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Injectable Polyethylene Glycol Terminated Poly(propylene fumarate)/ Acrylamide Biodegradable Materials for Cardiac Applications

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Abstract

Injectable and biodegradable hydrogel material based on polyethylene glycol terminated poly(propylene fumarate)(PEG-PPF-PEG)/ acrylamide have been developed and evaluated for cardiac applications. The present PEG-PPF-PEG resin undergoes fast setting with acrylamide with low exothermic temperature and mechanical properties equivalent to that of cardiac tissue. The chemically cross linked hydrogels undergo swelling in Ringer's solution prior to degradation. The present material are non cytotoxic to L-929 fibroblast and compatible with the cardiac fibroblast cells. Adhesion and proliferation of cardiac fibroblast cells has been appreciably good which is due to surface reorganisation and generation of synergistic hydrophilic-hydrophobic surface.

Key Words: Injectable and biodegradable hydrogel, Polyethylene glycol terminated pol(propylene fumarate)/acrylamide, Fast

INTRODUCTION

Natural and synthetic materials are explored as scaffold material for tissue engineering. Synthetic biodegradable polymeric materials are an attractive choice because of the controlled manner in which they can be prepared with required microstructure, mechanical properties and degradation profile. Biodegradable polymer could be designed to degrade *in vivo* in a controlled manner over a predetermined period. The advantages of degradable materials are (i) they do not have to be removed after use by secondary surgery because degradation products formed can be excreted from the body via natural pathways and (ii) progressive loss of degradable implant material will lead to regeneration of tissue.

Biodegradable polymers based on glycolide and lactide are investigated as implant for biomedical applications. These polymers have important drawbacks for use as scaffolds for soft tissue engineering such as incompatible mechanical properties and very high rates of degradation. Moreover there are challenges in the development of myocardial implant. To allow the development of myocardial tissue, the scaffold should be compatible to cell growth. The scaffold should be reproducibly produced into three-dimensional porous structures that are dimensionally stable under physiological conditions. Furthermore, the mechanical properties of the scaffolding material should be adequate to provide the correct micro stress environment for the cells to develop the required phenotype and

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orientation. Therefore the scaffold should be flexible to allow the contraction of the growing tissue and to withstand the contractions of the surrounding myocardium after implantation. The efficacy of biodegradable polymeric implant is basically dependent on the rate of biodegradation commensurate with heart tissue growth and also on the bioassimilability of degraded fragments. The major challenge is the development of suitable scaffold material for tissue engineering of myocardial tissue. The present paper deals with the injectable polyethylene glycol terminated poly(propylene fumarate)/acrylamide biodegradable materials for cardiac applications.

EXPERIMENTAL

Preparation and Evaluation of Polyethylene Glycol Terminated Poly(propylene fumarate) (PEG-PPF-PEG)

Biodegradable materials carboxy terminated poly (propylene fumarate) (CT-PPF) was prepared by reacting maleic anhydride (MA) with 1,2-propylene glycol (PG) with mole ratio MA/PG >1.0 under high temperature, 200°C and vacuum conditions for 2 h as per the general procedure published elsewhere [1]. CT-PPF was copolymerized with poly(ethylene glycol) $_{1000}$ by vacuum condensation at 160°C for 20 minutes to get polyethylene glycol terminated poly(propylene fumarate)(PEG-PPF-PEG). The setting properties (setting time and exothermic temperature of the resins) of PEG-PPF-PEG as an injectable material, were determined by crosslinking with comonomer acrylamide as per ISO 5833/1 – 1999 E standard.

Preparation and Evaluation of PEG-PPF-PEG/ Acrylamide Hydrogel Material

Injectable PEG-PPF-PEG/acrylamide hydrogel material was then prepared by cross linking with acrylamide using ascorbic acid and ammonium per sulphate and water at room temperature and casting and curing in a glass plate at 70±2˚C for 48 h.The crosslinked hydrogel samples (0.4mm diameter disc) were cleaned ultrasonically to remove unreacted acrylamide monomer and polyacrylamide oligomer. The hydrogels were subjected to freezing at -80˚C for 24 hours, freeze-dried at -110˚C for 48hours and subjected to ethylene dioxide sterilization for the evaluation. AT-IR spectral analysis of crosslinked PEG-PPF-PEG /acrylamide hydrogel material was carried out using a spectrophotometer (Nicolet, Impact 410, USA).

Cross link density and (apparent) number average molecular weight between cross-links (M_c) of the dry hydrogel material was determined by swelling the samples in distilled water and determining the maximum swelling coefficient (θ) using the modified Florry-Rehner's equation as per the general method reported elsewhere [2].

The hydrophilic and hydrophobic character of the dry hydrogel material was determined by measuring the contact angle dynamically using the KSV sigma 700 tensiometer in distilled water and Ringer's solution. The tensile strength of the swelled hydrogel was determined using dumbbell-shaped transparent flexible sheets (specimen type 5A) as per the standard ISO 527-2:1993 (E). The studies on *in vitro* aging of the dry hydrogel material were carried out in Ringers solution. Samples were aged in the medium (weight ratio of sample to medium 1:10) at 37°C for 57 days. The degree of swelling and biodegradation was estimated.

In Vitro Studies on L-929-Fibroblast Cell Interaction

In vitro cell response was studied by directly exposing the hydrogel material to L-929 mouse fibroblast cells as per ISO 10993-5. The test material was initially incubated in culture medium overnight at 37˚C to attain its maximum swelling. Then the

hydrogel sample was placed on a subconfluent monolayer of L-929 mouse fibroblast cells and incubated at 37 °C in a CO_2 incubator for 24h. The cell response around the test sample was microscopically evaluated. The hydrogel sample was tested for its affinity to adhere fibroblast cells on its surface using L-929 mouse fibroblast cells. L929 cells were seeded onto the samples and afterwards incubated at 37˚C for 24 h in a CO_2 incubator. The incubated cell sample was processed as per standard procedure for optical and scanning electron microscopic analysis. The test sample was then observed under the phase contrast and scanning electron microscopes.

Studies on Cardiac Fibroblast Cell Interaction

Cardiac fibroblast cell interaction and growth and proliferation of cardiac cells onto the hydrogel materials were evaluated. Cardiac fibroblast cells were isolated and cultured from new born rat as previously described by Nair and Gupta [3]. The sample was kept at the bottom of a petri dish and subjected to ETO sterilization. Then the required amount of cell suspension was delivered onto and around the sample. Fresh medium was also added into the seeded petri dish and kept for incubation. The cells were allowed to grow for 3 successive days. The incubated cell sample was processed as per standard procedure for scanning electron microscopic analysis. The test sample was then observed under the scanning electron microscope.

RESULTS AND DISCUSSION

Unsaturated polyester, poly(propylene fumarate) (PPF) is a nontoxic degradable polymer. The degradation products liberated during the biodegradation of PPF products *in vivo* are propylene glycol and fumaric acid which are non toxic and physiologically tolerable. Propylene glycol is commonly used as diluent in parenteral drug formulations. Fumaric acid is one of the metabolic substances of Krebs cycle. The degraded products, propylene glycol and fumaric acid could be excreted through normal physiological routes. As per the FDA regulations, cross-linked polyester resins may be safely used as articles or components of articles intended for repeated use in contact with food [4].

Poly(ethylene glycol) is flexible and blood compatible polymer. It modulates the level of cellular interaction between tissues and implants material. Poly(ethylene glycol) (PEG) has been used in the vasculature because for the molecular weight up to 20,000 it is not toxic. It has unique solubility properties and is an extremely hydrophilic polymer. The hydrophilic property of the PEG can increase the hydration of the material, as well as it can decrease the thrombogenicity and cellular response. PEG is biocompatible and intrinsically resistant to protein adsorption and cell adhesion [5,6]. It is not degradable, however, low molecular weight PEGs (<20,000 Da) are excretable. The use of PEG can enable swelling of the implant.

Though acrylamide is a potent neurotoxin for occupational workers, polyacrylamide polymers are non-toxic. Degraded acrylamide doesn't accumulate in the body. Acrylamide undergoes biotransformation by conjugation with glutathione [7,8] or reduction by microsomal cytochrome [9] P-450 with glutathione conjugation probably being the route of detoxification. The metabolites are non-toxic. Polyacrylamide based hydrogel is used as long term human breast implant; it is found to be non toxic, stable over time, nondegradable and diffusion and migration resistant [10].

Preparation and Characterisation of PEG-PPF-PEG

Biodegradable carboxy terminated poly(propylene fumarate) (CT-PPF) was prepared using mole ratio maleic anhydride/1,2-propylene glycol >1.0. CT-PPF

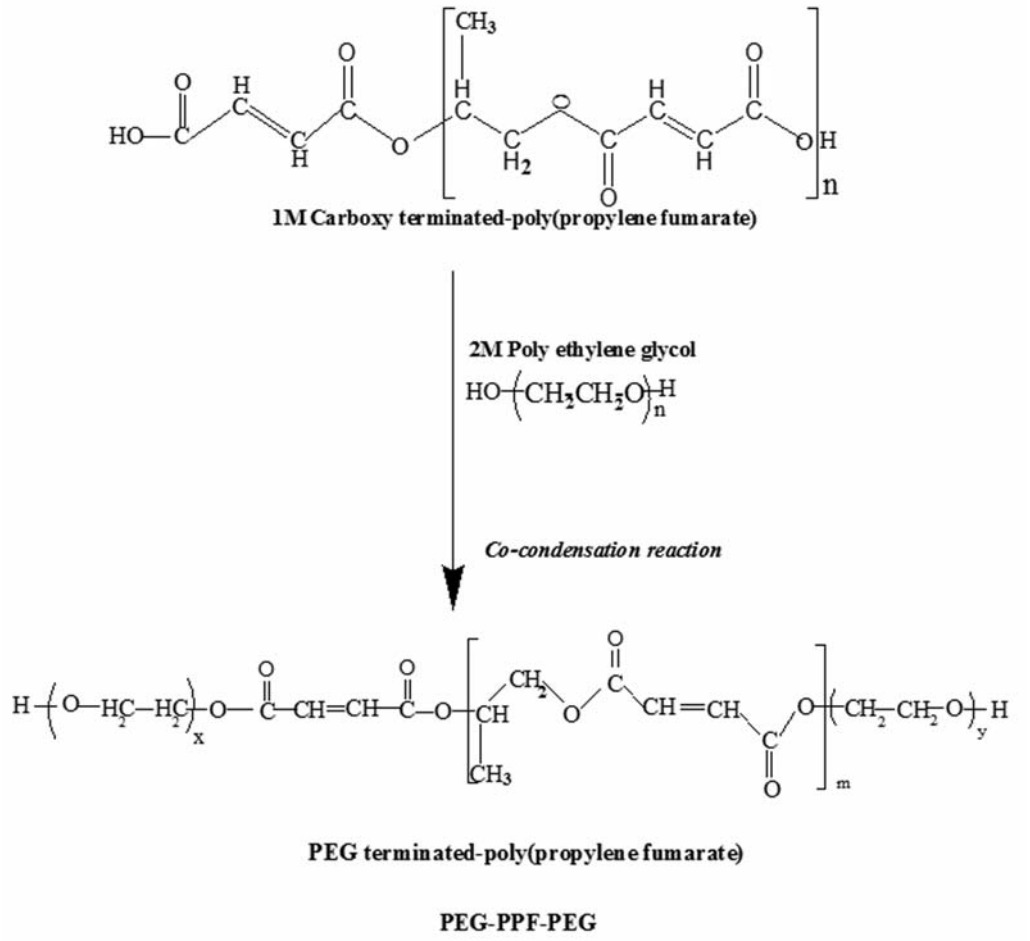


Figure 1. Synthesis of polyethylene glycol terminated poly(propylene fumarate) (PEG-PPF-PEG).

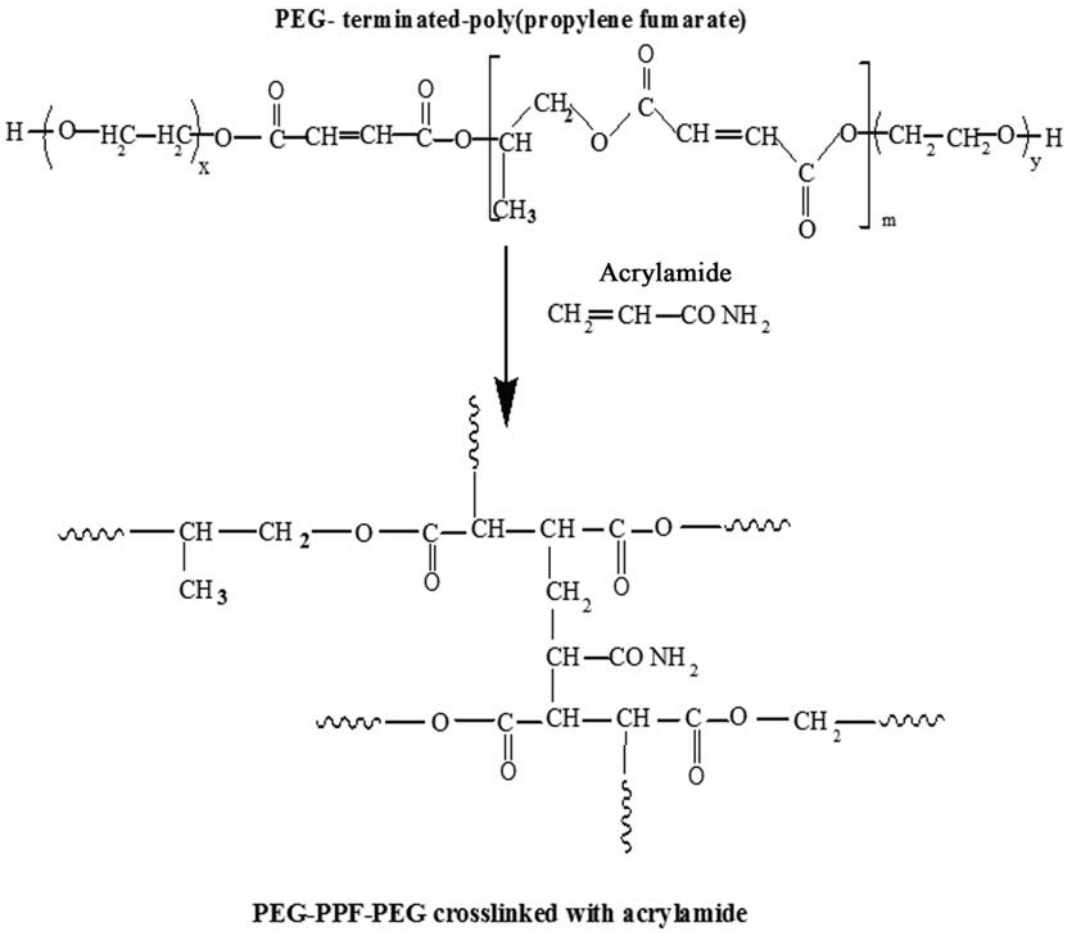


Figure 2. Cross linking of PEG-PPF-PEG with acrylamide.

was copolymerized with poly(ethylene glycol)$_{1000}$ to get polyethylene glycol terminated poly(propylene fumarate)(PEG- PPF-PEG) as shown in Figure 1. The cross linking reaction of PEG-PPF-PEG with acrylamide in the presence of water produces a three dimensionally cross linked material which can undergo gelling in water to a smart hydrogel. The cross linking reaction of PEG-PPF-PEG with acrylamide is shown in Figure 2.

The injectability and fast setting nature of the present copolymer is assessed with the setting characteristics. The copolymer, PEG-PPF-PEG undergoes rapid setting in less than 1 min without producing abnormally higher exothermic temperature (Table 1). The lower setting temperature observed in the present studies is more favourable for injectable tissue engineering applications. The mixture of PEG-PPF-PEG, acrylamide, water, catalyst and accelerator has low viscosity. The low setting time and temperature for the present system enables fast dispension of the mixture into the desired site.

The AT-IR spectral analysis of crosslinked PEG-PPF-PEG with acrylamide support the cross linking at the trans CH=CH double bonds. The spectrum reveals appearance of peaks at 1413.9 cm^{-1} (C-N stretching of amide), 1604.6 cm^{-1} (N-H bending of amide II), 1652.1 cm^{-1} (C=O stretching of amide I) and doublet at 3200-3400 cm^{-1} (N-H stretching of amide) due to cross linking of PEG-PPF-PEG with acrylamide (Figure 3).

Preparation and Evaluation of PEG-PPF-PEG/ Acrylamide Hydrogel Material

PEG-PPF-PEG on cross linking with acrylamide yields a three dimensionally crosslinked hydrogel. Swelling studies and the data on cross link density and molecular weight between cross links reveal the crosslinked nature of the present hydrogel material (Table1). The present hydrogel material is a chemically crosslinked material with effective covalent and apparent physical crosslinking (hydrogen bonding). The percentage of mass and volume swelling of present hydrogel are very high in water. The percentage of swelling, weight swelling 640±10 and volume swelling 500±15, in water is very high revealing the hydrogel character. The equilibrium swelling time is 20-25 min. The high

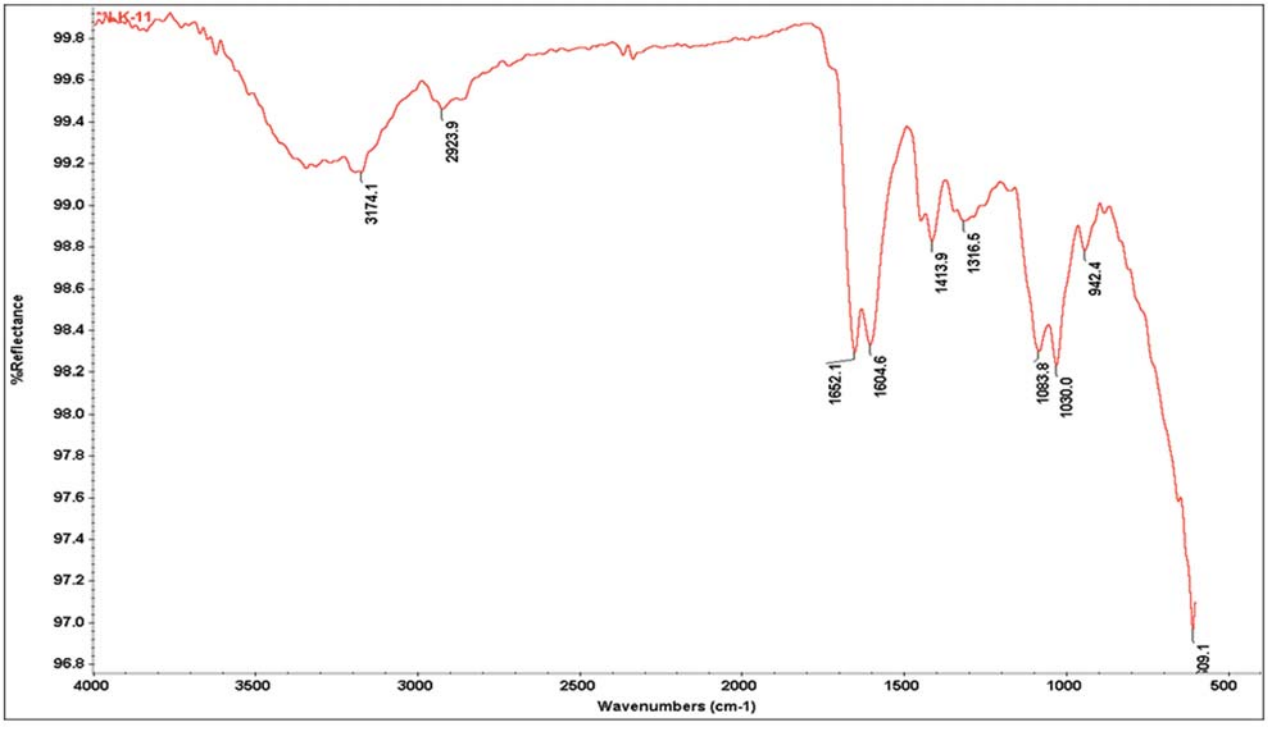


Figure 3. AT-IR spectrum of crosslinked PEG-PPF-PEG with acrylamide.

mass and volume swelling and fast swelling is attributed to the combine effect of diffusion process of water, capillary raise of water through the interconnected pores and hydration of the present copolymer. Contact angle studies on the crosslinked hydrogel materials reveal contact angle of 20 and 25 in distilled water and Ringer's solution respectively. This may be attributed to fast absorption of water and wetting of the surface leading to a flat droplet. Contact angle studies reveal synergistic hydrophilic and hydrophobic character (amphiphilic) of the crosslinked materials. Surface reorganisation in the present material is more feasible due to the hydrophilic tail end of PEG and hydrophobic main chain of PPF.

The present process of hydrogel, ultrasonic cleaning and freezing at -80°C, enable uniform distribution of water into the hydrogel network and two-dimensionally ordered interconnected pores in the hydrogel. Mechanical tests on the present hydrogel material reveal tensile strength 0.142±0.05 MPa and elongation 250±15% (Table 1). Yoshimatsu [11] have determined the tensile properties of human cardiac muscle from the myocardium of left ventricle; the ultimate tensile strength is 0.108Mpa in adult and ultimate percentage elongation is 63.8. The relatively higher tensile properties of the present hydrogel material is more favourable for tissue engineering of myocardial patch implant to augment with the process of degradation and loss of mechanical properties with time.

The biodegradation of a hydrogel *in vivo* physiological environment is largely influenced by the degree of crosslink density and molecular weight between crosslinks. The present hydrogel material has crosslink density around $8.24\pm0.5x10^{-3}$ mol/cm^3 and molecular weight between crosslinks around 121±5. Since the present hydrogel material is a crosslinked one, aging in Ringers solution leads to appreciable swelling (%) due to initial absorption of media. However the swelling (%) is comparatively lesser than that in water (Table 1). The aging studies and swelling data reveal that the present hydrogel materials may survive reasonably longer duration *in vivo* physiological environment.

Table 1. Properties of PEG-PPF-PEG resin and its hydrogel materials.

Properties	Value
Properties of the resin	
Setting temperature (°C)	47.5 ± 1
Setting time (sec)	60 ± 7
Properties of the hydrogel material	
Crosslink density (mol/cm^3) (x10^3)	8.24 ± 0.5
Molecular weight between cross links	121 ± 5
Weight swelling in water (%)	640 ± 10
Volume swelling in water (%)	500 ± 15
Weight swelling in Ringer's solution (%)	575 ± 15
Volume swelling in Ringer's solution (%)	455 ± 10
Tensile Strength (MPa)	0.142±0.05
Elongation at break (%)	250 ± 15
Contact angle in distilled water	20
Contact angle in Ringer's solution	25

In Vitro Studies on L-929 Fibroblast Cell Response with Hydrogel Material

The present hydrogel material was found to be compatible with the subconfluent monolayer of L-929 mouse fibroblast cells. The cells surrounding the material maintained their characteristic spindle like appearance and were confluent in nature as shown in Figure 4. Generally, cell adhesion is more favoured by the hydrophobicity of a material in comparison with the hydrophilic counterpart in serum-containing culture medium. It has been reported that PEG hydrogels are intrinsically resistant to cell adhesion [6]. However, cell adhesion is influenced by the synergistic hydrophilic and hydrophobic character and presence or absence of serum protein. Adhesion of human endothelial cells in serum-containing culture medium onto copolymers of hydroxyethyl methacrylate (HEMA)

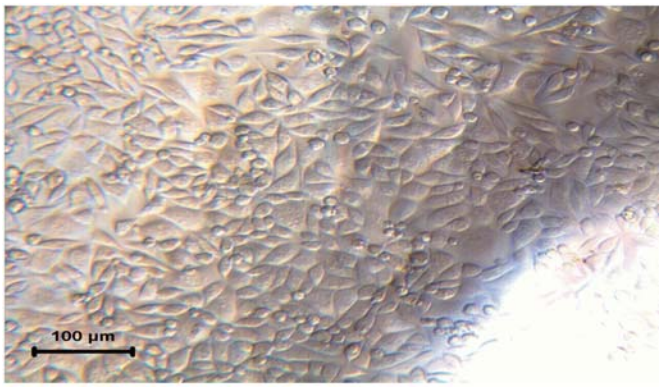


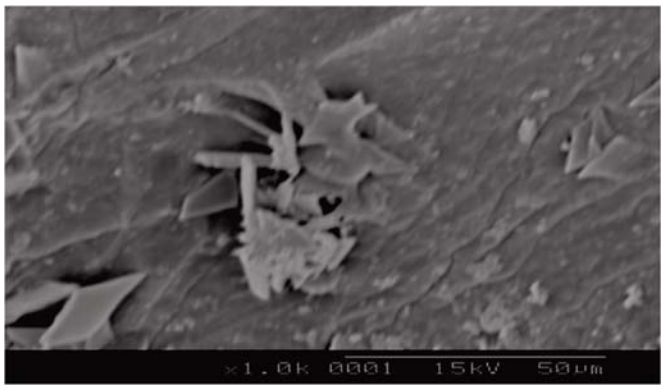
Figure 4. L 929 fibroblast cell response.
PEG-PPF-PEG/acrylamide hydrogel material placed on a subconfluent L-929 population and incubated for 24 h at 37°C in a CO_2 incubator. Micrograph depicting the viable cells surrounding the gel under the phase contrast microscope at (20X) magnification.

and methyl methacrylate (MMA) was found to be optimal on the moderately wettable copolymer (mol ratio 25 HEMA/75 MMA) [12].

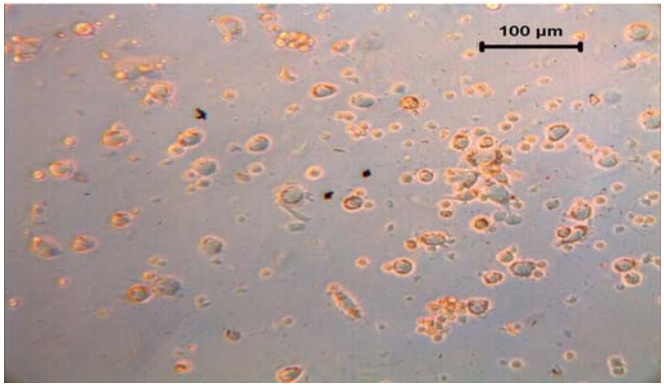
However, the adhesion of L-929 fibroblast cells on the present hydrogel material has been observed which may be due to the favourable synergistic hydrophilic and hydrophobic character for the adhesion of cells. Koyano et al. [13] has reported spherical morphology of L-929 fibroblast cells onto the poly(vinyl alcohol)/chitosan blend with lower chitosan content and spindle-shaped cells with the blend having more than 15 wt% chitosan content. Cell spreading with spindle like appearance was not seen in greater magnitude under the inverted phase contrast microscope (Figure 5). However, SEM photomicrograph reveal moderate cell spreading with extensive filopodia-like attachments which may be due to synergistic hydrophilic and hydrophobic character.

Studies on Cardiac Fibroblast Cell Response with Hydrogel Material

The studies on the interaction of cardiac fibroblast cells with the present candidate hydrogel material reveal cellular adhesion and also proliferation. Cardiac fibroblast cells adhered to the hydrogel material and spindle structure spread with filopodia-like attachment, as seen in the SEM pictures (Figure



(a)



(b)

Figure 5. L-929 fibroblast cell adhesion.
PEG-PPF-PEG/acrylamide hydrogel material incubated in DMEM for 24 h at 37°C in a CO_2 incubator and allowed to swell. Subsequently L929 cells were seeded onto the material and incubated for 24 h and then observed under the scanning electron microscope at 1.0 K magnification (a) and the inverted phase contrast microscope at 20X magnification (b).

6). The appreciable cellular growth with cytoplasmic spreading on the surface of the hydrogel material is due to the formation of synergistic hydrophilic-hydrophobic surface by surface reorganisation. The higher adhesion of cardiac fibroblast cells on the present hydrogel material can enable slow growth on and around the hydrogel material

CONCLUSION

The present polymer, polyethylene glycol terminated poly(propylene fumarate)(PEG-PPF-PEG), exhibits fast setting with comonomer, acrylamide and produces three dimensionally crosslinked porous smart hydrogel. The present hydrogel is compatible for cell growth and adhesion of L-929 fibroblast cells. Adhesion and proliferation of cardiac fibroblast

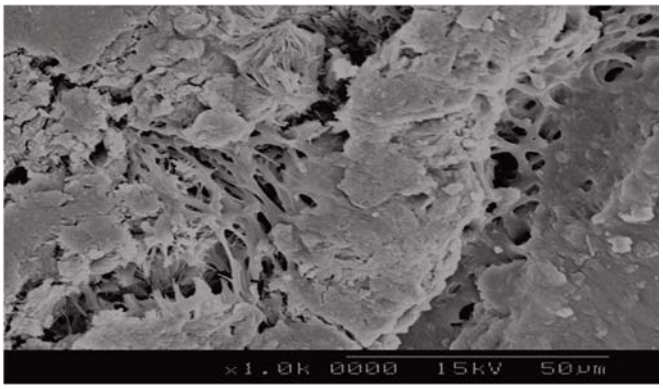


Figure 6. Cardiac fibroblast cell response.
Cardiac fibroblast cells grown on PEG-PPF-PEG / acrylamide hydrogel material as seen under 1.0 K magnification.

cells has been appreciably good which is due to surface reorganisation and generation of synergistic hydrophilic-hydrophobic surface. The present system is a promising injectable fast setting hydrogel material for cardiac tissue engineering applications.

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