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Preparation of Silk based Hydrogel and Sponges for Tissue Engineering Application in Cartilage Repair/Replacement

M. Annie Rosalyn[1] and R. Sridhar Skylab[1]*

[1]Department of Electronics and Communication Engineering,
Anna University, College of Engineering Guindy, Chennai - 600025, India.

*Corres. author: sridharskylab@gmail.com

Abstract: The major trouble which the society suffers these days is joint pain which is a hindrance in daily activities. It is a consequence of the wear and tear of cartilage or in other words, cartilage degeneration. Although various techniques, often surgical, are currently employed to treat this affliction, none have had a complete success. The main objective of this project is to develop a material that mimics the natural cartilage structure and functions that is worthy to be implanted in-vivo. This work is directed to develop a material that is a novel class of natural biopolymeric hydrogel composite and sponges. A Thermo-gelling polymer undergoes a reversible phase transition and thus solidifies after injecting it into the body reaching the body temperature. Injectable formulations have its major advantages such as possibility of a minimal invasion to reduce patient morbidity, moldability to fill any irregular shape and to incorporate therapeutic drugs. The prime materials chosen for this application are degummed silk (silk fibroin) and chitosan which are natural biopolymers and has good bio-compatibility for cells. The material formed is characterized to check the proper blending of the mixture with its functional groups using Fourier Transform Infra-Red Spectroscopy (FTIR), to show the thermal stability of the hydrogel using Thermo Gravimetric Analysis (TGA) and degradation of the material is studied by in vitro degradation.
Keywords: Silk; Silk degumming; Chitosan; Polyethylene glycol; Hydrogel.

1 Introduction

Cartilage is a tough and flexible tissue which supports the joints between the bones and can be moulded with its elasticity and act as a shock absorber to reduce friction. Cartilage, which is composed of chondrocytes that produces collagen fibers and proteoglycans, lacks blood cells that help in healing thus delaying the healing period. Cartilage is categorized into: (a) Articular cartilage: found in joints is elastic and tough, (b) Elastic cartilage: found in parts of the nose and pinna of the ear has flexibility and (c) Fibrocartilage: found in intervertebral discs and pelvic region manages a weight load and is toughest of all cartilages. As surgical and non-surgical techniques did not show successful results, it led us to the development of a material that can be used to replace or repair cartilage by implanting it. Chitosan, derived from chitin, is a cationic linear polysaccharide and a natural biopolymer that is biochemically similar to cartilage and can also be degraded by cell secreted enzymes and helps in cell attachment (1) (2). Chitosan has high biocompatibility, non-toxicity, supports cell growth and initiates immune response in vivo (2). Silk is biocompatible and biodegradable and is

FDA approved as an implant material for soft tissue repair. It mimics structural and mechanical properties of cartilage (3). Polyethylene glycol has attractive properties such as antigenicity, immunogenicity, protein resistant, less toxicity and minimal protein and cell adhesion (4).

1.1 Experimental Procedure

1.1.1 Materials

Chitosan was purchased from HiMedia (India) which is of 400kDa molecular weight, Bombyx mori silk was purchased from Bombyx mori silk farm (India), Polyethylene glycol, purchased from Merck (India), is of 9000kDa molecular weight, Lithium Bromide and Sodium Carbonate were purchased from Sigma Aldrich (India).

1.1.2 Degumming of silk and preparation of solutions for hydrogel formation

Silk was degummed by boiling it with 1.1 g/L Na2Co3 anhydrous followed by 30 minutes in water with 0.4 g/L Na2Co3. After extensive rinsing in boiling distilled water, it was air dried at room temperature. 9.3M LiBr was used to completely dissolve silk fibroin by heating it at 75^0C for 5 hours. The result solution was centrifuged thrice at 9000rpm for 20 minutes at 4^0C to remove the impurities and bubbles (5). The filtered solution is dissolved in water at 7 weight %. Chitosan was solubilized at a concentration of 5% (w/v) in 3% (v/v) aqueous acetic acid. PEG was dissolved at a concentration of 2.5% (v/v) in distilled water.

1.1.3 Fabricating Gel

Chitosan, silk and PEG were intensely blended in two ratios: 4:2:4 and 7:2:1 respectively. Silk and chitosan solution were blended under constant stirring at 7:3 ratio. These were labeled as hydrogels 1, 2 and 3 respectively. The solutions were continuously stirred using a magnetic stirrer for 5 hours for 3 consecutive days and was placed in an oven at 37^0C to maintain room temperature. Hydrogels, formed after 3 days, were washed with distilled water and stored at RT.

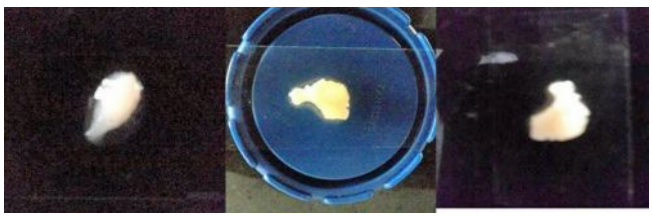

Fig. 1 (From left) hydrogels: 1. C:S:PEG (4:2:4), 2. C:S:PEG (7:2:1) and 3. C:S (7:3)

2 Results and Discussions

2.1 Characterization techniques

The hydrogel thus formed was subjected to FTIR, TGA and in vitro studies to characterize it.

2.1.1 In Vitro Degradation

Three hydrogels prepared were kept under observation for weight loss while in phosphate buffered saline (PBS) at 7.4pH at room temperature for 2 weeks and still on-going. With the available data we found that hydrogel C:S (7:3) was delayed in degrading when compared to hydrogels C:S:PEG (4:2:4) and (7:2:1). All the three hydrogels were degrading slowly and was found favourable as cartilage takes a longer duration to recover or repair.

2.1.2 FTIR Characterization

The functional groups of Chitosan-Silk-PEG (4:2:4) and Chitosan-Silk (7:3) hydrogels were confirmed by recording the FTIR spectrum in the range of 4000-400 cm-1. The peaks as shown in Fig. 2 and 3 confirm the presence of the functional groups of: chitosan with alcohol of high concentration O-H stretching, alkenes with C=C stretching, alkenes with C-H in-plane bend and alkyl halides with C-F stretching, silk fibroin with amides with N-H stretching, alkenes with C=C stretching, amines with N-H bending, carboxylic acids with C-O and

alkyl halides with C-Cl stretching. The peaks corresponding to hydrogen bonding, alkyne group and mono-substituted aromatic ring, showed the presence of PEG.

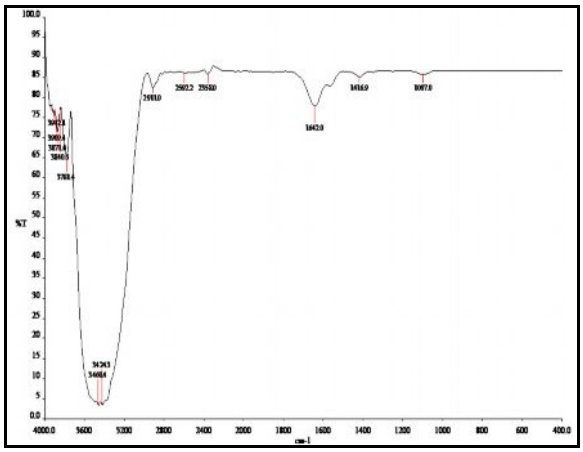

Fig. 2 FTIR spectrum of C:S:PEG (4:2:4)

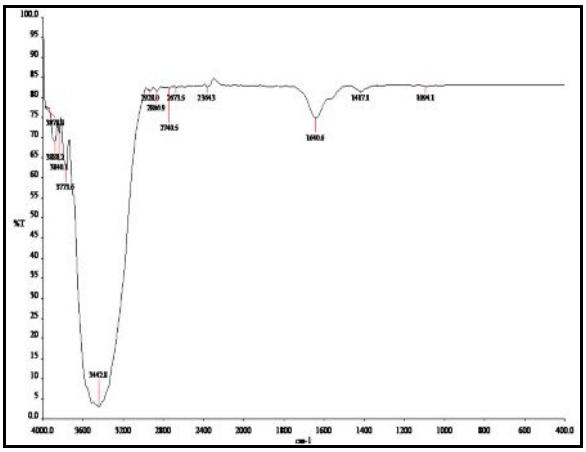

Fig. 2 FTIR spectrum of C:S (7:3)

2.1.3 TGA Result

The thermal stability of the hydrogel was interpreted by TGA using SDT Q600 analyser. The sample C:S (7:3) was analysed between the temperatures 37^{0}C and 800^{0}C at a heating rate of 10^{0}C/min. From the curve shown at fig.4, the material exhibits weight loss starting around 104^{0}C proving the compound to be stable up to 104^{0}C. The maximum weight loss was observed between 104.41^{0}C and 123.74^{0}C with the elimination of 83.17 % of the material.

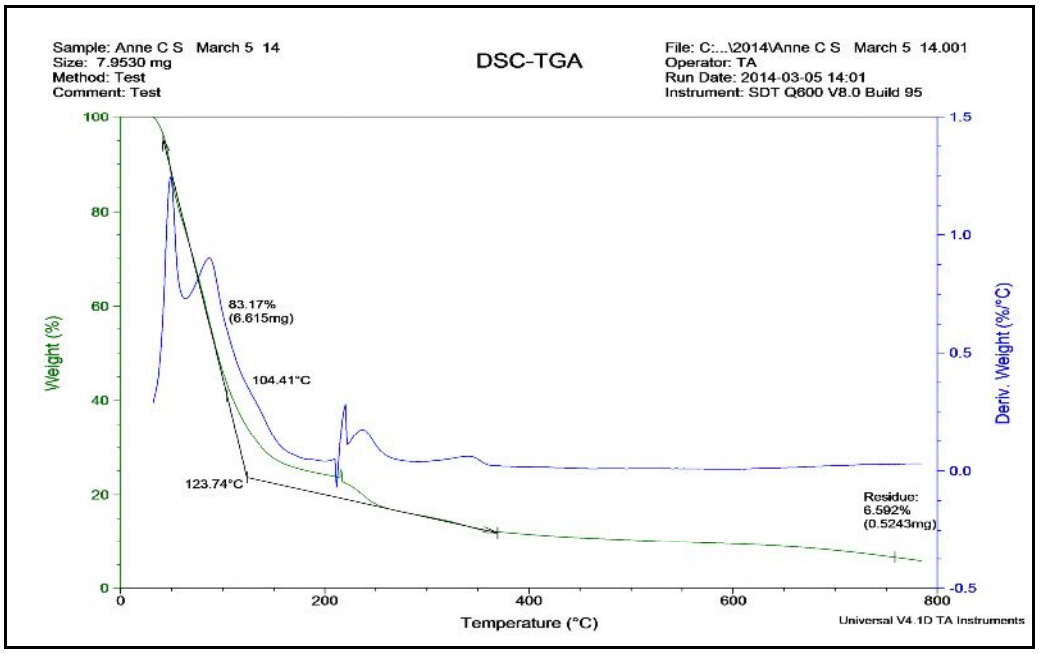

Fig. 4 TGA of Chitosan-Silk (7:3) showing the thermo stability of the material

2.1.4 Conclusion

The results show that a temperature responsive hydrogel can be prepared and is also thermo-stable. The hydrogel formed was elastic and thick as in cartilage. The elasticity and cell viability must be tested to prove the biocompatibility. Future work also aims the mode of delivery and the ability to repair the damaged cartilage by infusing drug in the material.

3. References

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