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MDPI

OBTAINING MESH SIZES FOR VISCOELASTIC ALGINATE-BASED HYDROGELS USING FLORY THEORY AND SAXS

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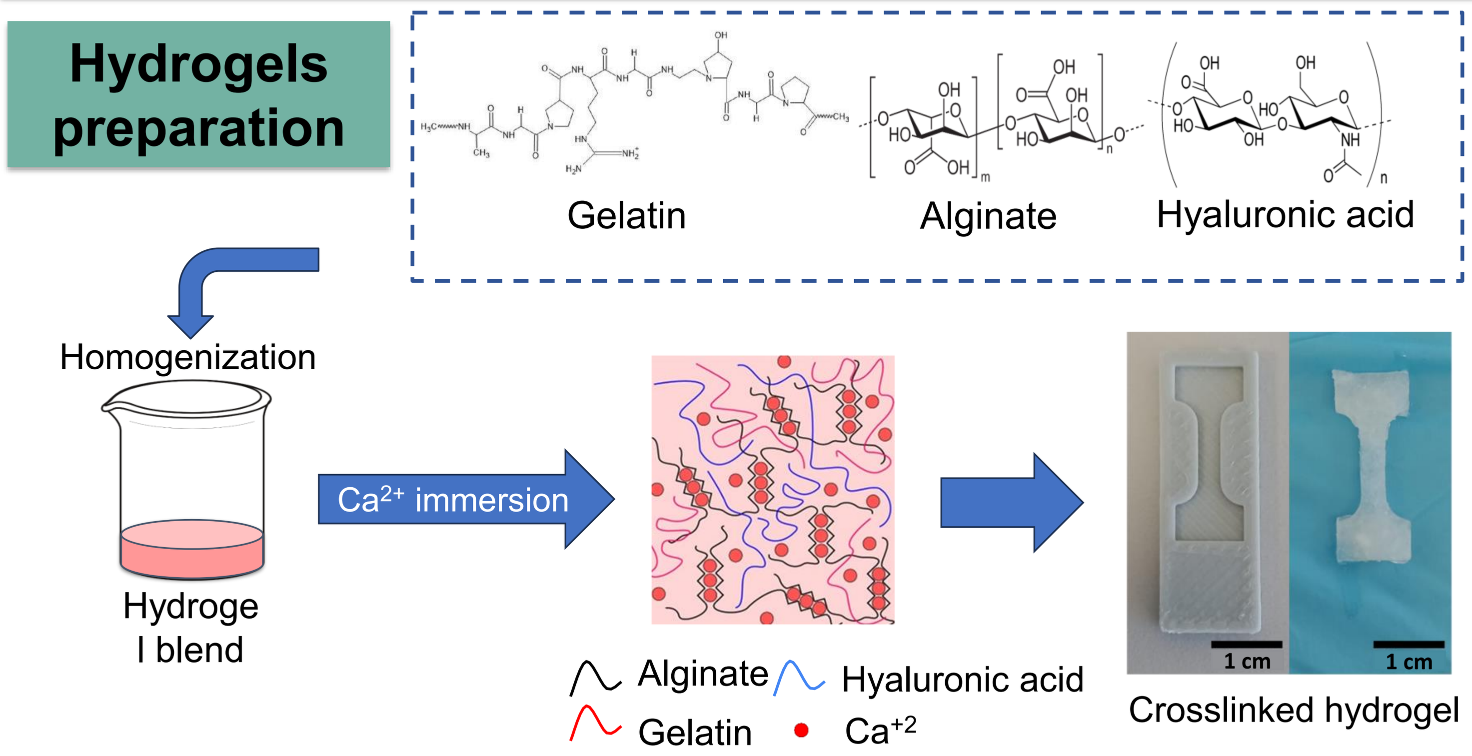
INTRODUCTION & AIM

Natural hydrogels are widely used to create scaffolds that emulate the extracellular matrix of different human tissues, due to their high-water content, good biocompatibility, and bioresorbability [1]. Cell adhesion is directly related to the properties of the scaffold on which the cells grow (artificial extracellular matrix). Some authors have shown that the tensile modulus and mesh size of the extracellular matrix are vital properties for cell proliferation [2][3]. This study aims to investigate the mesh size of a hydrogel composed of alginate (9% in 1X PBS), cross-linked with 0.5 M Ca^{2+}, and a hydrogel composed of 9% alginate, 4.5% gelatin, and 4.5% hyaluronic acid (AGH) [4]. The mesh size were estimated through mechanical test, the swelling behaviour and compared to SAXS analysis.

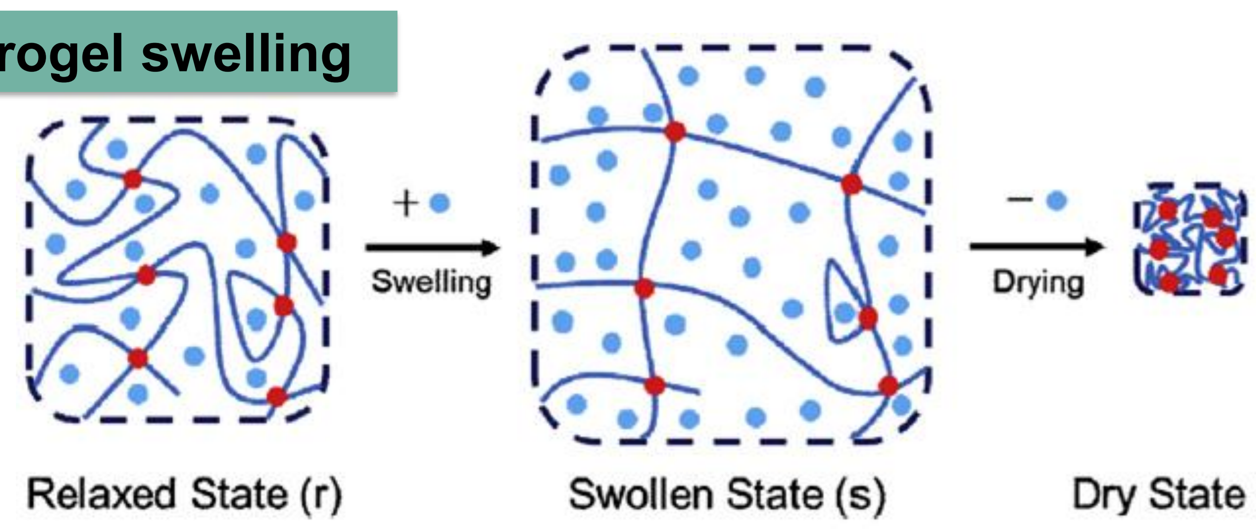
METHODS

Hydrogels preparation



The hydrogel is prepared by mixing 4.5% gelatin, 4.5% alginate, and 4.5% hyaluronic acid in 1× PBS (AGH), and then crosslinking with 0.5 M $CaCl_2$. Scaffolds were fabricated by molding in order to measure the mechanical properties and swelling.

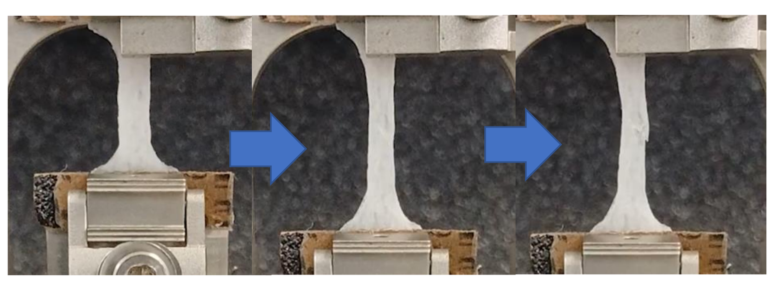
Hydrogel swelling



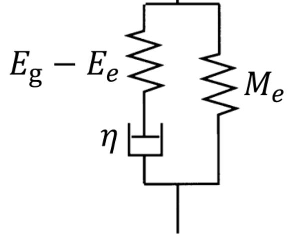
The swelling of the crosslinked hydrogels is measured by immersion in 1× PBS at pH 5. The scaffolds are weighed in the as-prepared, swollen, and dry states, and the polymer volume fraction (v) is calculated. Based on this value, and using Flory's theory, the mesh sizes are obtained.

CHARACTERIZATION RESULTS

Viscoelastic properties and M_c



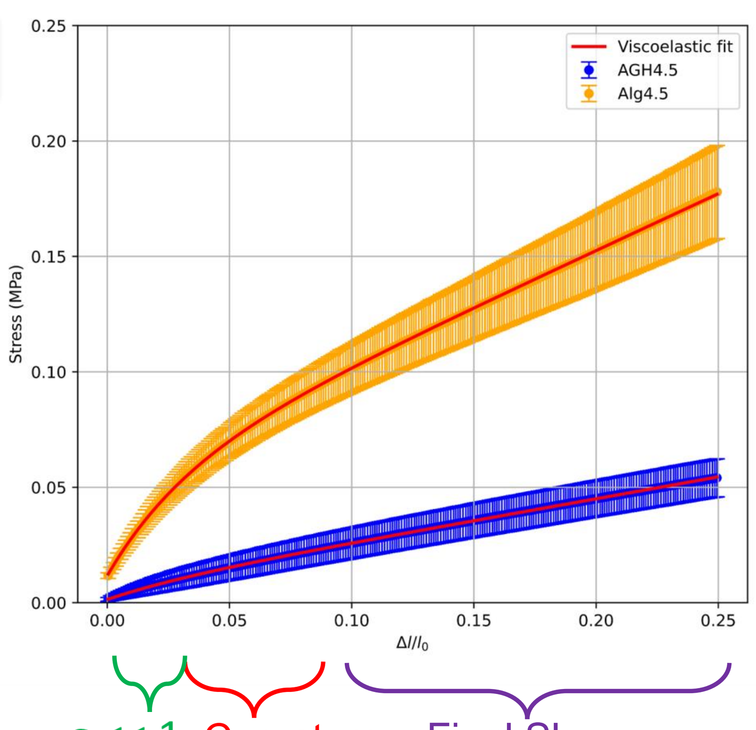
Mechanical Model



If ε << 1:

$$\sigma(\varepsilon) \approx \varepsilon E_g$$

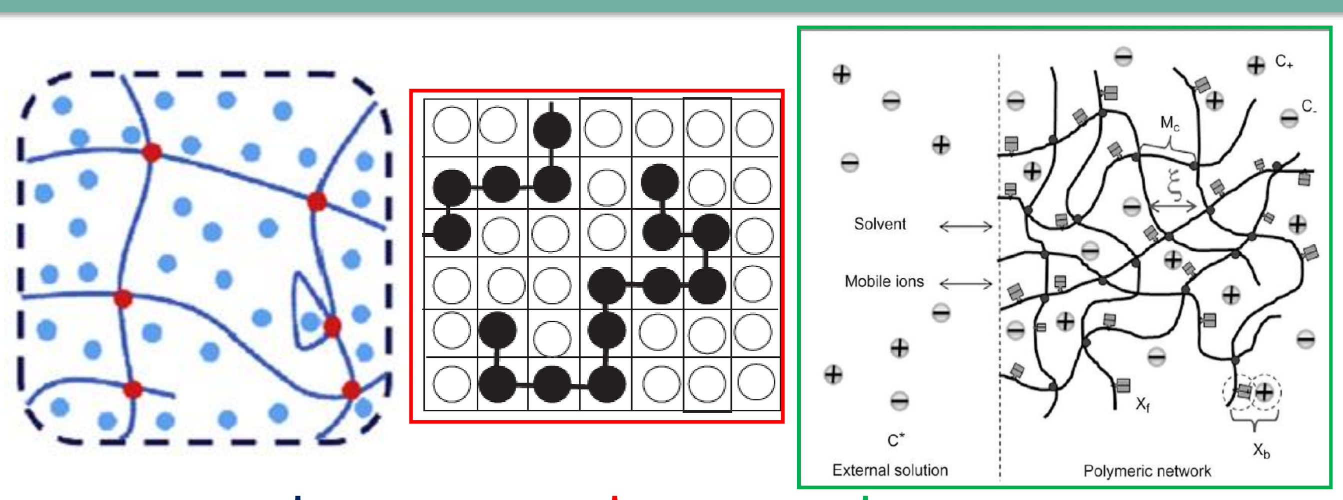
$$M_c \propto \frac{1}{E_g}$$



ε << 1 Curvature Final Slope

$$\sigma(\varepsilon) = (E_g - E_e)\,\tau\,v\,(1 - e^{-\frac{\varepsilon}{\tau v}}) + \varepsilon E_e$$

Flory theory for polyelectrolytes and the calculus of the mesh size



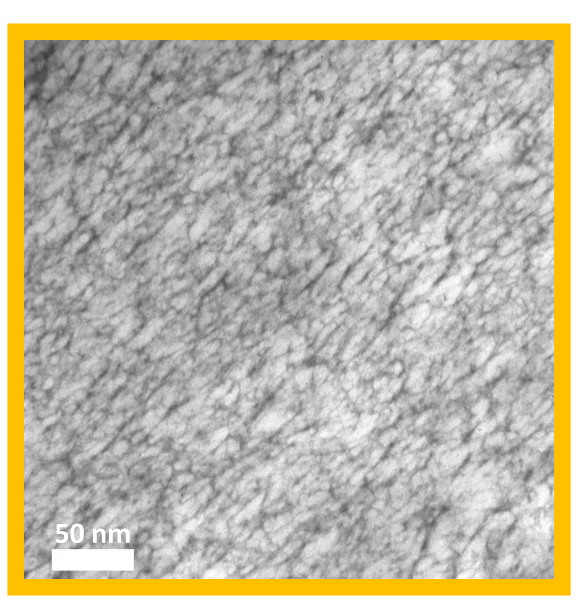
$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} + \Delta G_{ionic}$$

Mesh size

$$\xi(v) \propto v^{-\frac{1}{3}}\sqrt{M_c}$$

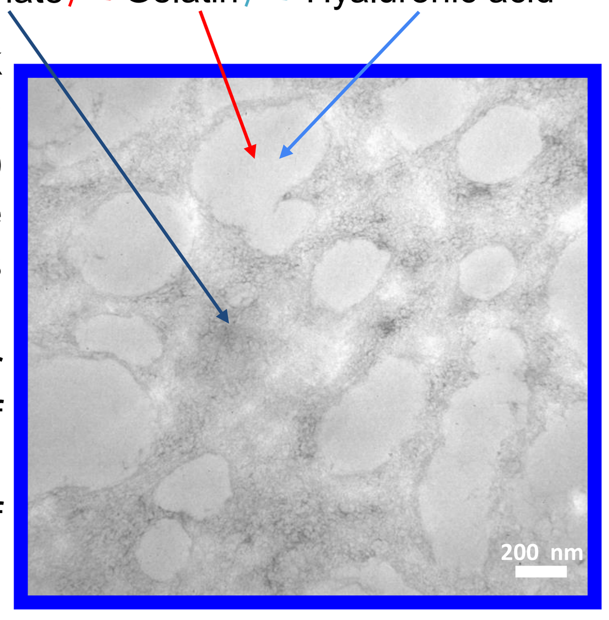
TEM: Morphological properties

Alginate



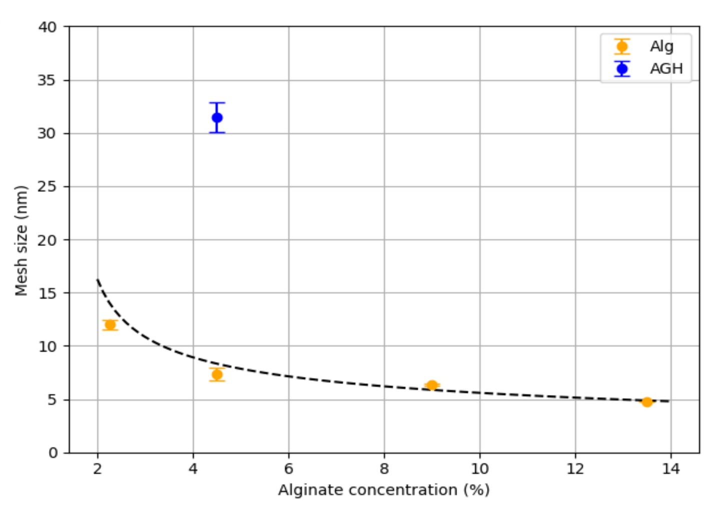
In the TEM images, dark regions with high electron density (Ca^{2+}-rich zones) are observed. For the alginate sample, structures with a size of 4.6±0.6nm are observed, whereas for the AGH sample cavities of gelatin and hyaluronic acid are observed, with sizes of 102±26 nm.

Alginate Gelatin Hyaluronic acid

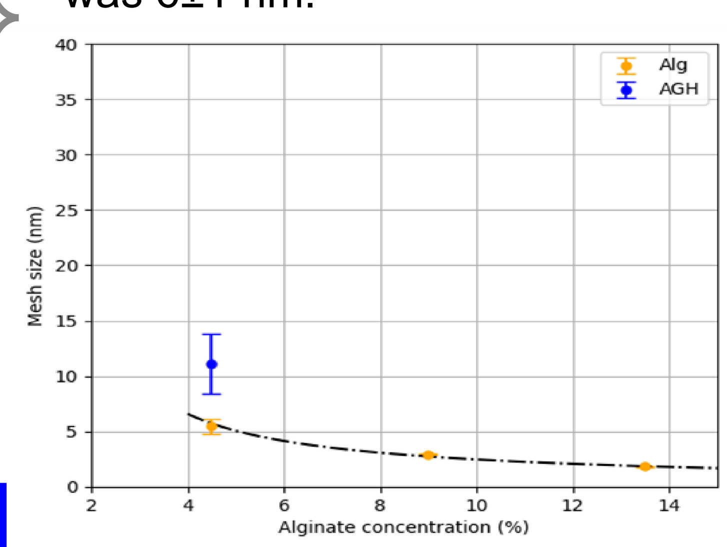


Mesh Size calculations

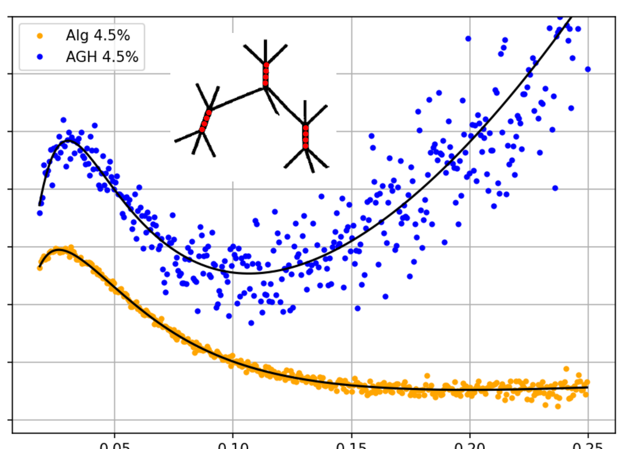
The mesh sizes were obtained for AGH and for alginate at different concentrations. The mesh size obtained for AGH was 32 ± 1 nm, whereas for 4.5% alginate it was 7.4 ± 0.6 nm.



The mesh sizes were obtained from swelling measurements using the Flory equations. The mesh size obtained for the AGH hydrogel was 12±3 nm, whereas for the 4.5% alginate hydrogel it was 6±1 nm.



SAXS Analysis: crosslinking sites size



Broken-rod Model

$$Iq^2 = A_1 q\left[\frac{J_0(qR_1)}{qR_1}\right]^2 + A_2 q\left[\frac{J_0(qR_2)}{qR_2}\right]^2 + A_3 q^n$$

Rod-like junction zones of radii R_1 and R_2

Free polymer strands

Sample	R_1 (nm)	R_2 (nm)
Alg	3.9±0.4	1.3±0.8
AGH	3.3±0.4	1.2±0.5

No correlation is observed with Alginate concentrations, hence R_1 and R_2 reported values for each sample correspond to the mean value over all %. The power law term reveals a greater proportion of uncrossliked polymer strands for the AGH sample than for pure Alginate.

DISCUSSION

In this work, different techniques were studied to obtain the mesh sizes of the polymers that make up an alginate hydrogel and the AGH hydrogel. The mesh sizes measured by swelling yielded values similar to those obtained by SAXS and can be associated with the alginate mesh sizes observed by TEM. Thus, both the swelling technique and SAXS can be said to probe structures on the order of tenths of a nanometer.

On the other hand, for the AGH hydrogel, the mesh size obtained from mechanical properties was 32 ± 1 nm, larger than the values obtained with the other techniques. This is reasonable, since TEM images show that in AGH not only mesh sizes of 4.6 ± 0.6 nm are present, but there are also cavities of 102 ± 26 nm. This indicates that the technique based on mechanical properties is more sensitive to larger cavities.

REFERENCES AND ACKNOWLEDGEMENTS

[1] Carrow J.K. et al., Polymers for Bioprinting, 2015, 1, 229-248.

[2] Ruppender NS et al., *PLOS One*, 2010, 5, e15451.

[3] Lee B-H. et al., *Acta Biomaterialia*, 2012, 8, 1693–1702.

[4] Palma J. et al., *Bioprinting*, 2024, 38, e00329.

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