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# Injectable Hydrogel for Cardiac tissue Engineering

**A.Janani[1] and R.Sridhar Skylab[1]***

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

***Corres. author: sridharskylab@gmail.com**

**Abstract:** An injectable bio polymeric scaffold to deliver cells is a novel approach developed in recent years in the field of cardiac tissue engineering. Injectable and degradable polymer-based materials, called thermogelling polymers, which are sensitive to temperature, undergo a sol-gel transition at 37˚C, are promising candidates in this field. Injectable formulations offer specific advantages such as: possibility of a minimally invasive implantation, an ability to fill a desired shape, and easy incorporation of various therapeutic agents. In this work, a novel class of two-component hydrogel namely Chitosan and Polyethylene Glycol (PEG) was prepared in various combinations.. The prepared hydrogel was characterized using FTIR, TGA and was subjected to in vitro degradation studies. Fourier transform infrared (FTIR) spectral analysis was used to confirm the presence of various functional groups in the hydrogel formed. Thermal stability of the hydrogel was established using the thermo gravimetric analysis.
**Keywords:** Chitosan; PEG; thermogelling polymers; Injectable hydrogel

## 1. Introduction

Chitosan, an amino polysaccharide obtained from the N-deacetylation of chitin, is known to have good biocompatibility, biodegradability, low immunogenicity, and biological activities. It has been shown that it is a potential injectable scaffold that can be used to deliver stem cells to an infarcted myocardium. It can increase cell retention and microvascular density and preserve cardiac function after myocardial infarction (1). On the other hand, PEG is a neutral, water soluble, non-toxic polymer. It is one of only a small number of synthetic polymers approved by the FDA for a wide range of biomedical applications (2). PEG based hydrogel has been investigated as a permanent tissue integrated scaffold in an infarcted heart (3). In this study, Chitosan-PEG based injectable, thermoreversible hydrogel is developed and the synthesised hydrogel is characterized using FTIR and TGA. The degradation rate of the hydrogel is also investigated.

## 1.1. Experimental Procedure

### 1.1.1. Materials

Chitosan was purchased from HiMedia (Mumbai, India), with an average molecular weight of 4,00,000 Da. PEG was purchased from Sisco Research Laboratories (Mumbai, India) with an average molecular weight of 9000 Da. All other chemicals were of analytical grade and used as received.

### 1.1.2. Preparation of Chitosan-PEG mixture

A clear solution of chitosan was obtained by dissolving 5g of chitosan in 3% (v/v) glacial acetic acid. 2.5% PEG solution (w/v) was prepared in deionized water. Then the Chitosan solution was added drop wise to the PEG solution under constant stirring for 30 minutes. The mixture was left overnight at 4˚C and centrifuged to remove air bubbles for 20 minutes. Approximately 5ml of the solution was stored in a 15ml tube and tightly capped. Hydrogel was formed when the solution was exposed to 37˚C. The hydrogels formed from varying ratios of Chitosan-PEG solution is shown in fig. 1.


**Fig.1.** Hydrogels formed from different Chitosan-PEG ratios

### 1.1.3. Sol-Gel transition behaviour

The sol-to-gel transition was determined by the test tube inverted method (4). Tubes containing 5ml of fresh chitosan-PEG mixture were kept in a hot air oven which was heated at 37˚C. Each sample was regarded as a gel in the event of no flow being observed. Gelation time was determined by tilting the tube at 90˚ angle for 1 min till no flow.

### 1.1.4. In vitro degradation studies

Two Chitosan-PEG hydrogels of 60/40 ratio of varying weights were kept in phosphate buffered saline (PBS) at pH 7.4 for 3 weeks at room temperature. Each day weight loss percentage was calculated and a graph was plotted. The weight loss percentage was calculated from the formula (5):

Weight loss (%) = $| W_A - W_B | \times 100 / W_A$, where $W_A$ – Initial sample weight, $W_B$ – Sample weight after period of degradation (Dried Weight).

### 1.1.5. Characterization techniques

The hydrogel formed was subjected to characterization techniques such as FTIR (Fourier Transform Infrared Spectroscopy) and thermal analysis. The functional groups of Chitosan-PEG hydrogel were confirmed by recording the FTIR spectrum in the range of 4000-400 cm-1 using Perkin-Elmer FTIR spectrometer (Spectrum RX1, Version 5.2.0) by the KBr pellet technique. The thermal stability of the hydrogel was established by thermo gravimetric analysis (TGA) using SDT Q600 analyser. The sample was analysed between the temperatures 81 and 800°C at a heating rate of 10˚C/min.

## 3. Results and Discussions

### 3.1. Fourier transform infrared spectroscopy

The FTIR spectrum of (60/40) Chitosan-PEG hydrogel is shown in Fig.2.The spectrum showed the main characteristic bands of chitosan at 3774.9, 1099.5 and 1641.1 $cm^{-1}$ which could be assigned to the saccharide structure of the Chitosan that is the O-H bending, C-O stretching and N-H bending respectively. The peaks at 3902.7, 2476.6 and 668 cm-1 corresponding to hydrogen bonding, alkyne group and monosubstituted aromatic ring, showed the presence of PEG. The peaks at 2107.5 and 1563 $cm^{-1}$ could be attributed to the alkyne group and N–H bending respectively. The bands at 3453.5, 2691.7 and 2359 $cm^{-1}$ are attributed to the H

bonded O-H stretching carboxylic acid. The C-H in plane bending vibration has been observed at 1413 $cm^{-1}$.

### 3.2. TGA analysis

The thermal stability of the (60/40) Chitosan-PEG hydrogel was established by TGA analysis and the thermogram is depicted in Fig.3. From the thermo gravimetric curve, the material exhibits sharp weight loss starting at 81.67˚C. Hence the compound is stable upto 81.67°C. The first stage weight loss was observed between 81.67 and 147.54°C with the elimination of 76.39 % of the material. The second stage weight loss was noticed between the temperature 276.37 and 311.06 °C. It incurs a weight loss of 4.501% of the material. The final stage weight loss was noted as 10.66% between the temperature 379.33 and 428.23˚ C. From the DTA trace, it is observed that there is an endothermic peak starting at 106.7°C. This is followed by two endotherm peaks at 293.40°C and 396.99°C, which may be attributed to the second and third decomposition temperatures of the hydrogel respectively.

### 3.3. In vitro degradation studies

In vitro degradation of two (60/40) Chitosan-PEG hydrogel of varying weights was studied and the weight loss % was plotted against time (days) which is shown in Fig.4. The plot suggested that both hydrogel 1 (0.9890 g) and hydrogel 2 (0.8649 g) degraded at the same rate and there was no significant difference till the 2nd week of the study.It was observed that the weight loss % of hydrogel 2 slightly increased after 14th day. The shape of both the hydrogels was well maintained till 18th day of the study.Thus at the end of 3rd week the degradation rate was approximately between 40-45 % and they are expected to degrade fully within next 2 weeks.

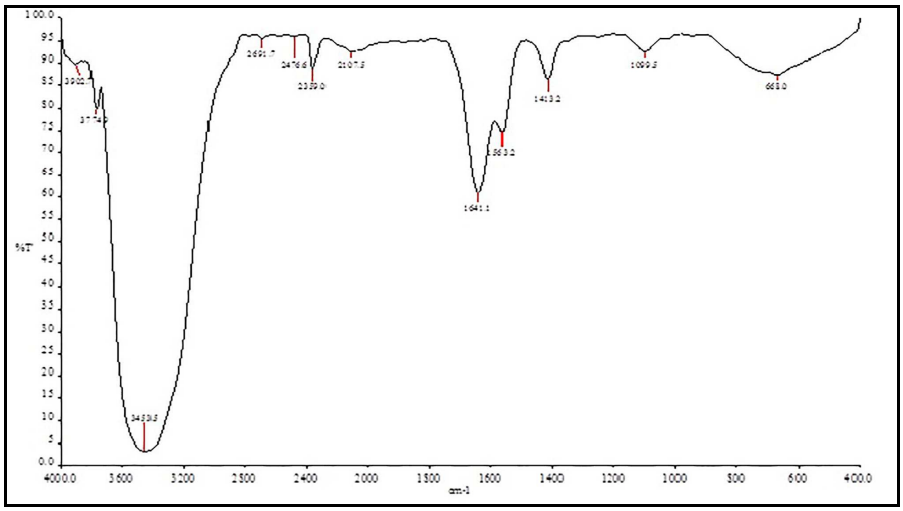


**Fig.2. FTIR Spectrum of (60/40) Chitosan-PEG hydrogel**

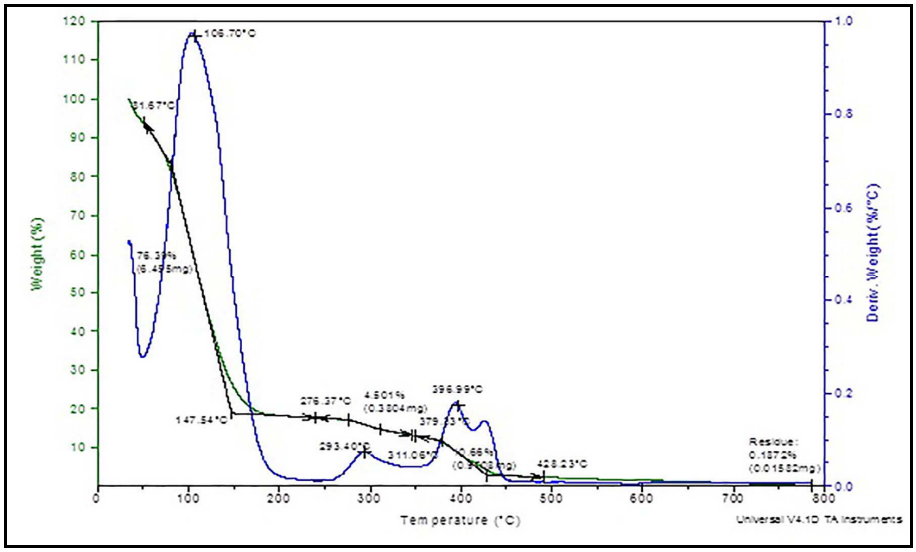


**Fig.3. TGA of (60/40) Chitosan-PEG hydrogel**

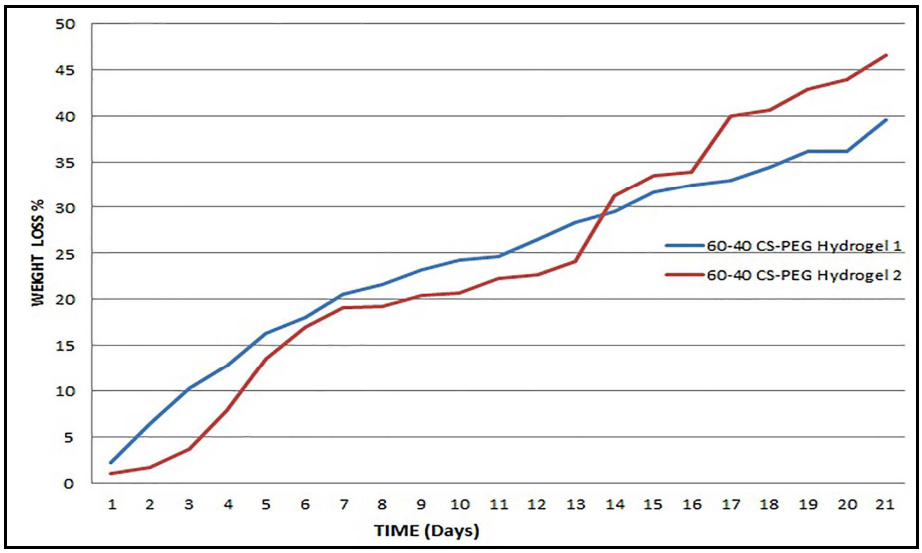


**Fig.4. Invitro degradation profile of (60/40) Chitosan-PEG hydrogel**

## 4.Conclusion

In summary,the results of this study indicate that a temperature responsive hydrogel can be formed from a combination of natural (Chitosan) and synthetic (PEG) biomaterials. The formed hydrogel was subjected to characterization techniques namely FTIR and TGA which gave satisfactory results.The in vivo degradation was also analysed. Future work should focus on the mode of delivery and biocompatability to illustrate the efficacy of Chitosan-PEG hydrogel to regenerate cardiac tissue after Myocardial Infarction.

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