.4 Cell and Contraction Assay

Rat neonatal cardiac fibroblast cells were cultured to confluence on 75 mm plates and then trypsinized to create a cell-suspension with an approximate concentration of 1×10^7 cells/mL. Collagen was concentrated to achieve a final 3% (w/w) after the addition of both the cells & HEPES solution. An equal volume of cell suspension and HEPES was added to equal volumes of the hydrogel solutions to provide uniform cell distribution and promote polymerization. After carefully mixing to avoid introduction of bubbles, the collagen-cell mixture, henceforth abbreviated Coll-NCF, was loaded into a mold, covered with 5% FBS

supplemented DMEM, and allowed to polymerize overnight in an incubator. The Col1-NCF gel disks were then removed from the mold and were placed into our real-time incubator based contraction chamber (Figure 7). The contraction chamber consisted of a transparent polystyrene culture dish in which a 3 mm thick polytetrafluoroethylene (PTFE) circular base was placed covering the entire bottom surface. A total of 10 needles (26-gauge) were inserted into the PTFE and then Col1-NCFs disks were gently placed on the PTFE allowing the needle to puncture the center, creating a biologically inert anchor point to maintain a consistent focal length for real-time imaging. Careful examination and manipulation of the Col1-NCFs ensured that no additional boundary conditions or tractions were applied and the Col1-NCF disks did not adhere to the syringe needle or PTFE. Disks were then cultured for more than 7 days, refreshing media every 3 days, with diameter and height measurements recorded daily using a harsh environment camera (EO-1312, Edmund Optics).

2.5 Theoretical Framework

Material points in the cylindrically shaped hydrogel are originally at (R, Θ, Z) in an unloaded reference configuration and mapped to (r, θ, z) in a axisymmetric deformed configuration (Figure 2) so that $r = \lambda R$, $\theta = \Theta$, $z = \Lambda Z$. The deformation gradient for this motion is given as

$$\mathbf{F} = \begin{bmatrix} \lambda & 0 & 0 \\ 0 & \lambda & 0 \\ 0 & 0 & \Lambda \end{bmatrix}, \quad (1)$$

with the right Cauchy-Green strain tensor represented by $\mathbf{C} = \mathbf{F}^T \mathbf{F}$. Compressible material behavior is apparent when the determinant of the deformation gradient, $\det \mathbf{F} = \Lambda \cdot \lambda^2$, is less than 1.

The Cauchy stress tensor for isotropic, homogeneous, elastic, and compressible, materials (i.e., the General Blatz-Ko, Special Blatz-Ko, and Neo-Hookean), is [23]

$$\boldsymbol{\sigma} = \frac{1}{\det \mathbf{F}} 2\mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T, \quad (2)$$

but for an incompressible material (Mooney-Rivlin model only) we have

$$\boldsymbol{\sigma} = -p\mathbf{I} + 2\mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T, \quad (3)$$

With p , the Lagrange multiplier used to enforce incompressibility and $\mathbf{I}$ the identity matrix.

The strain energy function W for a homogeneous isotropic elastic material is a function of the principal invariants of $\mathbf{C}$ so that:

$$I_C = \text{tr}(\mathbf{C}), \quad II_C = \frac{\text{tr}(\mathbf{C})^2 - \text{tr} \mathbf{C}^2}{2}, \quad III_C = \det(\mathbf{C}). \quad (4)$$

Following the chain rule [32], we have

$$\frac{\partial W}{\partial \mathbf{C}} = \frac{\partial W}{\partial I_C} \frac{\partial I_C}{\partial \mathbf{C}} + \frac{\partial W}{\partial II_C} \frac{\partial II_C}{\partial \mathbf{C}} + \frac{\partial W}{\partial III_C} \frac{\partial III_C}{\partial \mathbf{C}}, \quad (5)$$

and by definition

$$\frac{\partial I_C}{\partial \mathbf{C}} = \mathbf{I}, \quad \frac{\partial II_C}{\partial \mathbf{C}} = I_C \mathbf{I} - \mathbf{C}^T, \quad \frac{\partial III_C}{\partial \mathbf{C}} = III_C \mathbf{C}^{-T}. \quad (6)$$

Evaluating the remaining non-zero derivatives of (2) or (3) using (5) and (6), and when the appropriate form of W is chosen, (6) yields an expression for Cauchy stress. In the case of simple uniaxial compression, the only nonzero value of (6) is in the axial direction.

The General Blatz-Ko material model used to represent compressible foamed materials undergoing large deformations has the form [27,30]

$$W = \frac{\mu\alpha}{2} \left(I_C - 1 - \nu^{-1} + \frac{(1-2\nu)}{\nu} III_C^{\frac{-\nu}{1-2\nu}} \right) + \frac{\mu(1-\alpha)}{2} \left(\frac{II_C}{III_C} - 1 - \nu^{-1} + \frac{(1-2\nu)}{\nu} III_C^{\frac{\nu}{1-2\nu}} \right), \quad (7)$$

where the three material parameters are the shear modulus $\mu > 0$, an interpolation parameter thought to be related to the void fraction $\alpha \in [0,1]$, and the nonlinear Poisson's ratio-like parameter $\nu \in [0,0.5]$ [33]. Unlike the classic Poisson's ratio, we use a nonlinear form that relates the dependence on axial to radial/circumferential stretch ratios via

$$\lambda(\Lambda) = \Lambda^{-\nu}, \quad (8)$$

per the ad hoc assumption of [2] and later derived equivalently by [29,33]. It can be shown that for small strains equation (8) becomes the linearized and familiar form of Poisson's ratio $\epsilon_r = -\nu \epsilon_z$ with ϵ the linearized Green strain. This nonlinear form is applicable when the graph of $\ln(1/\lambda)$ versus $\ln(\Lambda)$, in tension or compression, results in a straight line (see, Figure 3) [29,33].

The physical meaning of α in compression was not immediately evident. For this investigation however, we explore the utility of assigning values to this parameter consistent with the work of other investigators [27,29,30]. First, we consider $\alpha = 0$ so that equation (7) becomes

$$W = \frac{\mu}{2} \left(\frac{II_C}{III_C} - 1 - \nu^{-1} + (1 - 2\nu) III_C^{-\frac{\nu}{1-2\nu}} \nu^{-1} \right), \quad (9)$$

and, when a value of $\nu = 1/4$ is assigned, (9) becomes the Special Blatz-Ko. On the other hand, with a value of $\alpha = 1$, equation (7) becomes

$$W = \frac{\mu}{2} \left(I_C - 1 - \nu^{-1} + (1 - 2\nu) III_C^{-\frac{\nu}{1-2\nu}} \nu^{-1} \right), \quad (10)$$

which is the strain energy function for a compressible Neo-Hookean material.

In contrast to the compressible forms (7), (9), and (10), an incompressible Mooney-Rivlin material can be found by taking the limit as $III_C \rightarrow 1$ and $\nu \rightarrow 1/2$ in equation (7). Then (7) becomes,

$$W = \frac{\mu\alpha}{2} (I_C - 3) + \frac{\mu}{2} (1 - \alpha) (II_C - 3), \quad (11)$$

The remaining free parameters μ (all models) and α (General Blatz-Ko and Mooney-Rivlin models only) were found via minimizing the residual of the objective function defined by

$$RMS = \sqrt{\sum_{n=1}^N (\sigma_n - \sigma_n^{\text{exp}})^2 / N}, \quad (12)$$

where n is the number of discrete measurements of N total measurements in each experimental dataset.

For the cell based gel contraction assay we calculated the stored elastic energy (microjoules) via integration of the strain energy function as [34]

$$\hat{W}(\mathbf{C}) = \int_{-H/2}^{H/2} \int_0^{2\pi} \int_0^R W(I_C, II_C, III_C) \cdot R \cdot dR d\Theta dZ, \quad (13)$$

with H the undeformed height of the disk. Equation (13) provides a metric of acute cell-based gel contraction provided the material is considered elastic. At long time scales (>3 days) plasticity of collagen hydrogels may be observed therefore we only considered acute deformations in the calculation of $\bar{W}$.

2.6 Statistical Analysis

All numerical values are expressed as the mean $\pm$ standard deviation with 3 to 5 samples in each group of the unconfined compression experiments and 9 samples in the cell based compaction experiments. An uneven two-way ANOVA with Tukey Post-Hoc analysis was performed to find significant differences at $p < 0.05$. Comparisons were made between the parameters (μ , ν , α) of the different constitutive models (General Blatz-Ko, Special Blatz-Ko, Neo-Hookean, Mooney-Rivlin) and for the stress-stretch (σ ; λ , Λ) relationships of different collagen treatments (0, 10^5 , 10^6 , 10^7 microjoules UV; MMP2) and formulations (2, 3, 4% w/w). RMS errors were also compared between these groups.

3. RESULTS

Confocal reflectance micrographs of a 3% (w/w) gel demonstrate both the initially random microstructural organization of collagen fibers and the inherent material porosity contained therein (Figure 1). The nonlinear form of the Poisson's ratio described by equation (8) yielded an excellent fit to all experimental data (Figure 3; $R^2 > 0.976$). No significant differences in nonlinear Poisson's ratio were observed between any experimental groups resulting in an average value of $\nu = 0.29 \pm 0.06$. Material parameters (Tables 1–3) determined from uniaxial unconfined compression testing (Figure 2) indicate that the shear modulus increases with w/w collagen (e.g., Special Blatz-Ko: $\mu = 0.198 \pm 0.09$ to 0.575 ± 0.04 kPa) and exposure to ultraviolet cross-linking (e.g., Special Blatz-Ko 10^7 microjoules UV: $\mu = 3.45 \pm 0.89$ kPa) while decreasing in the presence of proteases (e.g., Special Blatz-Ko: $\mu = 0.119 \pm 0.09$ kPa). Fitted shear modulus values were slightly higher in the Neo-Hookean ($\mu = 0.896 \pm 0.03$ kPa) than the General Blatz-Ko ($\mu = 0.609 \pm 0.01$ kPa) at 4% w/w. When used as a free parameter, α approaches 0 for all experimental groups except for the 2% gel (General Blatz-Ko: $\alpha = 0.973 \pm 0.05$). All other comparisons of μ or α between models failed to reach statistical significance.

A representative stress-stretch behavior of a 3% w/w of collagen hydrogel under unconfined compression can be observed in Figure 4. Here the General Blatz-Ko, Special Blatz-Ko, compressible Neo-Hookean, and incompressible Mooney-Rivlin models are plotted next to the raw experimental data and a linear fit. The General Blatz-Ko (RMS=0.019) and Special Blatz-Ko (RMS=0.017) provide the best representation of experimental data and, by virtue of similar values for ν and a low value for α in the General Blatz-Ko, are virtually indistinguishable. Subsequently, the compressible Neo-Hookean (RMS=0.057) provides the poorest fit of the nonlinear material models. Collectively the average root-mean-squared fitting error using the General Blatz-Ko material model (RMS=0.13 $\pm$ 0.07) was comparable to the Special Blatz-Ko (RMS=0.13 $\pm$ 0.07) and much improved over the Neo-Hookean (RMS=0.23 $\pm$ 0.10) or Mooney-Rivlin (RMS=0.18 $\pm$ 0.08) material models (Figure 5). An exception to this was observed for the 2% collagen gels using the Special Blatz-Ko. The

Cauchy stress vs. axial stretch ratio fit for all experimental groups using the Special Blatz-Ko is visualized in Figure 6.

Following an initial seeding period, the Coll-NCF contraction assay demonstrated progressive and isometric contraction for 7 days in culture (Figure 7) with no statistical significance between axial ($\Lambda = 0.85 \pm 0.8$) and radial ($\lambda = 0.88 \pm 0.03$) compaction but considerable volumetric changes were observed ($\det \mathbf{F} = 0.68 \pm 0.7$). During this time, gels did not need to be freed from the surrounding material as commonly observed in coated polystyrene dishes since both the PTFE base and stainless steel syringe provided little to no additional traction forces as demonstrated by the free and uniform contraction of these gels. The stored energy from acute deformations using the Special Blatz-Ko model was calculated to be $\bar{W} = 12.5 \pm 7.49 \mu\text{J}$.

4. DISCUSSION

Working within the finite strain theory of continuum mechanics, the present work describes the macroscopic mechanical response of collagen hydrogels under unconfined compression testing using collagen harvested and processed from a bovine corium. As expected the shear modulus μ was readily tunable using different collagen treatments and formulations thereby emphasizing the diverse application of this material for a variety of biomedical applications. A nonlinear form of the Poisson's ratio was calculated for each sample and not found to be significantly different between any of the experimental groups. Based on the overall nonlinear mechanical response, we compared four different, but related, constitutive models to find an optimal fit amongst candidates: the General Blatz-Ko, Special Blatz-Ko, compressible Neo-Hookean, and incompressible Mooney-Rivlin models. Of these, the Special Blatz-Ko provided the best overall fits with the minimum number of free parameters and was therefore used to represent experimental data in Figure 6.

The Special Blatz-Ko material model is simply a form of the General Blatz-Ko where it is assumed that $\alpha = 0$ and $\nu = 1/4$ [29,31]. This particular model was desirable since, after applying the constraints, μ becomes the only fitted parameter thereby adding considerable simplicity to the requisite mode of mechanical testing for parameter estimation. To this point, the experimentally measured value of the nonlinear Poisson's ratio (equation 8) $\nu = 0.29 \pm 0.06$ was close to the value prescribed in the Special Blatz-Ko model resulting in a good fit to most experimental data. This value was recorded directly in our experiments through measurements of axial and lateral dimensional changes. Other examples of hydrogel mechanics solve for the Poisson's ratio indirectly through an assumed constitutive behavior (linearized), or parameter fitting, rather than being measured directly [35].

The α parameter has been described as being related to the volume-fraction of voids in the material [30]. However, in the original work performed by Blatz and Ko, α was found to be 0 for a 47% void fraction polyurethane foamed rubber in tension while a value of 1 was used to describe a continuum rubber [27,29,30]. Although it was tempting to prescribe the value based on a direct or indirect measurement of the void fraction (e.g., as a structurally motivated model), the physical meaning is not as straight-forward. In our experimental procedure a lower percentage of collagen resulted in a better fit at higher values of α (see

Tables 1–3). This seemingly paradoxical behavior suggests that a better description is that it's value is assigned as an “interpolation parameter” [29,36]” and that the Blatz-Ko model is a purely phenomenological one. Regardless, the overall fitting error for the Special Blatz-Ko (RMS=0.13±0.07) was indistinguishable from the General Blatz-Ko material model (RMS=0.13±0.07) thus with these materials there was little advantage to leaving it as a free parameter - especially for the stiffer gels. We conclude therefor that the Special Blatz-Ko is the preferred model.

Our uniaxial compression measurements are qualitatively similar to those found in [21] at comparable collagen concentrations and loading rates. A notable difference was that the experimental data in that work was presented in terms of the First Piola-Kirchoff stress while ours was Cauchy stress. Note that these two measures of stress are related through the deformation gradient [22]. In general, the First Piola-Kirchoff stress would tend to overestimate the Cauchy stress in unconfined compression since the cross-sectional area in the deformed configuration would be greater. In reality we found the radial expansion to be considerable for all samples but surprisingly consistent as indicated by the lack of statistical significance of the nonlinear Poisson's ratio between experimental groups.

To modify the inherent stiffness, ultraviolet light exposure was used to cross-link the collagen molecules (Table 2) [20,37]. Many other methods of collagen hydrogel crosslinking have also been explored by others including: dehydrothermal treatment, glutaraldehyde, and carbodiimides, and their effects on cell mediated contraction, proliferation, and biosynthesis characterized. In contrast, we also quantified the effect of collagen degradation by the protease matrix metalloproteinase-2 (MMP2), which was found to cause a 37.2% decrease in the Blatz-Ko stiffness parameter (Table 3). MMP2 plays a major role in a variety of diseases and over/under-expression in the cardiovascular system can lead to complications such as aortic aneurysms and calcification [38,39]. A broad-reaching implication of these particular tests was that collagen hydrogels could be used as a test-bed to evaluate the efficacy of neutralizing MMPs to preserve tissue-analogue mechanics.

Although the models presented here capture well the mechanical behavior under these conditions of interest, they are inherently limited to quantification of the macroscopic mechanical response which may differ from the local mechanical environment experienced by adhered cells. Moreover, our focus was exclusively on the elastic (acute) portion of this response. We acknowledge that the operative time scale in hydrogel mechanics is extremely important to the mechanical behavior. Under rapid loading, viscous loss is significant due to the aqueous drag during flux, while at slow or static loading plastic deformation and creep occur as crosslinks can break down over time [40]. Ultimately, the appropriate theoretical framework and constitutive model depends on the anticipated application. For example, at small strains Neo-Hookean behavior is a sufficient descriptor of material behavior but at higher strains the Blatz-Ko models present a better approach to hydrogel mechanics.

Unconfined compression has been used to produced dense collagen matrices representing a more “tissue like” structure [5]. Evidence suggests that without these distinct fiber-alignment techniques that collagen gels are initially isotropic due to the random alignment of fibers within the gel during fabrication [26,41]. The fabrication process used in the present

work does not provide any strain- or chemically-driven fiber alignment in the hydrogels, thus allowing the formation of a random fiber network as shown in the confocal reflectance micrographs (see Figure 1). Additionally, we avoided preconditioning cycles to maintain the random alignment of fibers in our samples, to minimize fluid loss, and to promote consistency with potential applications (e.g., implantation, laboratory assay, drug delivery) [19].

Cell-based compaction of hydrogels primarily involve observation of the changes in size or shape of the hydrogel and estimation of cell-generated traction forces from the known material behavior [13,15,42–45]. As mentioned earlier, interpretation of experimental results depends on the selection of a suitable material model that can perform under the conditions of interest. As an illustrative example of the utility of the derived constitutive models, we embedded 3% collagen gels with neonatal rat cardiac fibroblasts (Col1-NCF) and observed compaction for one week. In our setup we used a virtually free floating gel with only an axisymmetric boundary applied via the syringe needle. It was clear that this setup enabled consistent and uniform isometric compaction (Figure 7). Although the magnitude of acutely stored energy among the compressible constitutive formulations for the Col1-NCF materials was virtually equivalent for small strains, larger deformations would likely introduce greater error and therefore utilizing the best constitutive model would generate more accurate results. Incompressible models, such as the Mooney-Rivlin as derived here, are not applicable as volume changes are significant ($\det \mathbf{F} = 0.68 \pm 0.7$).

The gel contraction assay could be used to better inform clinicians of certain cancer or cardiovascular pathologies [46]. For example, Marfan syndrome includes a loss of vascular smooth muscle cell (VSMC) contractility and dysfunctional remodeling due to increased MMP activity which contributes to the formation of aortic aneurysms [47]. With an aortic biopsy, clinicians could culture explanted VSMCs by placing cells within a collagen gel, with or without MMPs, and observe the cell-based compaction over time. In this manner, candidate therapeutics could be safely be evaluated in isolation while the contractile capacity of these cells is continually assessed. Accurate interpretation of these experiments however, requires the application of an appropriate constitutive model that captures the complex mechanical behavior.

To help explain and interpret tunable collagen mechanics we've employed a constitutive model that was developed for foamed polyurethane rubbers and applied this toward collagen hydrogel mechanics under uniaxial compression. This approach was motivated by experimental evidence that demonstrates the collapse of aqueous voids under compressive strains [45]. The Special form of the Blatz-Ko demonstrated results that are superior to the Neo-Hookean or Mooney-Rivlin models and comparable to the General form of the Blatz-Ko for all formulations of collagen concentrations greater than 2%. Unconfined compression testing may not be suitable at very low collagen concentrations that are outside of the scope of this paper. We conclude that the single parameter Special Blatz-Ko material model was sufficient for representation of a wide-class of collagen hydrogels but greater errors are introduced at low collagen concentrations. The parameters for this and the other material models are tabulated in this work and can easily be implemented into diverse experimental, analytical, and computational applications.

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Highlights

- Diverse type-I collagen hydrogel disks were tested using unconfined compression
- A nonlinear form of Poisson's ratio was consistent for all hydrogel formulations
- The Special Blatz-Ko model best represented the mechanics of hydrogel compression
- Using this model, the shear modulus was the only fitted-parameter
- Hydrogel mechanics are greatly simplified using the Special Blatz-Ko

Figure 1.
Confocal reflectance microscopy of a 3% collagen hydrogel. Image recorded with a 63X oil immersion objective to illustrate the random orientation of collagen fibers within the gel.

Figure 2.

Uniaxial unconfined compression testing of (3.5×10 mm height $\times$ diameter) type-I collagen hydrogel disks. (a) Setup illustrating compression testing in an aqueous solution. The image depicts a disk that has already undergone compression. (b) Schematic demonstrating the axial Λ and radial λ stretch ratios as a result of the force f applied during compression testing and a single representative dataset from a 3% collagen hydrogel showing (c) force vs. (–) axial displacement and (d) radial vs. (–) axial displacement.

Figure 3.

Experimental and fitted data illustrating the dependence of the radial extension on axial compression for (a) 2% ($R^2=0.996$), 3% ($R^2=0.987$), and 4% ($R^2=0.989$) collagen gels, (b) 3% gels following 0, 10^5 ($R^2=0.985$), 10^6 ($R^2=0.985$), and 10^7 ($R^2=0.976$) μJ of UV crosslinking, and (c) 3% gels (control) exposed to MMP2 ($R^2=0.993$). The slope of these lines are equal to the nonlinear Poisson's ratio for each group. Error bars $\pm$ standard deviation.

Figure 4.

Example of Cauchy stress vs. axial extension ratio of a 3% collagen disk using different constitutive models. Experimental data is indicated by ($\times$) and modeling results, using the General Blatz-Ko ($\mu=0.355$ kPa, $\alpha=0$, $\nu=0.29$), Special Blatz-Ko ($\mu=0.352$ kPa, $\alpha=0$, $\nu=1/4$), Neo-Hookean ($\mu=0.561$ kPa, $\alpha=1$, $\nu=0.29$), or Mooney-Rivlin ($\mu=0.371$ kPa, $\alpha=0$, $\nu=1/2$) materials, are illustrated by the continuous curves. A linear (Hookean) fit is shown for comparison. Note that compressive stress is indicated by a positive value.

Figure 5.

Fitting errors reported as Root-Mean-Squared (RMS) of experimentally measured stress using the General Blatz-Ko □, Special Blatz-Ko ■, Neo-Hookean ■, or Mooney-Rivlin ■ material models for all collagen hydrogels under unconfined compression. Error bars are $\pm$ standard deviation.

Figure 6.

Cauchy stress vs. axial extension ratio for all groups under unconfined uniaxial compression. Experimental data (symbols) and that data represented using the Special Blatz-Ko model (curves) for (a) 2%, 3%, and 4% collagen, (b) 3% collagen gels following UV 0, 10^5 , 10^6 , and 10^7 μ J of UV crosslinking energy, and (c) 3% gel exposed to MMP2. Error bars $\pm$ standard deviation.

Figure 7.

Gel contraction/compaction experiments using neonatal rat cardiac fibroblasts (Col1-NCF). (a) Incubator setup for real-time contraction/compaction measurements of Col1-NCF floating in culture media (n=9). (inset) Orientation of multiple Col1-NCF disks for batch recording. (b) The measured axial Λ and radial λ stretch ratios plotted next to $\det \mathbf{F} = \Lambda \cdot \lambda^2$ indicates a significant reduction in volume. (c) A sample image of a Col1-NCF after 7 days in culture. A 26-Ga (0.46 mm) syringe needle is located at the center of the disk and anchored in a layer of polytetrafluoroethylene (PTFE) to provide traction free boundaries.

Table 1

Material parameters for the General Blatz-Ko, Special Blatz-Ko, Neo-Hookean, and Mooney-Rivlin models using 2, 3, and 4% collagen gels.

		General Blatz-Ko	Special Blatz-Ko	Neo-Hookean	Mooney-Rivlin
2% collagen (w/w)	μ (kPa)	0.313 ± 0.14	0.198 ± 0.09	0.317 ± 0.10	0.285 ± 0.13
	α	$0.973 \pm 0.05^*$	0	1	$0.928 \pm 0.10^*$
	ν	0.313 ± 0.07	1/4	0.313 ± 0.07	1/2
3% collagen (w/w)	μ (kPa)	0.370 ± 0.07	0.353 ± 0.09	0.564 ± 0.16	0.372 ± 0.10
	α	0.136 ± 0.24	0	1	$2.9\text{E-}10 \pm 2.6\text{E-}11$
	ν	0.286 ± 0.05	1/4	0.286 ± 0.05	1/2
4% collagen (w/w)	μ (kPa)	$0.609 \pm 0.01^*$	$0.575 \pm 0.04^*$	$0.896 \pm 0.03^{*\S}$	$0.607 \pm 0.04^*$
	α	0.105 ± 0.18	0	1	$4.4\text{E-}12 \pm 4.8\text{E-}12$
	ν	0.332 ± 0.05	1/4	0.332 ± 0.05	1/2

* Denotes statistical significance ($p < 0.05$) between the experimental group and the 3% while

$\S$ indicates statistical significance between material model and the General Blatz-Ko model.

Material Parameters for General Blatz-Ko, Special Blatz-Ko, Neo-Hookean, and Mooney-Rivlin models using a 3% collagen gel at 10^5 , 10^6 , and 10^7 μJ of ultraviolet light.

Table 2

		General Blatz-Ko	Special Blatz-Ko	Neo-Hookean	Mooney-Rivlin
		μ (kPa)	α	ν	
10^5 (μJ) UV cross-linking	μ (kPa)	0.689 ± 0.26	0.635 ± 0.29	0.980 ± 0.41	0.677 ± 0.30
	α	0.207 ± 0.36	0	1	0.066 ± 0.11
	ν	0.321 ± 0.06	1/4	0.321 ± 0.06	1/2
10^6 (μJ) UV cross-linking	μ (kPa)	$1.92 \pm 0.37^*$	$1.90 \pm 0.38^*$	$2.89 \pm 0.62^*$	$1.98 \pm 0.39^*$
	α	$1.6\text{E-}10 \pm 2.0\text{E-}10$	0	1	$5.1\text{E-}11 \pm 7.9\text{E-}11$
	ν	0.332 ± 0.09	$1/4^{\S}$	0.332 ± 0.09	1/2
10^7 (μJ) UV cross-linking	μ (kPa)	$3.43 \pm 0.89^*$	$3.45 \pm 0.89^*$	$5.66 \pm 1.37^*$	$3.63 \pm 0.92^*$
	α	$3.7\text{E-}11 \pm 6.1\text{E-}11$	0	1	$4.0\text{E-}11 \pm 4.4\text{E-}11$
	ν	0.21 ± 0.03	1/4	0.214 ± 0.03	1/2

* Denotes statistical significance ($p < 0.05$) between the experimental group and the 3% gel without ultraviolet crosslinking while

$\S$ indicates statistical significance between material model and the General Blatz-Ko model.

Material Parameters for General Blatz-Ko, Special Blatz-Ko, Neo-Hookean, and Mooney-Rivlin models using a 3% collagen gel and exposure to MMP2.
No statistical significance was found between groups.

Table 3

	General Blatz-Ko	Special Blatz-Ko	Neo-Hookean	Mooney-Rivlin
MMP2	μ (kPa)	0.119 ± 0.09	0.297 ± 0.13	0.222 ± 0.09
	α	0	1	0.218 ± 0.19
	ν	$1/4$	0.285 ± 0.05	$1/2$