MECHANICAL BEHAVIOR UNDER UNCONFINED COMPRESSION LOADINGS OF DENSE FIBRILLAR COLLAGEN MATRICES MIMETIC OF LIVING TISSUES

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Bio-artificial tissues are being developed as replacements for damaged biologic tissues and their mechanical properties are critical for load-bearing applications. Reconstituted dense three-dimensional (3D) fibrillar collagen matrices are promising materials for tissue engineering, at the light of their interaction with fibroblasts. The mechanical properties of these fibrillar collagen matrices are now being characterized under unconfined compression loading for various strain rates and collagen concentrations. The data were compared to those obtained in the same conditions with a biological tissue, the rat dermis. The results show a very sensitive behavior to both the displacement rate, typical of biological soft tissues, and the collagen concentration varying between 5 and 40 mg/ml. The link between the mechanical properties and the microscopic structure of the collagen scaffolds show an increasing viscoelastic modulus with respect to the fibril density. It is found that the matrices at 5 mg/ml and the dorsal rat skin (DRS) exhibit similar stress–strain response when submitted to the same external unconfined compression load. Such results highlight the interest of these matrices as potential tissue substitutes.

Keywords: Collagen fibrils; 3D matrix; dermis substitute; viscoelastic; unconfined compression test.

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1. Introduction

Tissue substitutes developed as replacements for damaged biologic tissues need to show mechanical properties close to tissue function requirements. Collagen-made matrices, at high concentrations and order, are particularly interesting candidates, as this biopolymer is the major protein of vertebrates whose main function is to form structural networks in the extracellular space of living tissues. Collagen molecules assemble in fibrils and networks, having excellent mechanical resistance and giving their shape to highly specialized structures as bone, dermis, tendon, and cornea.

The success in performing tissue substitutes, in order to restore tissue function, is due to the growing interdisciplinary field of tissue engineering. Among different approaches, the use of three-dimensional (3D) matrices containing specific cells has been proposed as tissue equivalents. This is the case for deep burns for which fundamental research started in the 1970s and has now given rise to various industrial products. A commercialized epidermis layer, Epicel® is issued from the work of Rheinwald and Green on epidermal cell studies. Clinical observations showed that the keratinocyte cell layer needed to be deposited on a derm-equivalent layer for best reconstruction conditions. Two acellular derm substitutes are now commonly used in clinic under the label Integra®. Other prototypes propose floating collagen gels, including fibroblasts, as cell component. Lyophilized collagen sponges were also proposed. Although very successful results are already obtained with skin implants, surgeons nevertheless deplore insufficient mechanical properties of the substitutes, either during manipulation, or when implanted in tissue-bending regions. In this context, preparing materials with improved viscoelastic characteristics, as close as possible to living tissues, is of major interest. Ordered collagen assemblies have been highlighted with acid-soluble collagen solutions at high concentration ranges between 20 and 180 mg/ml. Type I collagen acid-soluble molecules spontaneously organize at long distance and the fluid state can be stabilized by a sol/gel transition at neutral pH.

Tissue-engineered products with excellent biological/chemical compatibility but those cannot withstand the mechanical loads incurred during typical conditions of use will not be clinically useful. In the present contribution, a very high collagen concentration range was chosen, much closer to tissue structures than classical hydrogels, combined to large range applied strain rates. In addition, the samples were analyzed in scanning electron microscopy (SEM) to observe the collagen fibril order and in transmission electron microscopy (TEM) to attest the presence of banded collagen fibrils. Combined compression-shear stresses are also presented. Current testing protocols for bio-artificial tissues vary widely and most of them do not consider viscoelasticity. Unconfined compression tests (i.e. where the sample edges are stress free) have been performed for different displacement rates of the impermeable platen (Fig. 1d): \( V_1 = 0.01 \text{ mm/s} \), \( V_2 = 0.1 \text{ mm/s} \), \( V_3 = 1 \text{ mm/s} \), \( V_4 = 10 \text{ mm/s} \). Under monotonic loadings, the stress–strain response is compared
to those obtained on living tissues with respect to collagen concentration, which implicitly is linked to the hierarchical structures established in optical and/or electron microscopy. The development of controlled collagen constructs requires a clear understanding of the mechanical properties of the biomaterial, and the aim of this study is the development of improved tissue analogs that meet specific mechanical demands.

Fig. 1. Specific device designed and manufactured in our laboratory (Fig. 1 b). The specimen is located between the two plates L and F (Fig. 1 a, c). (A) Horizontal stage for shearing action; (B) Aluminium plate interface of the translating device; (C) Plastic support of the shear load cell, (D) Shear load cell, (E) Connecting system (three cylindrical bearings); (F) Translating lower plate (one degree of freedom); (G) Two guide rails Schneeberger of tool steel of standard 1.3505 (hardness from 58 to 64 HRC) equipped with roller cages ensuring a smooth movement (the plate F rests on them); (H) Vertical stage is assembled on a square; (J) Adjustable support fixed at interface I; (K) Compression load cell attached to the support J on its upper side; (L) Compression upper surface. (Fig. 1d) A schematic figure showing the unconfined compression test. (Fig. 1e) Typical stress–strain curve for soft materials.
2. Materials and Methods

2.1. Preparation of collagen matrices

Type I collagen was extracted from rat tendon in 0.5-M acetic acid solution and adjusted at a concentration of 5 mg/ml. The solution was then centrifuged at 50,000 G up to 3 hr, best clearing time to eliminate collagen aggregates in the form of small pellets. Collagen solutions were poured into Petri dishes and progressively concentrated up to 20 or 40 mg/ml by slow evaporation of the solvent under a laminar flow bench in sterile conditions, during one week at 20° C. Collagen concentration was estimated at the end of the process by hydroxyproline filtration. The collagen solutions were at that time still acidic.

To prepare the matrices, 2 ml of acid-soluble collagen solutions at 5, 20, or 40 mg/ml were poured in polymethyl methacrylate molds of dimensions 4 × 20 × 24 mm. The samples were covered with a cover slip to avoid meniscus formation and gelated under concentrated ammonia vapors for 24 H at room temperature. The resulting matrices, 4.8 cm² in surface and 4 mm in thickness, were rinsed in PBS until the pH was 7.4 in order to mimic exact physiological conditions.

2.2. Collagen matrix analysis in SEM and TEM

In TEM, the collagen matrices were fixed in 2.5% glutaraldehyde for 1 hr, rinsed in three cacodylate buffer baths (0.05-M cacodylate, pH 7.4, 0.3-M saccharose) for 10 min, and post fixed in 2% osmium tetroxide for 1 hr. The samples were then dehydrated through successive ethanol baths (50%, 70%, 95%, and 100%), a propylene oxide bath, and embedded in araldite resin. An ultramicrotome (Ultracut Reichert-Jung) was used to section the block into 70-nm thin sections. The sections
were stained with phosphotungstic acid and observed with a Philips transmission electron microscope operating at 100 kV. For analysis in SEM, the collagen matrices were fixed as described above. The samples were dried by critical point technique. Samples were covered with gold pulverization under primary vacuum. SEM was performed on Jeol microscopes operating at 30 kV.

2.3. Mechanical testing

Temperature and humidity were controlled through an insulator thermostatic chamber whereas a “real-time” computer is used to acquire data and to drive the applied displacement rates. All specimens were maintained hydrated in sterile media and were compression-tested in the day following the preparation of the matrices at displacement rates ranging from $V_1 = 0.01$ to $V_4 = 10 \text{ mm/s}$. The mechanical tests were performed under a specific device designed and manufactured in our laboratory. (Fig. 1a, b, c). It consists in two parallel horizontal plates (L, B-C), both jointed to a Newport® monitored vertical (H) and horizontal (A) linear stage designed for self-supporting applications with particular travel ranges varying from 300 to 600 mm. Each plate is attached to a load cell, respectively 10 N for the shear stress (D) and 20 N for the compression stress (K), and their displacement rate is specified by the ESP300 1–3 Axis Motion Controller (Newport). To avoid any sliding of the samples at large strain, the two faces are in contact with water impermeable sandpaper that offers a sufficient granulometry in order to prevent any damage of the sample.

Suppose the original cross-sectional area of the prismatic sample is $A_0$ and the applied compressive load is $F$, then the axial component of the first Piola-Kirchhoff stress tensor $T$ is given by $T = F/A_0$. If the original length of the sample is $L_0$, the displacement is given as $\Delta L = L_0 (\lambda - 1)$, where $\lambda$ is the stretch ratio and $\Delta L \leq 0$ (see Fig. 1d). Many soft tissues have the same general nonlinear stress-strain curve as those we have seen for ligaments, tendons, blood vessels, and the cartilage solid matrix. This “J-shaped” stress-strain relationship is schematically illustrated in Fig. 1e; and the elastic modulus is deduced by measuring the slope of the stress-strain relationship, with respect to a stretch ratio $\lambda$ ranging from 0.9 to 1 (see Fig. 1e). One can also define the strain rate $\dot{\varepsilon}$, with regards to materials science, as the change in strain over the change in time and is denoted as $\dot{\varepsilon} = \delta \varepsilon / \delta t = (1/L_0)(dL/dt) = V/L_0$, where $V$ is the displacement rate of the impermeable platen (Fig. 1d).

3. Results

3.1. Microscopic characterization of the collagen matrices

Fibrillar collagen matrices are analyzed in SEM to observe the collagen fibril order and compaction. At low magnification, the differences in porosity of the samples appear as a function of concentration. At 5 mg/ml, the average diameter of pores is
Fig. 2. SEM of fibrillar collagen matrices at two concentrations of 5 mg/ml (a, c) and 40 mg/ml (b, d). At low magnification, the differences in porosity of the samples appear as a function of concentration (a, b). At high magnification, individual fibrils are seen. a, b: bar, 100 µm; c, d: bar, 1 µm.

50 µm (Fig. 2a); at 40 mg/ml, pores stay well below 10 µm in diameter (Fig. 2b). At high magnification, individual fibrils are seen (Fig. 2c, d) and confirm a difference in fibril diameter with respect to the associated concentration.

Fibrillar collagen matrices are analyzed in TEM. At low magnification, the differences in density and local alignments of the fibrils appear as a function of the two collagen concentrations of 5 mg/ml and 40 mg/ml (Fig. 3a, b). At high magnification, individual banded collagen fibrils are seen attesting the presence, at both concentrations, of a biomimetic fibrillogenesis assembly process (Fig. 3c, d).

3.2. Mechanical behavior of the collagen matrix under unconfined compression

Change the sample and redo the compressive tests reveal negligible scatter (Fig. 4a). The uniaxial compressive stress versus strain response at fixed concentration C2 = 20 mg/ml and at various applied displacement rates, V1 = 0.01 mm/s, V2 = 0.1 mm/s, V3 = 1 mm/s, and V4 = 10 mm/s offer a good repeatability. One can note the high sensitivity of the stress–strain response to the applied
displacement rate (Fig. 4a, b), typical of biological soft tissues viewed as multiphase materials. Apart from a number of other structural-related features, they are characterized by a rate-dependent material behavior (Fig. 4a, b), which is attributed to fluid–solid interactions as well as intrinsic viscoelastic properties of the solid matrix. The results of Fig. 5 are organized such that the applied displacement rate is fixed. Then, in each case, one notes: (a) an increase of the global stiffness of the biomaterial as collagen concentration increases. To be more precise, one can see that for a given macroscopic stretch ratio, the corresponding macroscopic stress is higher with respect to the increasing concentration; (b) the stress–strain response of both abdominal rat skin (ARS) and dorsal rat skin (DRS) are comprised between the lowest C1 and the intermediate C2 collagen’s concentrations except for the case where the applied displacement rate is $V_4 = 10 \text{mm/s}$. In particular, it is clearly shown that the ARS response is very close to the lowest concentration response $C1 = 5 \text{mg/ml}$. It is also possible to superpose our data at different velocities as it is sometimes done in rheology experiments. Thus, one can appreciate the strain rate effect upon the stress–strain response with respect the concentrations C2, C3, and the ARS and DRS Fig. 6. One can observe that for tissue strains exceeding 10%,
Fig. 4. (a) Repeatability of the axial stress–axial stretch ratio curves obtained at the same concentration $C_2 = 20\, \text{mg/ml}$ and four ramp displacement rates $v_1 = 0.01\, \text{mm/s}$, $v_2 = 0.1\, \text{mm/s}$, $v_3 = 1\, \text{mm/s}$, and $v_4 = 10\, \text{mm/s}$. Each test is performed twice and one can appreciate the repeatability of our tests. The ramp displacement effect is clearly shown denoting the viscoelastic character of our biomaterial. (b) Ramp displacement rate effect upon the axial stress–axial stretch curves for the lowest collagen concentration $C_1 = 5\, \text{mg/ml}$. One can see the sensitivity to the ramp rate displacement. For a given concentration, the stress level increases with the specified ramp displacement rate.
Fig. 5. (a) Axial stress–axial stretch response for the ramp displacement rate $v_1 = 0.01 \text{ mm/s}$. The response of the concentration $C_1 = 5 \text{ mg/ml}$ is close to the one exhibited by the ARS in the same conditions. (b) Axial stress–axial stretch response for the ramp displacement rate $v_2 = 0.1 \text{ mm/s}$. The response of the DRS is comprised between $C_1 = 5 \text{ mg/ml}$ and $C_2 = 20 \text{ mg/ml}$ in the same conditions.
Fig. 5. (c) Axial stress–axial stretch response for the ramp displacement rate $v_3 = 1$ mm/s. The response of the lowest concentration $C_1 = 5$ mg/ml is close to the one exhibited by the ARS, whereas the DRS response is still comprised between concentration $C_1 = 5$ mg/ml and concentration $C_2 = 20$ mg/ml, in the same conditions. (d) Axial stress–axial stretch response for the ramp displacement rate $v_4 = 10$ mm/s. The response of the lowest concentration $C_1 = 5$ mg/ml is also close to the one exhibited by the ARS, whereas the DRS response is lower than those presented by the lowest concentration $C_1 = 5$ mg/ml and the intermediate concentration $C_2 = 20$ mg/ml, in the same conditions.
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Unconfined compression test
Axial stress - Axial stretch response at various applied displacement rate for (C2=20 mg/ml)

Unconfined compression test
Axial stress - Axial stretch response at various applied displacement rate for (C3=40 mg/ml)

Fig. 6. Ramp displacement rate effect upon the axial stress–axial stretch ratio. (a) C2 = 20 mg/ml, (b) C3 = 40 mg/ml, (c) ARS, and (d) DRS.
Unconfined compression test
Axial stress - Axial stretch response at various applied displacement rate for (ARS)

Unconfined compression test
Axial stress - Axial stretch response at various applied displacement rate for (DRS)

Fig. 6. (Continued)
the deformation enters the nonlinear elastic region of the stress–strain curve. As the
load is increased, the exponential stress–strain behavior suggests a strain hardening
effect. This strain hardening has also been observed in elasticity measurements of
anterior cruciate ligaments, the aorta, psoas major tendon, and pericardium. Moreover, the critical stretch ratio $\lambda_{Cr}$ at which the stress–strain curve makes the
transition from the initial modulus to the strain-hardened final modulus is calcu-
lated by intersecting the initial and final tangents to each curve (Fig. 1e) and is
given in Table 1. An important question that arises from this study: for some cases,
does “strain softening” occur? The answer is not obvious because conditioned by
the complexity of studied biomaterials. What is well argued is that deformation-
induced softening is an inelastic phenomenon frequently accompanying mechanical
testing of soft biological tissues; and Mullins effect is a well-known softening
phenomenon in rubber-like materials? Strain softening also occurs: (a) in polymer
solutions and melts, because of the “freeing up” of interactions between long
molecules and microstructures in the tissues when larger strains are applied and (b)
in central nervous system tissue, as a result of reductions in intermolecular forces
as they move apart, failure of microscopic adhesions, or loosening of entanglements
between adjacent cells and their processes.

Few particular curves exhibit singular point from which a change in the curva-
ture is operated (strain softening). This point of inflexion associated to the stretch
ratio $\lambda_{If}$ has been measured in each case: (Fig. 5b, C3, $V_2$, $\lambda_{If} = 0.522$); (Fig. 5c, C3,
$V_3$, $\lambda_{If} = 0.578$); (Fig. 5d DRS, $V_4$, $\lambda_{If} = 0.475$). We assume that the inflection
points are mainly due to the combination of complex fibril deformation mecha-
nisms. Indeed, what is well known is that collagen fibrils are stiff in tension but
buckle in compression explaining their negligible resistance as the compression force
stands parallel to the fibril direction. The fibrils are linked to each other via
cross-links, and they can stretch, compress, or rotate at these points. Collagen fib-
rils in the samples are typically thin and long, as shown in our SEM and TEM
observations, with a diameter ranging from 50 to 100 nm and a length between 1
and 10 $\mu$m.

From our J-shaped stress–strain curves, an initial linear elastic region of tissue
stress–strain curves was observed for strains up to 5–10%, and from which the
elastic modulus is calculated (see Fig. 1e and Table 2). The results of the Fig. 7a
reveal the variation of elastic modulus $E$ as a function of the strain rate, for various
concentrations and both ARS and DRS. The measures of the tests are summarized

<table>
<thead>
<tr>
<th>Critical stretch $\lambda_{Cr}$</th>
<th>$V_1$</th>
<th>$V_2$</th>
<th>$V_3$</th>
<th>$V_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.62</td>
<td>0.731</td>
<td>0.739</td>
<td>0.755</td>
</tr>
<tr>
<td>C2</td>
<td>0.721</td>
<td>0.743</td>
<td>0.850</td>
<td>0.882</td>
</tr>
<tr>
<td>C3</td>
<td>0.750</td>
<td>0.799</td>
<td>0.884</td>
<td>0.957</td>
</tr>
<tr>
<td>ARS</td>
<td>0.616</td>
<td>—</td>
<td>0.626</td>
<td>0.649</td>
</tr>
<tr>
<td>DRS</td>
<td>—</td>
<td>0.666</td>
<td>0.644</td>
<td>0.776</td>
</tr>
</tbody>
</table>
Table 2. Measured elastic modulus.

<table>
<thead>
<tr>
<th>Young modulus E (kPa)</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.0847</td>
<td>0.583</td>
<td>1.8562</td>
<td>3.4285</td>
</tr>
<tr>
<td>C2</td>
<td>8.3404</td>
<td>14.722</td>
<td>16.553</td>
<td>40.057</td>
</tr>
<tr>
<td>C3</td>
<td>21.515</td>
<td>27.950</td>
<td>29.372</td>
<td>77.644</td>
</tr>
<tr>
<td>ARS</td>
<td>0.520</td>
<td>—</td>
<td>0.609</td>
<td>4.099</td>
</tr>
<tr>
<td>DRS</td>
<td>—</td>
<td>2.831</td>
<td>6.176</td>
<td>15.304</td>
</tr>
</tbody>
</table>

in log-log representation because the values of the strain rate seem to span three decades and leads to a better qualification of the power-law-type dependence of the elastic modulus $E$ versus applied strain rate $\dot{\varepsilon}$. Thus, one can write:

$$E = A \dot{\varepsilon}^n \Rightarrow \log(E) = n \log(\dot{\varepsilon}) + \log(A),$$

where both the exponent $n$ and the coefficient $A$ have been identified and reported in Table 3. One can see Table 3 that the coefficient of determination $R^2$ is weak for two cases: the concentration C3 and the ARS samples.

Concerning the elastic modulus, our measurements are summarized in Table 2, and one can try to compare the orders of magnitude found here to data from the literature. Simple 1D ultrasound elasticity measurements were previously performed on muscle and liver and compared with independent and established mechanical measurements (Instron universal testing instrument) to investigate both the accuracy and consistency of ultrasound elasticity measurements. Under unconfined compression, it was found that the average ultrasound and Instron elastic modulus of muscle samples was $2.12 \pm 0.91 \text{kPa}$ and $1.53 \pm 0.31 \text{kPa}$, respectively, with an average relative error of 35%. The average ultrasound and Instron elastic modulus of liver samples was $0.62 \pm 0.24 \text{kPa}$ and $0.94 \pm 0.65 \text{kPa}$ with an average relative error of 29%. The average ultrasound and Instron elastic modulus of PVC samples was $33.77 \pm 5.49 \text{kPa}$ and $39.97 \pm 12.09 \text{kPa}$ with an average relative error of 16%. Conventional mechanical test machines are used to perform uniaxial tensile and compressive stress versus strain tests at low to medium strain rates ($10^{-3} \text{s}^{-1}$ to $10 \text{s}^{-1}$). Lee et al. have investigated the effects of four cross-linking methods on the compressive stiffness of collagen–glycosaminoglycan (CG) matrices and the interaction between adult canine articular chondrocytes and the matrix: dehydrothermal treatment (DHT), ultraviolet irradiation (UV), glutaraldehyde treatment (GTA), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC). The uniaxial unconfined compressive stress–strain relation was remarkably linear with coefficients of determination above 95%. The slope of the linear fit to the stress–strain data was used to define the “apparent compressive modulus” of the matrices. This method yielded moduli ranging from $1.45 \pm 0.23 \text{kPa}$ (mean SEM) for the DHT matrices to $11.17 \pm 1.09 \text{kPa}$ for the EDAC matrices. Compared to the minimally cross-linked DHT matrices, the compressive modulus was doubled by UV and GTA cross-linking protocols and increased another threefold by the EDAC cross-linking protocol. More recently, Myung et al. presented the systematic development of
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Elastic modulus versus Strain rate

![Graph showing elastic modulus versus strain rate](image)

Log$_{10}$(Strain rate) s$^{-1}$

(a)

Elastic Modulus versus Concentration

![Graph showing elastic modulus versus concentration](image)

(b)

Fig. 7. (a) Elastic modulus versus axial strain rate for various collagen concentrations and rat skin (ARS: abdominal rat skin, DRS: dorsal rat skin). (b) Elastic modulus versus collagen concentration for various ramp displacement rates.
mechanically enhanced interpenetrating polymer network (IPN) hydrogels with elastic’s moduli, obtained from tensile tests, rivaling those of natural load-bearing tissues. The modulus enhancement ranged from two-fold to over 10-fold depending on the synthesis conditions used. Variation of the network parameters and swelling conditions enabled “tuning” of the hydrogels’ physical properties, yielding materials with water content between 58% and 90% water, tensile strength between 2.0 and 12.0 MPa, and initial elastic’s modulus between 1.0 and 19.0 MPa. Under physiologic pH and salt concentration, these materials attain “biomimetic” values for initial elastic’s modulus in addition to high-tensile strength and water content. As such, the authors claim that they are promising new candidates for artificial replacement of natural tissues such as the cornea, cartilage, and other load-bearing structures.

The stress–strain response of the cat spinal cord to compressive indentation has been investigated in vivo in a single study. In that study, Hung et al. reported a characteristic “J-shaped” stress–strain relationship, similar to studies of spinal cord under tension. They found a region of linear stiffness (approximately 5 kPa) at displacements below approximately 0.5 mm, and no apparent change in stress–strain response with increasing strain rate; however, they used very slow (<0.0084 s\(^{-1}\)) strain rates.

The variation of elastic modulus \(E\) as a function of the collagen’s concentration at different strain rates is presented in Fig. 7b, and one can observe that whatever the specified strain rate, the variation remains linear with comparable slopes, except for the highest strain rate. Taking advantage of the above results, let the elastic modulus be a linear function of the collagen’s concentration as \(E = \alpha C + \beta\), where the coefficients \(\alpha\) and \(\beta\) have been identified and given in Table 4 with respect to their coefficients of determination \(R^2\).

<table>
<thead>
<tr>
<th>Samples</th>
<th>(n)</th>
<th>(\text{Log}(A))</th>
<th>(A)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.5324</td>
<td>0.4612</td>
<td>1.586</td>
<td>0.9452</td>
</tr>
<tr>
<td>C2</td>
<td>0.2095</td>
<td>1.4586</td>
<td>4.300</td>
<td>0.9216</td>
</tr>
<tr>
<td>C3</td>
<td>0.1694</td>
<td>1.7209</td>
<td>5.590</td>
<td>0.7996</td>
</tr>
<tr>
<td>ARS</td>
<td>0.2611</td>
<td>0.282</td>
<td>1.326</td>
<td>0.6385</td>
</tr>
<tr>
<td>DRS</td>
<td>0.3664</td>
<td>1.0298</td>
<td>2.801</td>
<td>0.9981</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Displacement rate (V)</th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>0.6148</td>
<td>-3.3407</td>
<td>0.9916</td>
</tr>
<tr>
<td>V2</td>
<td>0.7754</td>
<td>-2.3814</td>
<td>0.9897</td>
</tr>
<tr>
<td>V3</td>
<td>0.7783</td>
<td>-0.9365</td>
<td>0.9853</td>
</tr>
<tr>
<td>V4</td>
<td>2.1074</td>
<td>-5.2841</td>
<td>0.9944</td>
</tr>
</tbody>
</table>
Fig. 8. Axial stress–axial stretch ratio response for the highest ramp displacement rate $v_4 = 10 \text{ mm/s}$ affected by two specified shear ramp displacement rates. One can note the effect upon the stress level that remains insensitive to the specified shear rates.

Finally, taking advantage of our experimental device, we have performed an axial unconfined compression until 20% of deformation and then added a specified shear load for two lateral displacement rates. Quite interestingly, it appears in Fig. 8 that the presence of the shear stress leads to the increasing of the global stress in comparison to the uniaxial compression load alone. In addition, one can note that the shear rate induced a difference, as observed for some biological tissues undergoing slight changes versus rates of deformation effect. The combined shear with compression stresses is viewed as arising from surgical manipulation (suture technique) and/or particular situations, such as arteries that are subjected to axial forces, not necessarily uniform, caused by the surrounding tissue and to blood flow-induced shear forces due to wall friction.

4. Discussion

Collagen is known as a good candidate for many applications, as it is a biodegradable and natural material that may undergo tissue remodeling and ultimately be replaced with neo-collagenous tissue in vivo. However, a tissue-engineered product with excellent biological/chemical compatibility but which cannot withstand the mechanical loads incurred during typical conditions of use will not be clinically useful.\textsuperscript{13} The development of controlled collagen gel/scaffold constructs requires a clear understanding of the mechanical properties of the biomaterial, and data reported in this work should therefore enable the development of improved tissue analogs that meet specific mechanical demands. The present experiments allowed to characterize the elastic properties of 3D fibrillar collagen matrices, as a biomaterial
for tissue-engineering applications, with respect to both strain rate and/or collagen concentration and to compare them to rat dermis. Interestingly, SEM and TEM data allow direct correlations with material stiffness (elastic modulus). Moreover, microscopic analyses revealed that changes in collagen concentration affect both fibril length and diameter. In particular, the increase of the linear modulus with concentration was related to a decreased fibril diameter.

What is well known is that the sensitivity to applied strain rate is predominantly due to the fact that the collagen is known as a biphasic composite consisting of a fibrillar dense lattice structure filled with >99% fluid. Its mechanical behavior is then governed, in a large part, by the inherent viscoelasticity of the fibrils and their interaction with the fluid content. Indeed, unconfined compressive loading results in the rapid expulsion of the fluid phase to produce scaffolds with improved mechanical properties potentially suitable for direct implantation and suturing. Compressive properties are known as important to biological tissues, such as bone and articular cartilage. The present research was concerned with soft connective tissues such as skin, for which tensile properties prevail. Our goal was here to investigate complementary compressive properties of dense collagen matrices in addition to data sated in tension. Compression tests are pertinent in automotive accident research, and thoracic trauma and injury in particular. For example, in car accidents, one can note that concerning the elastic force caused by tissue compression, the percentage of the total force is larger in the belt loading case than in the impact loading case. Other applications range from predicting the penetration pressure of skin by a hypodermic needle\textsuperscript{24,25} or a high-speed liquid jet\textsuperscript{26} in order to administer an injection, to soft tissue damage due to car crashes and stabbing incidents.\textsuperscript{25} Only the strain rate associated with these applications ranges from less than 0.1 s\textsuperscript{-1} to above 1000 s\textsuperscript{-1}. There is a pressing need within the pharmaceutical industry to develop \textit{in-vitro} substitutes for skin in order to develop and test devices for drug delivery for example.\textsuperscript{24–27} It has been also reported by Hanley \textit{et al.}\textsuperscript{28} that CG scaffolds with equiaxed pores were mechanically isotropic under compression but were significantly stiffer in tension in the plane of the sheet (in compression $E_C = 2.08$ kPa; in tension $E_T = 20.00$ kPa), similar to previous investigations.\textsuperscript{29,30} The comparison between dry and hydrated (CG) scaffolds is given in the Table 5 with respect to different mean pore sizes. One can note that our measurements are of the same order magnitudes to those given in the above literature. The uniaxial compressive

<table>
<thead>
<tr>
<th>Mean pore size (µm)</th>
<th>Dry E (kPa)</th>
<th>Hydrated E (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>31.7 ± 3.9</td>
<td>2.06 ± 0.36</td>
</tr>
<tr>
<td>110</td>
<td>28.5 ± 3.4</td>
<td>1.76 ± 0.41</td>
</tr>
<tr>
<td>121</td>
<td>29.8 ± 3.9</td>
<td>2.21 ± 0.47</td>
</tr>
<tr>
<td>151</td>
<td>39.4 ± 4.5</td>
<td>2.29 ± 0.22</td>
</tr>
<tr>
<td>Mean pore size</td>
<td>30.0 ± 3.9</td>
<td>2.08 ± 0.41</td>
</tr>
</tbody>
</table>
responses of silicone rubber (B452 and Sil8800) and pig skin have been measured over a wide range of strain rates \( (0.004 - 4000 \, \text{s}^{-1}) \).\textsuperscript{31} The experiments reveal that pig skin strain hardens more rapidly than silicone rubbers and has greater strain rate sensitivity: pig skin stiffens and strengthens with increasing strain rate over the full range explored, whereas silicone rubber stiffens and strengthens at strain rates in excess of \( 40 \, \text{s}^{-1} \).

Christiansen \textit{et al.}\textsuperscript{32} analyzed collagen fibril diameter distributions and mechanical properties of the fibers formed under different incubation conditions. Their results indicated that fibril diameters grow \textit{via} the lateral fusion of discrete subunits (\( \sim 4 \, \text{nm} \)), and that fibril diameters correlate positively with low tensile strain modulus. Taking advantage of the fact that the relationship between molecular properties and tissue properties remains a scarcely explored aspect of collagen materials, Buehler\textsuperscript{33} used a molecular multi-scale approach and confirmed the significance of cross-links in collagen fibrils in improving its mechanical stiffness. Further, it is found that cross-links influence the nature of its large deformation and fracture behavior.\textsuperscript{33–35}

This research involving preparation of dense collagen tissue-like samples and their microscopic characterization and analysis by mechanical tests bring original data and offer validation before clinical applications. The widespread growth of numerical modeling in biomechanical research has placed a heightened emphasis on accurate data for soft biological tissues.\textsuperscript{36} The present results become also important for model's validation aspect. For one, quantification of viscoelastic stiffness is made with respect to rat dermis and it is clearly shown that our biomaterial has sufficient mechanical properties for direct implantation applications in tissue engineering and that they can even be sutured. In good agreement, preliminary (unpublished) data already have shown the good resistance of sub-cutaneous animal implants after four weeks. Theoretical approach should be possible in a near future to quantify the non-linearity using non-linear elasticity including the rate of deformation.\textsuperscript{37} Understanding and controlling mechanical properties, specifically softness, are important for appropriate physiological function in numerous contexts. The mechanical properties of the substrate on which, or within which, cells are placed can have as large an impact as chemical stimuli on cell morphology, differentiation, motility, and commitment to live or die.\textsuperscript{38} Tissue characterization and modeling based on biological samples need also to be investigated in order to provide more realistic results in virtual-based surgical simulations.

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2. Helary C, Ovtracht L, Coulomb B, Godeau G, Giraud-Guille M, Dense fibrillar col-