# Mechanical Stiffness Testing of Collagen Products

**Introduction:** Mechanical stiffness is a critical parameter to consider when designing a cell culture system. In the context of 2D or 3D cell culture, matrix stiffness can impact a wide variety of pathways including stem cell differentiation[1], cancer metastasis[2], and cardiac tissue function[3]. Furthermore, depending on the application, the structural integrity of a construct may be of importance, particularly in load bearing scenarios. Thus, as culture systems become more tissue specific, the need to quantify the mechanical stiffness of biomaterials becomes more present.

Among the most common methods for evaluating the mechanical properties of cell culture substrates is oscillatory shear rheology (OSR) which applies a sinusoidal shear stress profile to a sample and measures its complex shear rigidity ($G^*$) which can be separated into elastic storage (G') and viscous loss (G'') moduli. This method is especially well suited for soft extracellular matrices (ECM) samples and hydrogels as those substrates are designed to model viscoelastic tissues within the body. Another method commonly used to test biomaterial rigidity is nano-indentation by atomic force microscopy (AFM). Here, a micro-fabricated tip is indented into the surface of the sample. The resulting deflection of that tip from its principle axis is then measured and converted into a force-indentation curve by the AFM. This curve is used to calculate a tensile modulus (E) using an appropriate model. Because of its small scale, negative "squeezing" effects on the gel hydration do not arise in AFM and the sample can be hydrated throughout testing. In addition to OSR and AFM, contactless methods (CLM) for assessing material stiffness and other rheological properties offers a non-destructive and continuous means for material testing.

Advanced BioMatrix offers a wide variety of ECM products with a variety of mechanical stiffness' following polymerization of hydrogel. To aid in determining the right material for your application, we performed OSR, AFM, and CLM mechanical characterization to measure the shear and tensile moduli of several popular collagen products and determine what factors matter the most in selecting a culture substrate.

**Methods:** Relevant properties of Type I collagen products under test are summarized below (Table 1).

| Product Name/ Cat No. | Concentration (mg/mL) | Telopeptide or atelo peptide |
|---|---|---|
| PureCol® 5005 | 3 | atelo |
| Nutragen® 5010 | 6 | atelo |
| FibriCol® 5133 | 10 | atelo |
| TeloCol®-3 5026 | 3 | telo |
| TeloCol®-6 5225 | 6 | telo |

*Table 1: Description, concentration and form of collagen products.*

Dynamic Shear Rheometry was performed on a Bohlin CVO Rheometer (Malvern Panalytical) with a 20mm parallel plate geometry. Material was deposited within a 1mm gap between the stage and geometry and allowed to fully polymerize undisturbed at 37°C on the rheometer stage. All plateau moduli were captured within the material's linear viscoelastic region. Moduli were evaluated at 1Hz for all materials with the exception of PureCol® which was evaluated at 0.1Hz. Atomic Force Microscopy was conducted on an MPF-3D BIO AFM (Asylum Instruments) with a silicon nitride cantilever with a length, mean width and thickness of 200 µm, 28 µm and 0.5 µm respectively. Samples were formed on glass slides as per manufacturer instructions and submerged in deionized water for the duration of testing. Gels were indented at 2µm/s in 20µm X 20µm grids at multiple locations per sample. Young's modulus was determined by a Hertz fit applied to force/indentation curves. Contactless measurements (CLM) were carried out on an ElastoSens Bio2 (Rheolutions Instruments).

2 mL (PureCol®) and 4mL (all other) of sample was loaded into a custom cartridge and incubated within the ElastoSens at 37°C while continuous measurements were taken. Moduli were determined within the plateau region following full polymerization.

**Results:** Dynamic shear rheology was utilized to determine the material storage and loss moduli across a range of applied strains and frequencies. The resulting storage moduli G' are plotted below (Figure 1). Gel stiffness is largely governed by

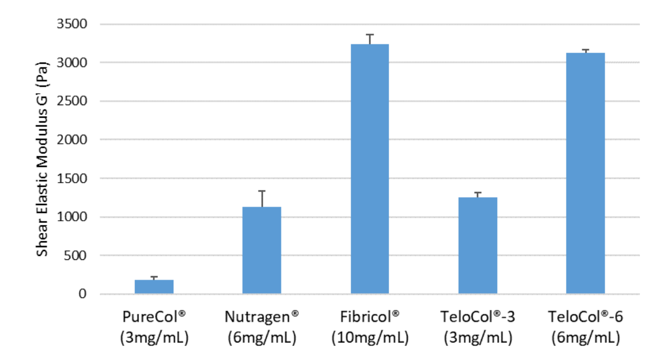


*Figure 1: Plateau storage modulus taken from parallel plate dynamic shear rheometry. All materials evaluated at 1Hz with the exception of PureCol® which was evaluated at 0.1Hz. Bars represent mean ± S.D. for n≥2 replicate samples.*

collagen concentration and the presence or absence of the telo-peptide region of the collagen molecule with low concentration materials such as PureCol® (3 mg/mL) exhibiting substantially reduced moduli. In contrast, high concentration products such as FibriCol® (10 mg/mL) show elevated moduli and produce stiffer gels. Telopeptide inclusive products (TeloCol®-3 and TeloCol®-6) also show increased moduli when compared to products of equivalent concentration by nearly an order of magnitude.

To determine the tensile behavior of collagen materials, nano-indentation was performed by AFM (Figure 2). The results similarly demonstrate a concentration dependence on gel tensile stiffness while TeloCol® products exhibited higher moduli than atelo products of equivalent concentration. Contactless measurement with the ElastoSens Bio2 (Figure 3) also validated the larger trend of conventional rheometry that collagen concentration and presence or absence of the telopeptide play and important role in gel stiffness.

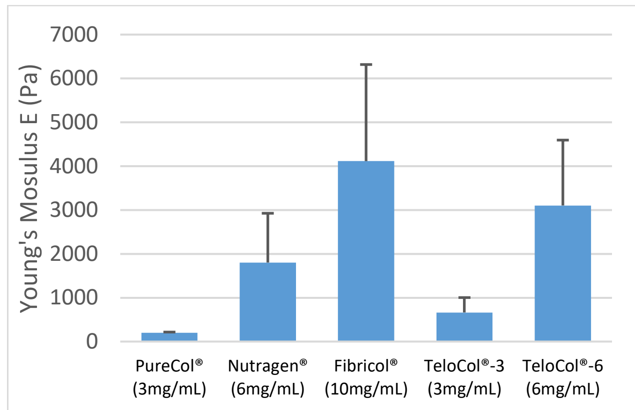


*Figure 2: Young's moduli determined by AFM stiffness testing. E calculated through application of a Hertz fit to force-indentation curves. Bars represent mean ± S.D. for n≥50 individual curves taken from two or more technical replicates.*

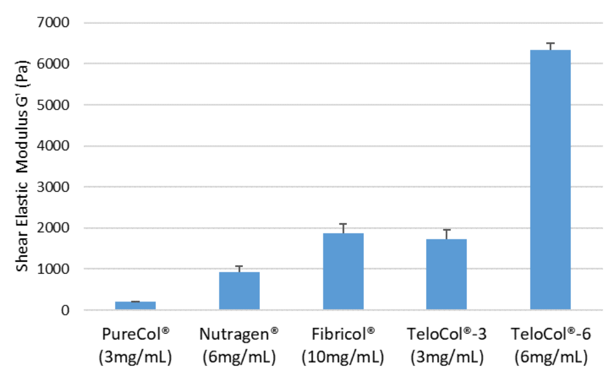


*Figure 3: Contactless measurement (CLM) of material rigidity with ElastoSens Bio2. Samples gelled within the ElastoSens unit and evaluated at the post-polymerize plateau region of G'. Bars represent mean ± S.D. for n≥2 replicates.*

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**Discussion:** This data demonstrates two key factors in selecting a collagen product based on its stiffness for cell culture applications. The first is a strong dependence on product collagen concentration which can attenuate the final stiffness of the gel in use. The second suggests that preservation of the telopeptide regions of collagen can also increase modulus possibly due to additional crosslinking between fibers[4]. Thus, a lower concentration can produce a stiffer gel if a telo-peptide containing collagen product is used.

Mechanical moduli are dependent on the stress conditions under which the material test was carried out. As such, OSR and ElastoSens (CLM) provide moduli in terms of shear stress (G) while AFM provided tensile moduli (E). Conversion between these parameters is given by the equation: $2G(1+\nu) = E$ where $\nu$ is the poisson ratio of the material. Converting the OSR and ElastoSens data into E by assuming a poisson ratio of 0.5, we note relative agreement between methods. Table 2 provides comparative results of Elastic modulus (G') and Young's modulus (E) of the rheological methods used to test mechanical strengths of collagen hydrogels.

| Method | OSR | CLM | OSR | CLM | AFM |
|---|---|---|---|---|---|
| **Units** | G' | G' | E | E | E |
| PureCol® | 184 | 195 | 552 | 585 | 201 |
| Nutragen® | 1127 | 931 | 3381 | 2793 | 1802 |
| FibriCol® | 3236 | 1874 | 9708 | 5625 | 4415 |
| TeloCol-3® | 1255 | 1738 | 3765 | 5214 | 664 |
| TeloCol-6® | 3122 | 6329 | 9366 | 18987 | 3103 |

*Table 2: Comparison of Elastic modulus (G') and Young's modulus (E) of OSR, CLM and AFM of various collagen products.*

The mechanical stiffness of collagen gels ranges between Young's moduli of 100Pa to 20kPa which covers many soft material applications and will guarantee a solid gel that can be manipulated if necessary. However, for stiffness in excess of 10kPa other materials may be required. Advanced BioMatrix offers higher concentration collagen as well as several photocrosslinkable ECMs and hydrogels better suited for high stiffness culture systems in the range of 10kPa to 100kPa as listed in Table 3. Taken together these data provide a basis for selecting a collagen product that abides by the mechanical necessities of your cell culture system.

| Product Name/ Cat. No. | | Description |
|---|---|---|
| PhotoCol® | 5201 | Methacrylated Collagen |
| PhotoHA® | 5220 | Methacrylated Hyaluronic Acid |
| PhotoGel® | 5215 | Methacrylated Gelatin |
| TeloCol®-10 | 5226 | Telopeptide Collagen, 10 mg/ml |

*Table 3: Photocrosslinkable and high concentration extracellular matrix (ECM) products*

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**References:**

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