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Evaluation of hydrogel composing of Pluronic F127 and carboxymethyl hexanoyl chitosan as injectable scaffold for tissue engineering applications

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ABSTRACT

This study demonstrated a novel hydrogel system composing of Pluronic F127, carboxymethyl hexanoyl chitosan (CA) and glutaraldehyde (GA) for encapsulating fibroblasts (L-929). The thermal behavior of the hydrogel was evaluated using TGA, the swelling behavior of the hydrogel was evaluated in Dulbecco's Modified Eagle's medium (DMEM), and the mechanical properties were determined through dynamic mechanical analysis. Cells were encapsulated by simple mixing, and the viability of encapsulated cells was determined using alamar blue cell viability assay and the cells morphology was examined using fluorescent imaging. The results indicated that the T_{gel} of this system was around 30 °C, where sol-gel transformation occurred within 90 s. Although the addition of CA and GA reduced the shear moduli slightly, the F127/CA/GA gel was able to remain in gelling state in the medium for more than 1 month. *In vitro* cell culture study revealed that F-127/CA/GA hydrogels were non-cytotoxic. Moreover, the viability of encapsulated L929 was 106% after incubation for 5 days. Based on these results, these F127/CA/GA hydrogels can be used to encapsulate cells for tissue engineering applications.

1. Introduction

Hydrogels have received a significant attention in medical area because of their high water content, good biocompatibility and their ability to mimic tissue environment. Many researchers used hydrogel for cell encapsulation, injectable scaffold and drug carrier. The results indicated that hydrogel could enhance cell behavior such as the rate of cell growth [1–6], the specific differentiation marker in the bone cells [7–9], bone formation [10,11], and vascular anastomosis [12]. Beside enhancing cell behavior, hydrogel can also enhance the absorption of drug in the breast tissue as drug carrier [13]. Natural polymers such as alginates, collagen, hyaluronic acid and chitosan, and synthetic material such as poly(ethylene glycol) (PEG), poly(propyl fumarate) (PPF) and poloxamers have been used to form hydrogels in biomedical field.

Pluronic F127 is a poloxamer that consists of polyoxyethylene (PEO) and polyoxypropylene (PPO) with two 99-unit hydrophilic PEO blocks surrounding one 65-unit hydrophobic PPO block. The F127 has a molecular weight approximately 12,600. At temperature above lower critical solution temperature (LCST), F127 will lose the solubility of PPO block which leads to the formation of micelles, and the solution transforms into gel [14–17]. F127 has been widely used in biomedical application due to its gelling behavior and biocompatibility. However, F127 hydrogel has some undesirable characteristics such as poor mechanical stability, lower stability and integrity in aqueous environment [18,19]. In order to address this problem, many researchers have modified poloxamer with other material such as hyaluronic acid [17,18,20], carbopol [21], methylcellulose [22], chitosan [5,23–25], alginate [20] and others [16,26–30].

Plu-ALA-L was obtained by chemically photo-crosslinked of pluronic, N-methacryloyl-1-alanine, lactic acid and irgacure 2959. The solution was mixed with bone marrow-derived mesenchymal stem cells-loaded gelatin, then injected into the medial diaphyseal cortex of the tibiae. Hydrogel transformed into gel because of the increase in temperature and the irradiation of UV A (2 min; $I = 3000\ mW/cm^2$; $\lambda = 365\ nm$). A significant difference was found between the bone substitute conditions and control defects for the size of the periosteal reaction and callus formation. After 8-weeks implantation, most defects were filled with new bone in the hydro-

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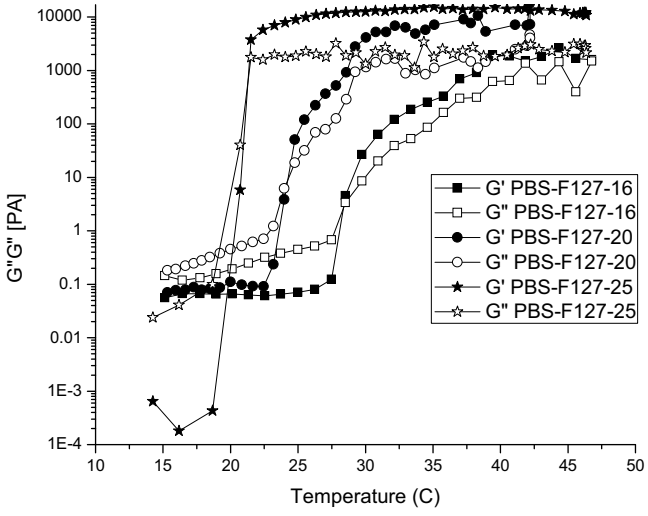
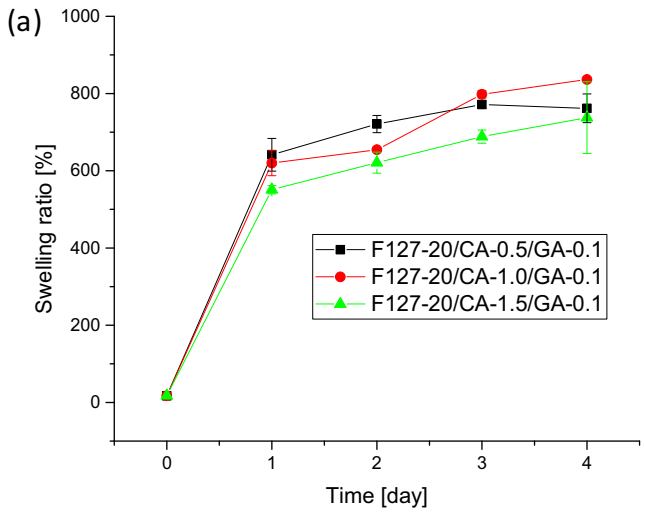


Fig. 1. Elastic and viscous moduli of hydrogel.



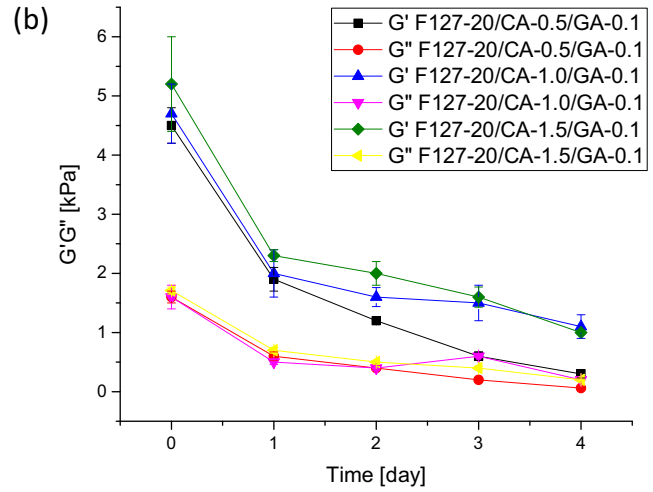


Fig. 2. (a) Swelling behavior and (b) dynamic moduli of F127/CA/GA hydrogel in DMEM.

gel system. This system was successful to encapsulated stem cell to enhance the bone regeneration [10].

F127 was photoiniatiator crosslinked with 0.01% irgacure 2959 to produce an injectable intraocular lens (IOL) hydrogel. Hydrogel was injected into the capsular bag of rabbit eye and the entire eye was irradiated by UVB (300 nm) for 5 min to induce gelation formation. The result showed no inflammation and no toxicity was observed in the conjunctiva, cornea, iris, vitreous and retina [31].

The other researchers used chitosan to enhance the properties of poloxamer in several ways. Chitosan-pluronic (CP) graft copolymer was obtain by crosslinking monocarboxy pluronic (MP) with chitosan. The mixing of chondrocytes pellet and CP was injected into the well and incubated for 15 min for gel formation. The viability of cell remained good until 28-day incubation [5].

Pluronic with hydroxyl terminal group was chemically modified with acryloyl choride to construct diacrylated pluronic. Before chitosan was mixing with modified pluronic, hydroxyl groups of chitooligosaccharide (COS) were reacted with glycidyl methacrylate to obtain glycidyl methacrylated COS. Then, human dermal fibroblasts (HDF) solution was mixed together with diacrylated pluronic, glycidyl methacrylated COS, basic fibroblast growth factor (bFGF), heparin and irgacure 2959. The solution was irradiated by UV (320–500 nm) irradiation for 15 s, and then incubated for 30 min for gel formation. The results showed the integrity of hydrogel increased. The cell viability in the 20% COS showed higher viability than in 50% COS because of the higher content of COS [23]. Higher content of modified chitosan can decrease cell viability due to the increasing intracellular reactive oxygen species [32].

The common methods to induce gelation of hydrogel are through chemical crosslinking or photoinitiator crosslinking. These methods may occur *in vivo* or *in vitro*. Hydrogel compounds, cells and crosslinking agents or photoinitiator are mixed together before crosslinking [7,8,10,23,31]. However, chemical crosslinking is expensive and some chemicals used in the crosslinking are toxic to organism. On the other hand, most photoinitiator requires UV exposure to crosslink the hydrogels. UV exposure may damage the DNA of cells. To overcome these limitations, we adopted thermally induced strategy to obtain injectable hydrogel scaffolds.

Our previous work tried to make an *in situ* forming gel consisting of F127 and chitosan. In this system, hydrogel was prepared by simple mixing in the acidic solution. The result indicated that hydrogel exhibited biocompatibility of osteoblast cells, and exhibited T_{gel} close to the body temperature [24]. However, the gel disintegrated rapidly in the medium, making it unsuitable for cells encapsulation. Furthermore, the low pH (5–6) in the system can decrease cell viability. Literatures stated that pH will influence the cell viability at the longer incubation time [33]. In this work, we tried to resolve the integrity and pH problem by changing the chitosan with a water-soluble chitosan derivative and adding GA in the of F127 hydrogel.

Carboxymethyl hexanoyl chitosan, commonly referred as Chitosonic® Acid (CA), is a new kind of chitosan derivative that is dissolvable in water at neutral pH. Its molecular weight is approximately 160 kD. CA exhibits antimicrobial activities against gram-positive bacteria, gram-negative bacteria and fungi. CA also exhibits high hydration capability for absorbing and retaining water molecules with its hydrophilic groups. It also has moderate DPPH radical scavenging activity, and has no cytotoxicity to cells [34].

The aim of this study is to develop an injectable F127-based hydrogel with short gelation time, stable structure in medium, and non-cytotoxicity for therapeutic applications. The gelation temperature, gelation time, mechanical strength, thermal behavior and swelling behavior of the hydrogel were measured. Furthermore, the viability of cells encapsulated in the hydrogel was also evaluated.