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Pore Size of Lyophilized Atelocollagen Hydrogels

Highlights

- Higher collagen concentration (10 mg/ml) results in smaller pore sizes than lower concentrations (3 and 6 mg/ml).
- By varying collagen concentrations, pore size can be tailored to meet the specific requirements of different tissue engineering applications.

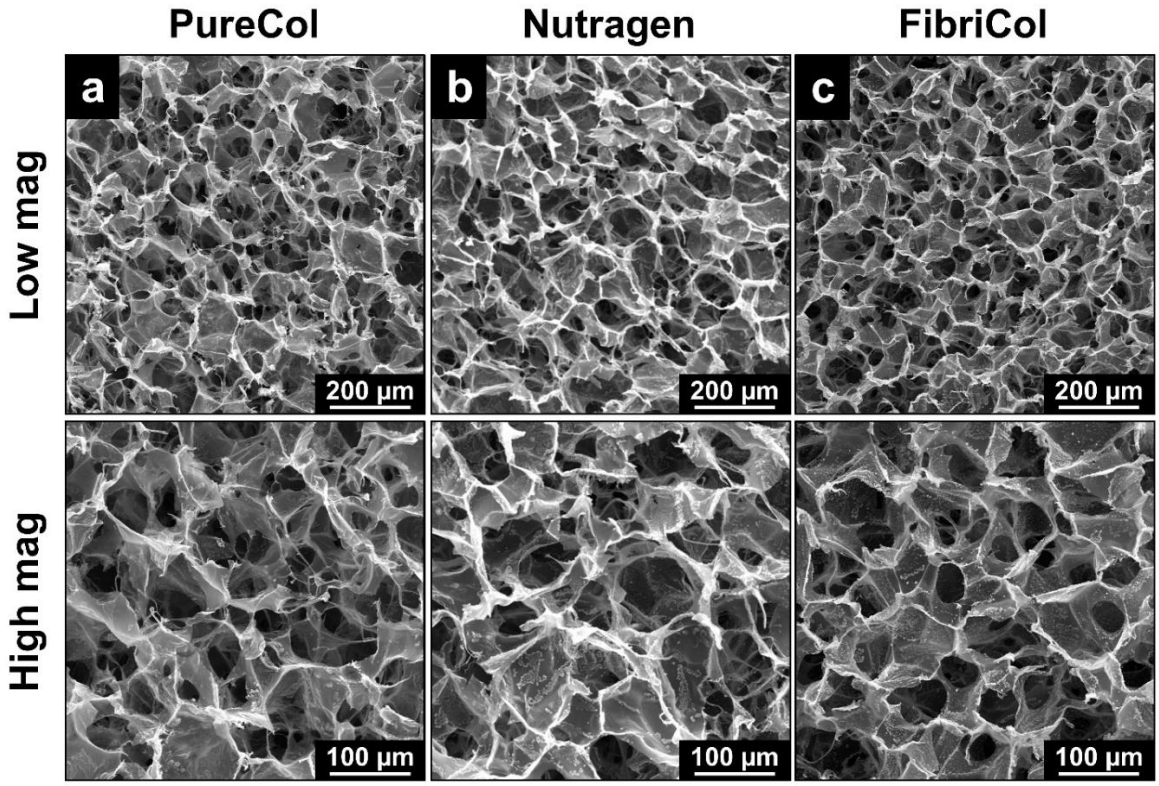

Figure 1. Scanning electron microscopy images showing the interconnected pore structures of (a) PureCol (3 mg/ml), (b) Nutragen (6 mg/ml), and (c) FibriCol (10 mg/ml) collagen hydrogels.

Introduction

Collagen hydrogels are widely utilized in tissue engineering due to their biocompatibility, biodegradability, and ability to mimic the extracellular matrix.[1] One critical factor influencing their performance for tissue engineering applications is pore size, which affects matrix stiffness and mechanical strength[2], cell infiltration[3] and migration[4], nutrient and waste diffusion[3], cellproliferation[4], and tissue formation and vascularization[5,6,7]. This study focuses on the evaluation of pore size in three distinct type I bovine atelocollagen hydrogels with varied

concentrations: PureCol® (3 mg/ml), Nutragen® (6 mg/ml), and FibriCol® (10 mg/ml). By combining scanning electron microscopy (SEM) imaging with quantitative pore size analysis, this study aims to provide insights into how collagen concentration influences the hydrogel microstructures and offers reference for selecting optimal pore sizes to enhance cell behavior in tissue engineering applications.

Results and Discussion

The SEM images shown in Fig. 1 illustrate the porous microstructure of three lyophilized collagen hydrogels: PureCol (3 mg/ml), Nutragen (6 mg/ml), and FibriCol (10 mg/ml). At both low and high magnifications, all samples exhibit interconnected porous networks typical of collagen scaffolds. PureCol and Nutragen present a relatively open structure with a looser pore network, exhibiting larger pore sizes and incomplete pore edges. In contrast, FibriCol, with the highest collagen concentration, displays a well-defined pore structure with the smallest pore sizes and a less fragmented pore edge, suggesting improved structural integrity.

To quantify pore sizes, we applied a measurement mask template to the SEM images (see Supplemental Material), placing lines across the pores and counting the number of pores intersected by these lines. As summarized in Fig. 2, PureCol had an average pore size of **129.28 ± 15.49 µm**, Nutragen had a similar average pore size of **131.86 ± 17.05 µm**, and FibriCol exhibited a smaller average pore size of **114.99 ± 19.19 µm**. While the mean pore sizes of PureCol and Nutragen were comparable, FibriCol showed a noticeably smaller average pore size with a broader distribution. It is important to note that these pores result from ice crystal growth during the freezing and ice sublimation during the lyophilization process, leading to larger pore sizes compared to other fabrication and measurement methods. For instance, Chen et al. [8] reported FibriCol pore sizes ranging from 0.8 to 5.7 µm at various concentrations, highlighting the influence of processing conditions and characterization on pore size outcomes.

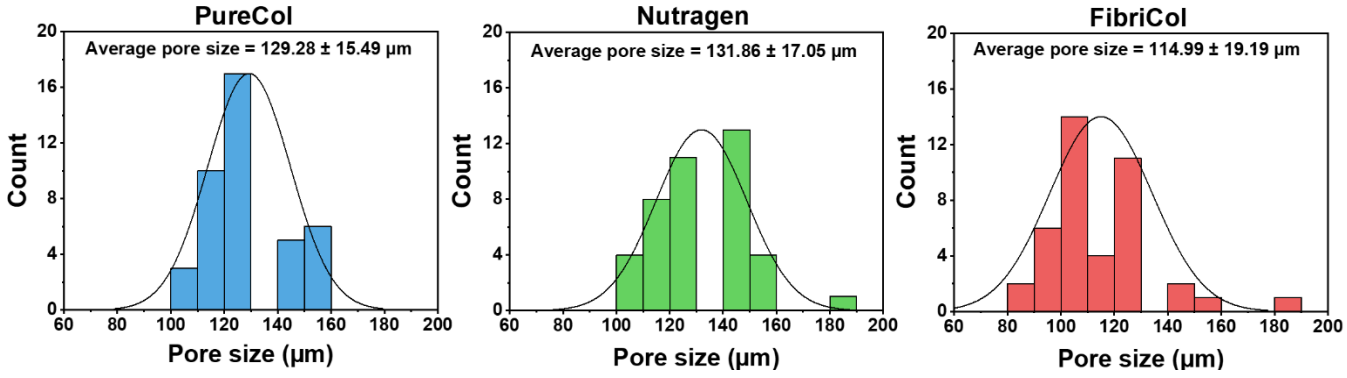

Figure 2. Measured and calculated pore sizes of PureCol, Nutragen, and FibriCol collagen hydrogels.

Aligned with our SEM observations, these results suggest that while PureCol and Nutragen appear to have relatively open structures with larger pores and thinner fibrils, FibriCol exhibits a denser appearance with thicker collagen strands and smaller pores. The smaller pore size in FibriCol may be attributed to its higher collagen concentration, which likely promotes greater fibril packing and reduced pore dimensions. Conversely, PureCol and Nutragen, with lower collagen concentrations, retain more open and less compact networks.

Summary

This study demonstrates that collagen concentration significantly influences hydrogel pore structure. SEM imaging and pore size analysis revealed that higher collagen concentrations, such as FibriCol (10 mg/ml), result in smaller pores, while lower concentrations, like PureCol (3 mg/ml) and Nutragen (6 mg/ml), produce larger pores with more open pore structures. The combination of these structural and quantitative findings highlights that the pore size of the collagen hydrogels can be tailored by different collagen concentrations. The varied pore size in collagen hydrogels could influence key properties such as cell infiltration[3], nutrient diffusion[3], and mechanical stability[2], which are crucial for tissue engineering applications. Therefore, we advise customers to carefully choose the right formulation to generate the appropriate pore size tailored to their specific application needs.

Materials and Methods

Collagen Gel Preparation

Type I bovine atelocollagen gels of PureCol®, Nutragen®, and FibriCol® from Advanced BioMatrix were prepared following their respective directions-for-use. Briefly, one part of chilled 10X PBS was added to eight parts of chilled collagen solution with gentle mixing or swirling. The pH of the mixture was then adjusted to approximately 7.4 using 0.1 M NaOH. Milli-Q water was added to bring the final volume to a total of ten parts. The neutralized gel solutions were then cast into a glass-bottom 24-well plate with 2 mL of solution per well. At least three samples were prepared for each collagen type. The solutions were allowed to gel at 37°C for 1 to 2 hours to ensure complete gelation, cooled at 4°C for 2 hours, followed by freezing at -20°C overnight (> 12 hours) before lyophilization.

Gel Lyophilization

After freezing, the gels were placed in a Magnum industrial lyophilizer (Millrock Technology, Inc., Kingston, NY) and subjected to a programmed freeze-drying cycle: (1) shelf freezing at -40°C for 60 minutes; (2) freeze-drying at -40°C with a gradual temperature increase to -20°C at a rate

of 1°C/min; (3) further temperature increase to 0°C; and (4) a final ramp to 24°C until all moisture was removed. The instrument continuously monitored the moisture level, and the samples were confirmed to be fully dried before being removed from the lyophilizer.

SEM Imaging

The freeze-dried gels were carefully removed from the well plate and sectioned into round discs approximately 2 mm thick using scalpels. Representative discs were collected from the top, middle, and bottom regions of the gels for SEM imaging. The discs were mounted onto aluminum stubs using conductive tape and sputter-coated with Pt-Au. Imaging was performed using an FEI Quanta 450 SEM at an excitation voltage of 20 kV. For each sample, three fields of view (FOV) were captured, with images acquired at 200× and 400× magnifications.

Pore Size Measurement

The SEM images at 200× magnification were imported into Microsoft PowerPoint, where a 6 × 7-inch rectangular mask template was applied. This template featured five evenly spaced horizontal lines with each 1.5 inch apart. The actual length of the line was normalized to the original scale bar in the image. The number of open pores (N) intersected by each line was recorded, and the pore size was calculated using the following formula:

$$\text{Pore size} = \frac{\text{Normalized line length } (\mu\text{m})}{\text{Number of pores, N}}$$

Data Analysis

The calculated pore sizes for each collagen type were plotted using Origin Pro 8.5 to illustrate the pore size distribution. The final average pore size was reported as the mean ± standard deviation (SD).

Acknowledgement

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Supplementary Material

Pore size analysis mask template: