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Temperature induced self-healing properties of alginate gelatin hydrogels

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ABSTRACT

One of the main challenges in tissue engineering is developing constructs that restore damaged tissues. This has led to the growth of classes of materials with tuneable mechanical and absorption properties. Among others, hydrogels are particularly fascinating because they can be functionalized to self-repairdamage like the native living tissues. This work proposes an improved thermo-responsive alginate-gelatine (SA-Gel) hydrogel capable of self-repairing, whose mechanical properties are enhanced by the addition of optimal concentration of graphene oxide (GO). The initial results show that the novel hydrogel's formulation improves self- healing and mechanical properties making them a potential candidate for biomedical applications.

1. Introduction

Tissue engineering aims to create biomaterials mirroring natural tissues, with self-healing being a crucial goal. This involves using polymers-based hydrogels, due to their analogy with the extracellular matrix (ECM) [1]. Self-healing hydrogels are intelligent biomaterials that repair themselves by crosslinking damaged polymer chains [2], through external triggers like temperature, pH or light. Typically, they are derived from natural or synthetic polymers through dynamic covalent and non-covalent bonding of polymer chains in a mobile phase. To the best of our knowledge, in the state-of-the-art collagen-based hydrogels are presented as the best solution to achieve self-healing properties[3,4]. However, Gelatine (Gel) being a protein and its ease of modification is a best substitute for collagen [4]. In recent years, carbon based materials like graphene oxide (GO) due to its excellent physical properties, and biocompatibility are used as fillers to enhance the mechanical property of hydrogels [5,6]. The layered structure and multiple reactive sites on GO form hydrogen bonds with alginate and interactions with Ca^{2+} results in interconnected flexible polymer network. Moreover, the toxicity of the graphene oxide is reduced when by dispersing them within polymers to create polymer nanocomposites [7]. The primary aim of this research is to optimise a material combination that is easily available and can yield mechanically tough self-healing hydrogels compliant for biomedical applications.

2. Materials

Sodium alginate (SA) and Calcium chloride (CaCl2) were purchased from Sigma-Aldrich. Gelatine was purchased from VWR Chemicals and graphene oxide (GO) was purchased from Cambridge Graphene Centre.

3. Hydrogels preparation

For this study, four samples with different concentrations of graphene oxide (GO) were prepared; the concentrations used were: 0 %; 1 %; 2 % and 3 %. A 5 % (w/v) solution of SA was prepared by mixing SA in distilled water until it became homogeneous and then degassed to remove the air bubbles.

To prepare the gelatine solution, 2 g of gelatine were dissolved in 20 mL distilled water by stirring for 15 min at 30 °C. To this solution, added 5 mL of 5 % (w/v) SA water solution and mixed for 30 min. GO was added to this solution and stirred until it was homogeneous mixture. All the solutions were degassed and transferred into Teflon moulds. Next, the moulds were immersed for 3 h in a 2.5 % (w/v) calcium chloride water solution to cross-link. Finally, the hydrogels were placed in distilled water.

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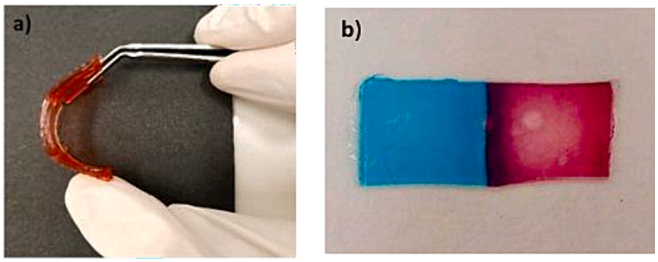

Fig. 1. A) bended healed hydrogels; b) two healed hydrogel fragments with food dye.

4. Methodology

4.1. Healing test

To test the healing properties of the proposed material, the SA hydrogels were cut into half and placed in contact with each other in a Petri dish. Temperature was used as an external stimulus, the hydrogel segments were heated at body temperature, i.e., 37 °C. The self -healing resulted from the reversibility of hydrogen bonds and the molecular diffusion of physical crosslinks when the cut surfaces are kept in contact. This test was repeated and confirmed using all the samples.

4.2. Tensile test

The tensile test on dumbbell-shaped samples (ASTM D412 standard) was used to understand the effect of varying GO concentration in the SA-Gel hydrogel. The Uniaxial SHIMADZU AUTOGRAPH AGS-X tensile machine measures the force and the displacement with a crosshead speed of 0.02 mm/s. The load data were converted into stress and strain by measuring the dimensions of the samples. Stress (σ) and strain (ε) were then calculated as

$$\sigma = \frac{F}{A} \tag{1}$$

$$\varepsilon = \frac{\Delta l}{l_0} \times 100 \tag{2}$$

where *F is* the force, *A* the sample area calculated as thickness × width, and Δl is the difference between the final and the initial (*l0*) length of the sample. The Young's modulus or modulus of elasticity was calculated from the slope of the linear part of the stress–strain curve, as

$$Y = \frac{\sigma}{\varepsilon} \tag{3}$$

4.3. Molecular structure test

The molecular structure of the pre-healed and post-healed hydrogels was analysed with the Fourier transform infrared (FT-IR) spectroscopy, FTIR Jasco 6600 (Japan) with a wavelength range from 400 to 4000 cm^{-1}. This was performed on the samples without GO, since the dark samples, makes it impossible to use the FTIR.

4.4. Swelling ratio

This test was used to investigate the absorption properties. To measure the swelling ratio, samples dried for 24 hrs, determined the dry weight (W_d). The samples were soaked in distilled water for 10 min and weighed as W_s, weight of swollen sample. This procedure was repeated every ten minutes until one hour. The swelling ratio was calculated as

$$\text{Swelling ration}\,(\%) = \frac{W_s - W_d}{W_d} \times 100 \tag{4}$$

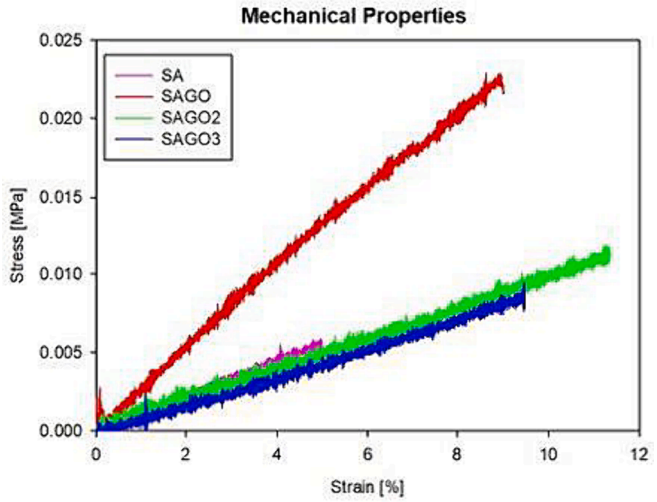

Fig. 2. Mechanical properties: Tensile curves of the self-healed hydrogels.

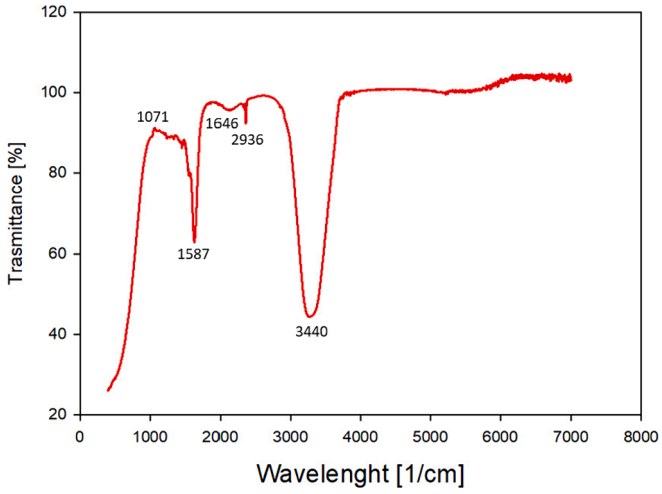

Fig. 3. FTIR of the SA-Gel hydrogels.

5. Results

5.1. Healing test

The temperature assisted self-healing ability were confirmed from the attached hydrogel segments. The healed hydrogel was obtained within body temperature 36–40 °C in less than 10 min. Theself-healed hydrogel displayed the characteristics of a single sample rather than two fragments and do not separate when bent (Fig. 1).

5.2. Tensile test

The tensile test data in Fig. 2 depicts that GO addition alters the hydrogel mechanical properties. The polar groups of alginates interact with the oxygen-containing groups of GO in the form of hydrogen bonds and Ca^{2+} via ionic interactions, improving the mechanical strength of hydrogel [5]. This result in an interconnected flexible polymer network. Increasing GO concentration increases the repulsion between the COO^- groups of SA and GO showing weak mechanical properties.

5.3. Molecular structure test

Fig. 3 shows the FTIR spectrum of the SA healed hydrogels, the

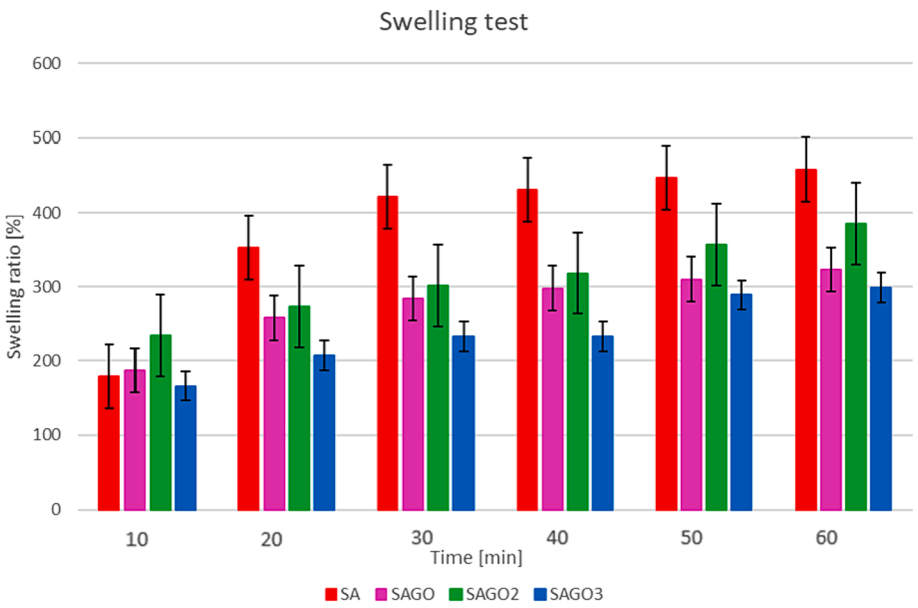

Fig. 4. Swelling test of the self-healed hydrogels.

spectra of samples before healing overlap with the spectra of the post-healing sample. The coincidence of the two graphs point out that the healing process did not interfere with the internal composition of the material.

The peak at 1646 cm^{-1} corresponds to the functional group of sodium alginate, resulting from the interaction of the carboxylic groups with Ca^{2+} ions. The peak at 1587 cm^{-1} corresponds to the stretch vibration of $–COO^{-}$ [8] and are specific to ionic binding [9]. The peaks at 2936 cm^{-1} and 3440 cm^{-1} correspond to the functional groups of gelatine, from the aliphatic –CH and –NH stretching vibrations respectively [10].

5.4. Swelling test

The swelling ratio of more than 500 % after 1 h for the SA-gel hydrogels are shown in Fig. 4, whereas the hydrogels with GO represents a ratio of 300 % in the same time interval. An accelerated increase of the swelling ratio is observed in SA-Gel hydrogel in comparison to the swelling ratio of hydrogels with different GO concentrations.

6. Discussion

Self-healing hydrogels are intelligent biomaterials that can remend by themselves at the damage site [1]. The experiments presented in this study, depict the self-healing properties of a SA hydrogel using temperature stimuli. Gelatine-based material makes the hydrogels time and cost convenient[11,12]. In the healing test, SA hydrogel fragments integrated to confirm their self-healing property. Mechanical properties were assessed via tensile testing, resulting in greater elasticity or Young's modulus compared to a vein (0.0053 MPa) [13]. The FTIR spectrum corroborates the presence of SA and gelatine even after the healing process. The swelling test confirmed the absorption property of hydrogels with SA hydrogel having a higher swelling ratio. These results prove that SAGO1 hydrogel shows improved rigidity and elasticity and the swelling ratio increases at a slower rate with the concentration of GO.

7. Conclusions

In this study, a set of experiments were conducted to investigate the self-healing properties of a new and improved SA based hydrogel. Since the mechanical properties is one of the most important feature for tissue engineering, this study found that embedding of GO in hydrogel, increase the mechanical integrity without compromising the biocompatibility. The best solution is given by the SAGO1 sample. Besides, another advantage is the preparation time (i.e., less than 10 mins), hydrogels' cost effectiveness and the possibility of using body temperature as stimuli. In conclusion, the hydrogel SAGO offers an optimal balance between the desired properties: biocompatibility and mechanical strength.

Data Availability Statement:

All dataset analysed in this study are included in the article. The raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

Author contributions

IG and AVG contributed to the design and conception of the study. IG and AVG carried out measurements. IG, AVG, CV and CSI drafted the article. IG AVG and CV created tables and figures. IG, AVG, CV and CSI participated in the revision process of the article and gave final approval of the submitted version. IG, AVG and AMB revision the manuscript and the revision process.

CRediT authorship contribution statement

Immacolata Greco: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Anet Vadakken Gigimon:** Writing – review & editing, Writing – original draft, Methodology. **Carolina Varon:** Writing – original draft, Validation. **Ana Madevska Bogdanova:** Writing – review & editing. **Carlo S. Iorio:** .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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