Evaluation of the Influence of Physical and Chemical Factors on the Properties of Alginate-Gelatin-Chitosan Hydrogel for Tissue Engineering Application

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1 Introduction

The current research aims to investigate hydrogel biomaterials containing natural polymers for potential use as nerve conductors. Alginate (Alg), chitosan (CS), and gelatin (Gel) are often used to create materials that aim to mimic the natural extracellular matrix of native tissue[1]. They are biocompatible and biodegradable, at the same time their concentration, ratio and technological conditions of use have a huge impact on the physicochemical properties of the created hydrogel systems. Combining these biopolymers and selecting technological conditions for their creation provides an optimal composition that meets the requirements for materials of the specified direction.

2 Methods

The samples were synthesized with an Alg:Gel 3:1; 1:1; 1:3 ratios and at different pH values of their solutions: Alg/Gel = 10.0/4.0 and 7.0/7.0. The ultrasonic treatment applied during the synthesis increases the viscosity of the gelatin-containing emulsion and strengthens the interaction of hydrogen bonds and the hydrophobic interaction between gelatin proteins. The lyophilization process makes obtaining a mechanically stable porous polymer matrix possible. Cross-linking of polymer chains occurred through the interaction of their functional groups with Ca^{2+} ions and protonated NH^{3+} amino groups of CS (Fig.1)

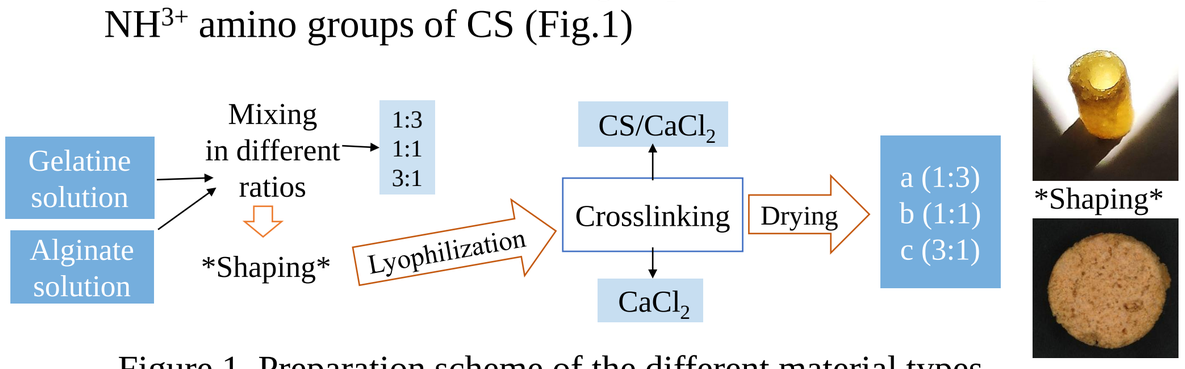

Figure 1. Preparation scheme of the different material types.

3 Results

An increase in the proportion of Gel leads to a decrease in the swelling degree (SW) from 550% (for Alg:Gel=3:1) to 400% (for Alg:Gel=1:3) in samples cross-linked simultaneously with CS and Ca ions. In samples cross-linked exclusively by Ca^{2+}, a sinusoidal curve of swelling in physiological solution is observed, which is related to the participation of Ca^{2+} in ion exchange processes and the reverse diffusion of Ca^{2+} due to the concentration gradient. SWs of these samples are generally smaller than that of samples cross-linked with CS and are 260-360%. However, the degradation degree (DD) is significantly higher than for samples cross-linked with CS. Degradation of samples synthesized at pH 7 begins after 24 hours, and samples synthesized at pH 4/10 - after 96 hours. Increasing the crosslinking time with Ca^{2+} from 20 minutes to 24 hours significantly increases the time before the degradation of the samples begins.

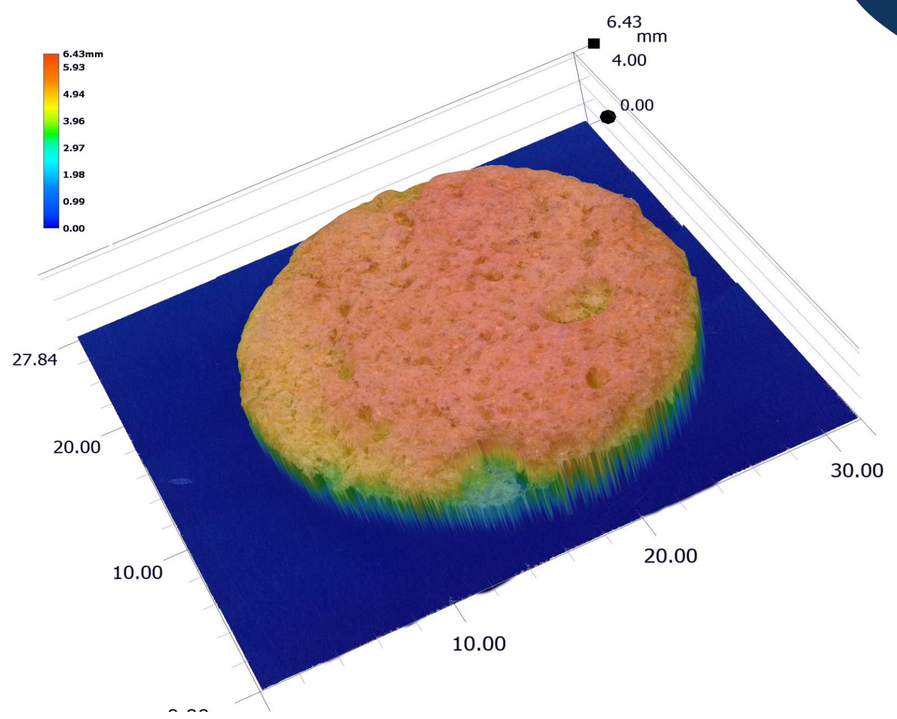

Figure 2. Digital microscope image of obtained composite.

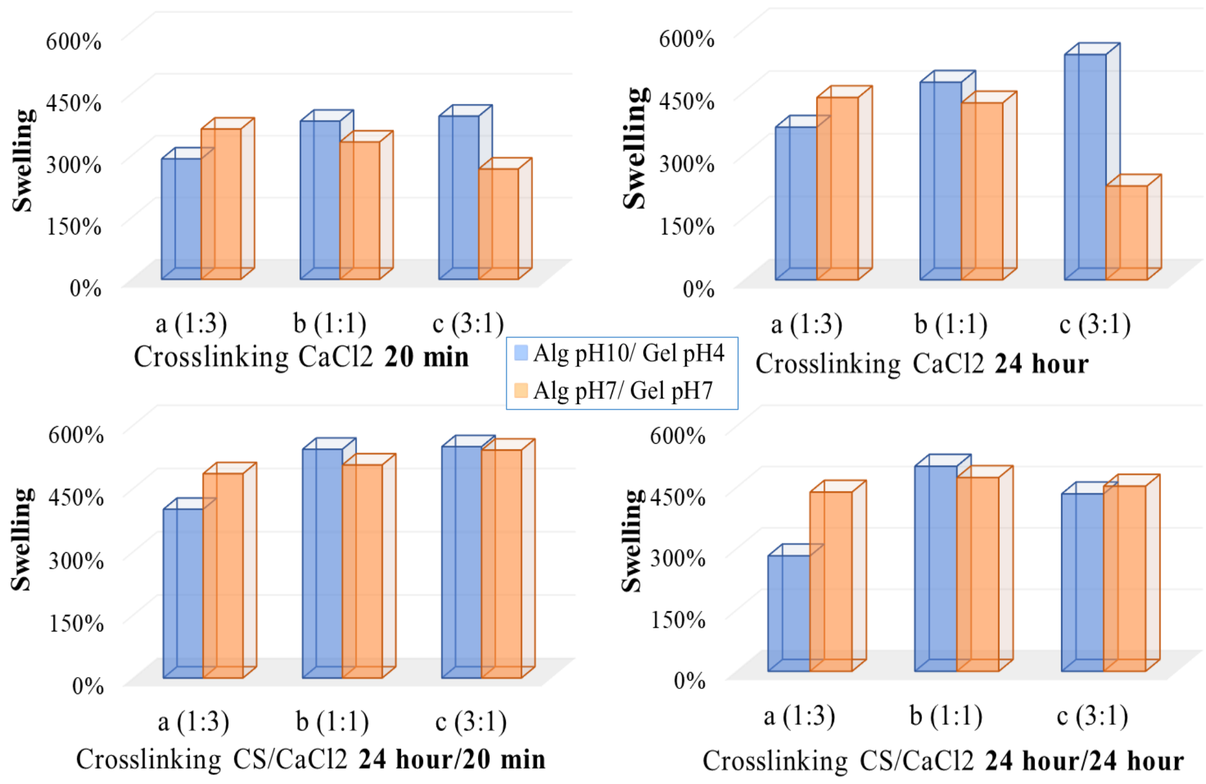

Figure 3. Influence of pH value and crosslinking method on the swelling degree of experimental samples.

Table 1. Time to the beginning of the degradation process during swelling

	pH value of components	Alg pH10/ Gel pH4. Time, h			Alg pH7/ Gel pH7. Time,h		
	Sample	a	б	c	a	б	c
Crosslinking	$CaCl_2$ 20 min	96	96	96	96	96	48
	$CaCl_2$ 24 h	144	144	336	96	144	96
	$CS/CaCl_2$ 24 h/20 min	96	144	96	96	96	144
	$CS/CaCl_2$ 24 h/24 h	336	336	336	96	96	144

4 Conclusion

The combination of the protein material gelatin, natural polysaccharides Alg and CS, as anionite and cationite, respectively, the selection of technological conditions for their combination ensures the optimal composition and shape of the composite, which meet the requirements for materials of the specified direction. The Alg: Gel 1:1 samples, crosslinked by CS and Ca^{2+} for 24h are especially noteworthy, which show shape stability long time (2 months and more).

5 References

1. *Serafin A., Culebras M., Collins M. N.* Synthesis and evaluation of alginate, gelatin, and hyaluronic acid hybrid hydrogels for tissue engineering applications // Int J Biol Macromol.-2023.-**233**.-123438.

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