Long-term Stability of PureCol® and Nutragen® Hydrogels in Cell Culture Medium

Highlights

- **Higher collagen concentration enhances stability:** PureCol lost 48% stiffness by 9 d, while Nutragen declined 24%.
- **Swelling affects stiffness:** PureCol's looser network absorbed more water, leading to decreased stiffness.
- **Hydrogels remained intact:** No visible loss of structural integrity despite stiffness reduction.

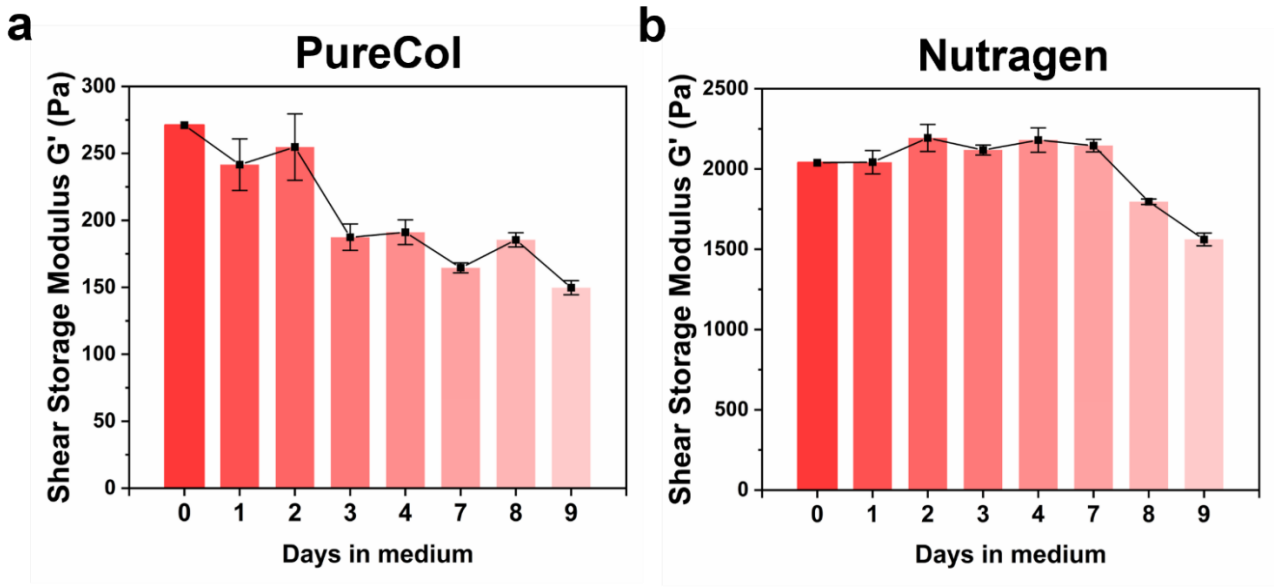

Figure 1. Stiffness change of crosslinked (a) PureCol and (b) Nutragen gels in Dulbecco's Modified Eagle Medium (DMEM) over 9 days.

Introduction

Collagen, a primary extracellular matrix component, provides structural support and biochemical cues that regulate cell behavior.[1] However, in physiological conditions, collagen hydrogels may degrade or undergo structural changes over time, affecting their mechanical properties, porosity, and bioactivity.[2] The long-term stability of collagen hydrogels in cell culture medium is critical for their effectiveness in tissue engineering, regenerative medicine, and in vitro modeling.[3,4] Ensuring hydrogel stability is essential to maintain a consistent cellular microenvironment, support long-term cell viability, and accurately model in vivo conditions.[5]

In this study, we evaluated the long-term stability of PureCol (3 mg/mL) and Nutragen (6 mg/mL) type I bovine collagen hydrogels in cell culture medium by monitoring changes in stiffness over a 9-day incubation period. Both hydrogels remained structurally intact, with no visible signs of disintegration. However, both hydrogels exhibited a gradual decrease in stiffness over time. Particularly, PureCol, the lower-concentration gel, experienced a more pronounced stiffness loss of approximately 48%, whereas Nutragen maintained greater mechanical stability due to its higher collagen concentration.

Results and Discussion

As shown in Fig. 1, the stiffness measurements revealed distinct differences in the stability of the two collagen hydrogels. Following complete gelation at 0 d, PureCol exhibited an initial shear storage modulus (G') of approximately 250 Pa, while Nutragen, with its higher collagen concentration, started at around 2000 Pa. During incubation, both hydrogels showed a decline in stiffness. PureCol experienced a significant reduction, with G' decreasing by approximately 48% by 9 d. In contrast, Nutragen maintained relatively stable stiffness for the first 7 d, displaying only minor fluctuations. However, a noticeable decline was observed on 8 d and 9 d, resulting in an overall G' reduction of approximately 24%.

The observed stiffness reduction in both collagen hydrogels is likely attributed to water absorption, as both are susceptible to swelling. PureCol, with its lower collagen concentration, absorbed more water due to its relatively loose polymer network, leading to a greater loss of gel stiffness over time. In contrast, Nutragen's denser network provided better resistance to swelling, contributing to its comparatively greater mechanical stability during prolonged incubation. While swelling may have caused the softening of the gels, it could be advantageous for applications that benefit from hydrogel swelling, such as controlled drug release, growth factor delivery, and wound healing.[6] In future studies, we will quantify the degree of collagen gel swelling and analyze its correlation with the stiffness data obtained in this study.

In addition, the solution pH is a critical factor influencing gel stiffness, because pH affects the electrostatic interactions and charge density of collagen molecules, thereby influencing the gel's mechanical properties.[7] For collagen, a higher polymerization pH typically results in greater stiffness within the optimal gelation pH range of 7.0 to 7.4. In this study, the pH of both PureCol and Nutragen solutions was adjusted to ~7.15 prior to initial gelation, ensuring consistent conditions for comparing their stability.

Despite the reduction in stiffness, both hydrogels remained structurally intact, showing no visible signs of loss of structural integrity (Fig. 2). These results highlight the critical role of collagen concentration in maintaining hydrogel stability in the absence of cells, with higher concentrations offering greater resistance to mechanical degradation over time. Future studies will incorporate cells in the collagen gels to further explore how cell-material interactions influence hydrogel degradation and stability.

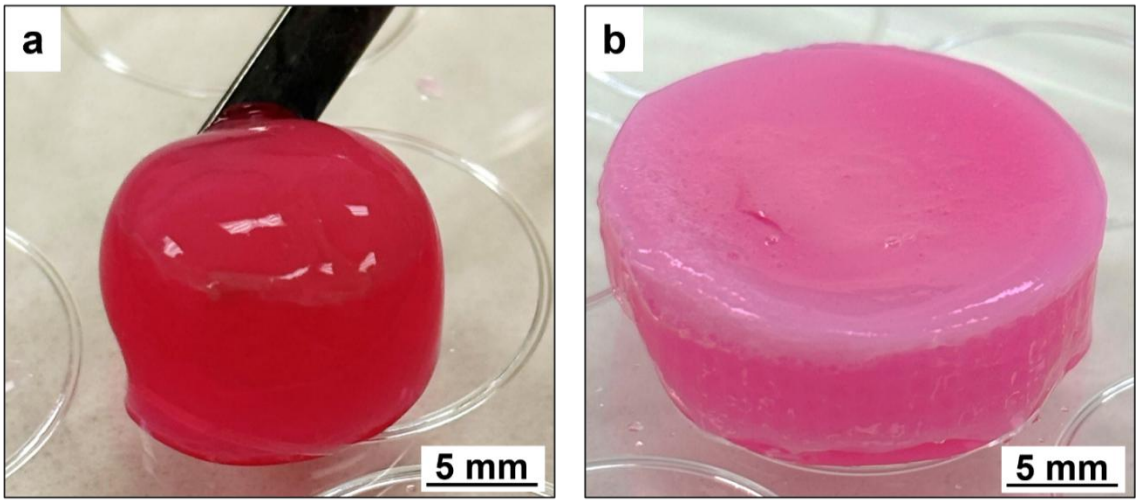

Figure 2. Physical appearance of (a) PureCol and (b) Nutragen gel after 9 days of incubation in DMEM. PureCol gel absorbed more DMEM and showed a darker color than Nutragen gel.

Conclusion

In this study, we evaluated the long-term stability of type I bovine collagen hydrogels at different concentrations in cell culture medium. The results indicate that hydrogel stiffness decreases over time in culture medium, with lower collagen concentrations (PureCol) exhibiting greater stiffness loss than higher concentrations (Nutragen). This suggests that collagen density plays a critical role in maintaining mechanical stability, which should be considered when selecting hydrogels for long-term cell culture applications.

Materials and Methods

Sample Preparation

PureCol® (Advanced BioMatrix, Cat #5005) and Nutragen® (Advanced BioMatrix, Cat #5010) working solutions were prepared according to the product direction-for-use. Briefly, 4 g of PureCol® or Nutragen®

solution was dispensed into a 15 mL test tube placed in an ice bath. Then, 0.5 g of 10X PBS was added to dilute the solution. The pH was adjusted to approximately 7.0–7.5 using 0.1 M sodium hydroxide. The tube was securely capped and gently inverted several times to ensure thorough mixing. The neutralized solution was then stored in the ice bath until use.

Rheology Measurement

For initial gelation at 0 d, the neutralized PureCol® or Nutragen® solution was dispensed into the measurement cup of ElastoSens Bio™ rheometer, and the cup was loaded into the rheometer for contactless rheology measurements at the constant temperature of 37°C (Table 1).

Table 1. ElastoSens testing sequence for initial gelation

Set temperature (°C)	Duration (min)	Reading step (min)
37	120	1

After complete gelation, the PureCol® and Nutragen® gels remained in their respective cups, with 0.5 mL and 1 mL of DMEM added to each, respectively. The cups were then incubated at a constant temperature of 37°C for 9 days. Every 24 hours, the cups were removed from the incubator, and the media was aspirated. The gels were then loaded into the rheometer for endpoint stiffness measurements (Table 2).

Table 2. ElastoSens testing sequence for endpoint stiffness reading

Set temperature (°C)	Duration (min)	Reading step (min)
37	10	1

Data Collection and Analysis

After complete gelation at 0 d, the stiffness of the same hydrogel sample was measured, with ten measurements taken at each time point. The final stiffness data are presented as the mean ± SD of all collected values.

References

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