Experimental Characterisation of the Alginate Gelation Process for Rapid Prototyping

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Hydrogels have received much attention due to their potential use in a wide variety of biomedical applications, including tissue engineering scaffolds, drug delivery, contact lenses, corneal implants and wound dressing. This research work focuses on a new route to produce three-dimensional scaffolds in alginate hydrogels for medical applications, through the use of a biomimetic rapid prototyping system. This system replicates some natural procedures used by some marine brown algae, namely *Laminaria Hyperborea*, to produce alginate used as a structural component of the algae, in accurate chemical conditions. The biomanufacturing of optimised alginate scaffolds requires the control of the gelation process in order to obtain improved mechanical and biological properties and appropriate surface morphology for cell attachment, proliferation and differentiation. This paper investigates the influence of sodium alginate of both sodium alginate and calcium chloride on the gelation kinetics.

1. Introduction

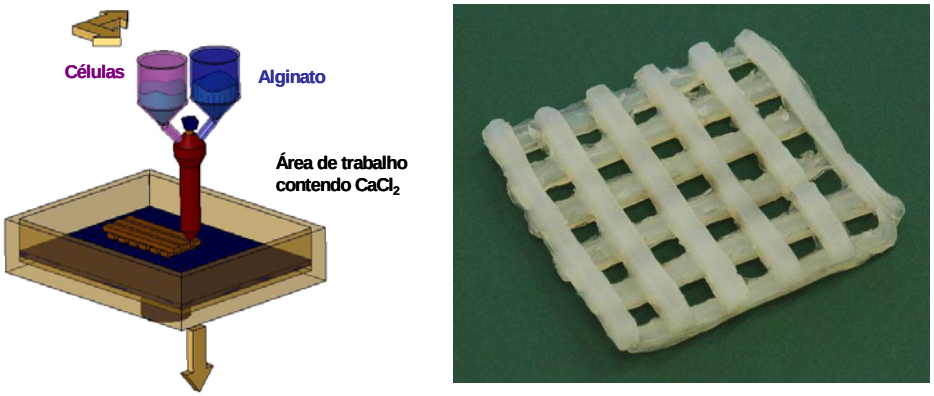
Tissue engineering is an interdisciplinary field that combines the use of living cells with either natural or synthetic extra-cellular structures (scaffolds) to develop body parts or devices that will enable the restoration, maintenance or enhancement of living tissue and organs. Three-dimensional scaffolds play an important role in promoting and guiding tissue regeneration. Usually, these scaffolds have high porosity (macro-porosity), appropriate surface morphology (micro-porosity), large surface area, suitable pore size and highly connected pore structure. They must also be biocompatible and biodegradable.

Rapid prototyping represents a new group of non-conventional techniques with great potential to produce scaffolds with customised external shape and predefined internal morphology (Leong et al., 2003, p.2363). These processes also allow controlling both pore size and distribution. Ideally, rationally designed tissue engineering scaffolds promote natural wound healing and regeneration. Therefore, we sought to develop a biofabrication system, specifically designed to soft tissue repair applications.

This paper focus on the concept of rapid prototyping to produce alginate scaffolds for medical applications. The effect of the gel composition in terms of both gelation and surface morphology is investigated.

2. Rapid Prototyping for alginate scaffolds

An alginate-based rapid prototyping system has been developed to produce alginate scaffolds by extruding, layer-by-layer, a solution of sodium alginate into a calcium chloride solution (Figure 1). The system comprises two nozzles, one for the sodium alginate and the other for the calcium chloride deposition (Bártolo, 2006, p.56).

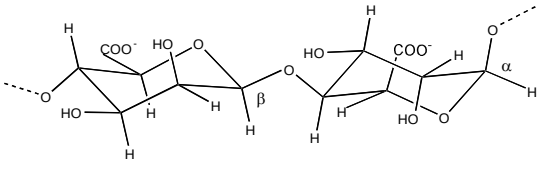


a) b)

Figure 1 – Alginate-based rapid prototyping system (a), alginate scaffold structure (b).

3. The chemistry of alginate

Alginate is an anionic copolymer composed (Figure 2) of homopolymeric regions of 1,4-linked β-D-mannuronic (M blocks) and α-L-guluronic acid (G blocks), interspersed with regions of alternating structure. The industrial manufacture of alginate is based on the extraction of a polymer from brown algae. The seaweed grows in nature mainly in temperate areas, but large amounts are also cultivated in other regions like the Far East, the coast of China or Japan. The seaweed is extracted with a dilute alkaline solution which solubilises the alginic acid present. Free alginic acid is obtained treating the resulting viscous material with mineral acids, being then converted to a salt. Sodium alginate is the major form currently used.



β-D-Mannuronic acid α-L-Guluronic acid

Figure 2 - Structure of an alginate showing a linkage between the M and G acids.

Gelation occurs when divalent ions (Ca^{2+}, Ba^{2+}, Fe^{2+}, Sr^{2+}, etc.) or trivalent ions (Al^{3+}, etc.) take part in the interchain ionic binding between G-blocks in the polymer chain giving rise to a three dimensional network. Such binding zones between the G-blocks are often referred to as “egg boxes”. These ions act as cross-linkers that stabilise alginate chains forming a gel structure, which contains cross-linked chains interspersed with more freely movable chains that bind and entrap large quantities of water. The gelification process is characterised by a re-organisation of the gel network accompanied by the expulsion of water (Serp et al., 2002, p. 253).
Gels made of M-rich alginate are softer and more fragile, and may also have lower porosity. This is due to the lower binding strength between the polymer chains and to the higher flexibilities of the molecules. The gelification process is highly dependent upon diffusion of gelification ions into the polymer network. Trasmittancy, swelling and viscoelasticity of alginate structures are highly affected by the M/G ratio.
Alginic acid and its sodium and calcium salts are non-toxic and biocompatible, being widely used in the medical, pharmaceutical, cosmetic and food industry (Gombotz and Wee, 1998, p.267). In tissue engineering, alginate has been used as a delivery vehicle or supporting matrix.

4. Material

Sodium alginate was purchased at Panreac (Barcelona, Spain). Calcium chloride was supplied by Carlo Erba (Milano, Italy). All solutions were prepared with pure water, with conductivity of 0.054 μS/cm. Alginate solutions were prepared by addition of weighted portions of sodium alginate to measured volumes of water. Due to their high viscosity, these solutions were agitated by orbital shaking for three hours at 50 ºC to ensure good homogeneity. Calcium chloride solution 5% (w/v) was obtained dissolving the salt in water. This solution was diluted to obtain solutions containing different concentrations of calcium chloride.

5. Results

To evaluate the kinetics of the gelation process solutions, containing different concentrations of both alginate and calcium chloride ($CaCl_2$), were prepared and mixed at room temperature. The effect of the alginate concentration is shown in Figure 3, which describes the weight loss as a function of gelation time for solutions containing 1% and 2% (w/v) mixed with a solution of 5% (w/v) $CaCl_2$. Figure 4 shows the variation of weight loss as a function of gelation time for a solution containing 2% (w/v) of alginate mixed with solutions containing different concentrations of $CaCl_2$. The experimental data were fitted using a sigmoidal equation. To correlate the experimental data and the values obtained from the sigmoidal equation, a numerical routine using the Marquardt-Levenber multivariable non-linear regression method was employed. A good correlation was achieved by controlling the number of steps, the increment and a small tolerance parameter, corresponding to the difference of values at the step n+1 and values at the step n, which is used for convergence purposes.

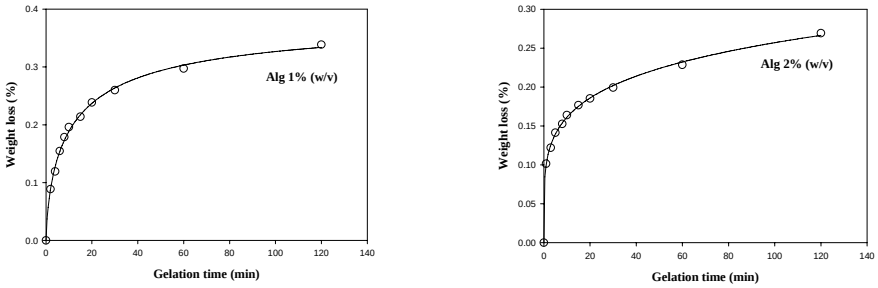


Figure 3 - Weight loss vs gelation time for two solutions containing different concentrations of alginate mixed with a solution of of 5%(w/v) of $CaCl_2$.

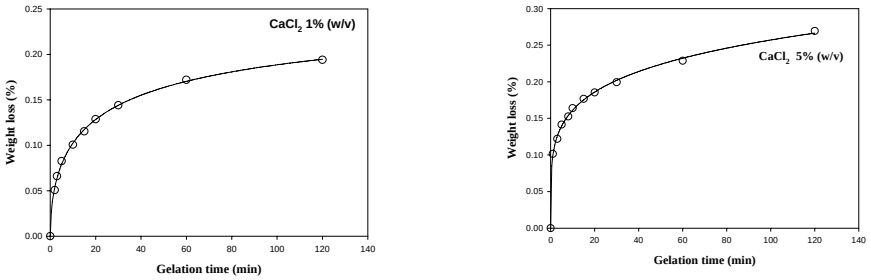


Figure 4 - Weight loss versus gelation time for a solution containing 2% (w/v) of alginate mixed with solution containing different concentrations $CaCl_2$.

It can be observed that the weight loss increases with time and is more significant for samples produced by solutions containing low contents of alginate and high contents of calcium chloride. This is due to two main reasons:

- the amount of water present in the initial alginate solution, which is higher in more dilute solutions;
- the kinetics of the gelation process, which is higher whenever solutions containing higher concentrations of $CaCl_2$ are used.

The calcium divalent ions act as cross-linkers that stabilise alginate chains forming a gel structure. Therefore, increasing the concentration $CaCl_2$ present in the solution increases the cross-linking of polymeric chains and the expulsion of water (Mendes et al., 2003, p.419). The effect of the gel composition can be observed in Figures 5 and 6.
The concentration of both sodium alginate and $CaCl_2$ determines not only the kinetics of the gelation process, but also the internal (not shown in this paper) and surface morphology of alginate gels as indicated in Figure 7. This Figure indicates that gels obtained from solutions containing higher sodium alginate and low $CaCl_2$ contents have smooth surfaces.

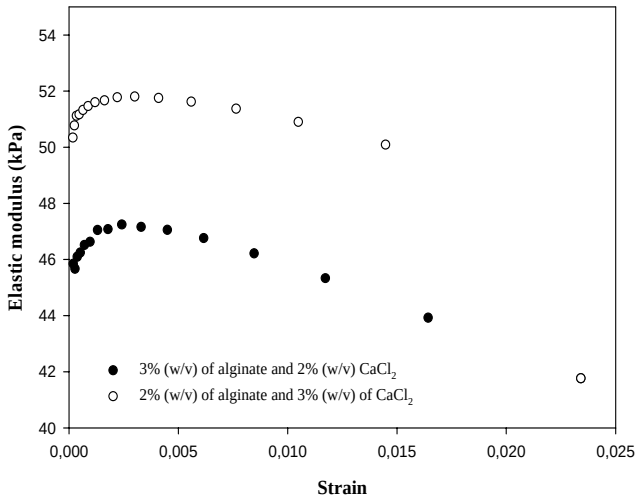


Figure 5 – Elastic modulus versus strain for two different alginate gels.

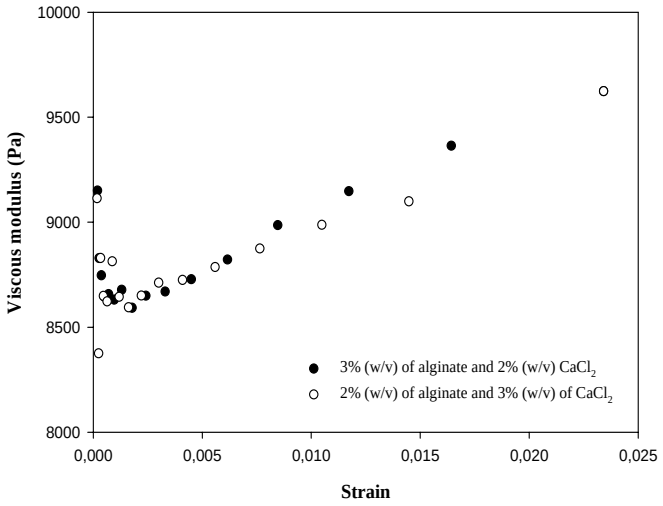
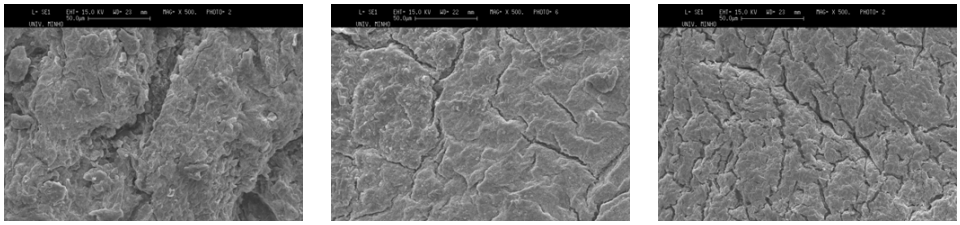


Figure 6 – Viscous modulus versus strain for two different alginate gels.

6. Conclusion

Alginate is a biodegradable and biocompatible biopolymer that can be used in tissue engineering as a scaffold that promotes wound healing. This paper presents a new rapid prototyping approach to produce three-dimensional alginate scaffolds for tissue engineering. This kinetics of the gelation process to obtain such scaffolds is discussed. It was found that the gelation mechanism is strongly dependent on both the alginate and calcium chloride concentrations. Additionally, the concentration of both sodium alginate and calcium chloride determine the mechanical behaviour of alginate gels and its surface morphology.



a) b) c)

Figure 7 – SEM for gels containing 2% alginate and 1% $CaCl_2$ (a), 3% alginate and 1% $CaCl_2$ (b) and 2% alginate and 2% $CaCl_2$ (c).

7. Ackowledgements

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8. References

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