Study on the Composite with Sequential and Sustained Release of Multiple Growth Factors for Bone Repair

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ABSTRACT

Previous studies on the drug carrier of single growth factor for bone repair have encountered a challenge that the regeneration of the new bone was not completely restored [1]. Thus, the drug carrier systems with multiple growth factors have emerged [2]. The current studies of drug carrier with multiple growth factors still face the difficulties that the growth factors needed to be sequential and sustained released. To achieve the goal of sequential and sustained release of multiple growth factors, here we propose an injectable drug-loaded implant including poly(lactide-co-glycolide) (PLGA)/poly(vinyl alcohol) (PVA) microspheres and chitosan-based hydrogel. A fluorescent dye, fluorescein isothiocyanate (FITC), was employed as a model drug which was encapsulated in the PLGA/PVA microspheres and hydrogel. Optical microscope, scanning electron microscope, and laser confocal microscope were applied to analyze the material structure and morphology of microspheres. Fourier transform infrared spectroscopy was applied to characterize the bonding of chitosan-based hydrogel. The release profile of FITC released from the PLGA/PVA microspheres and hydrogel were studied. The cell viability of the proposed implant was also examined. It was found that the PLGA/PVA microsphere-embedded hydrogel demonstrated a sequential and sustained release profile, and the composite achieved a high cell viability. Most importantly, the composite could be applied through an injectable style, which accommodates to clinical use. This composite can then be applied to encapsulate and release multiple growth factors for bone repair and bone tissue engineering.

Keywords: injectable hydrogel, growth factor, sequential release, bone repair, bone tissue engineering

1 INTRODUCTION

To the repair of bone defect, any kinds of materials, such as hydroxyapatite and polymer materials, for utilization must be tailored to control its hardness and degradation to a specific degree so as to generate appropriate osteoconduction and osteoinduction [3]. These bone-repairing materials need to combine specific growth factors to promote the repair of bone resembling the natural bone-repairing process. The growth factors involved in bone repair is not only one type [4]. If only single growth factor is exerted, the repair of bone is not perfect and the consequently use of large amount of growth factor may lead to inflammations and complications. On the contrary, the combination of multiple growth factors can regulate doses of growth factors and attain to long-term medication.

In bone repair, the applying of conventional material will be prepared by following the hardness and the composition of normal bone as standard. However, these materials confront the difficulty of degradation [5] and the regeneration of bone. In recent years, a scaffold with an injectable style is employed to solve these problems, but a scaffold carrying growth factors alone can not control the release of growth factors to a long period of time, while a drug carrier is easily contolled [6]. Therefore, the combination of a bone-repairing injectable scaffold with a micosphere of sustained-releasing drug to achieve sequential and sustained release of multiple growth factors resembling the natural bone repair is an important issue.

2 MATERIALS AND METHODS

2.1 Reagents

All the reagents were purchased from Sigma-Aldrich, USA. For cell culture, phosphate buffer saline (PBS), Dulbecco's Minimum Essential Medium (DMEM), penicillin, streptomycin, amphotericin, sodium pyruvate, glutamine, and 0.25% trypsin – EDTA were purchased from GIBCO, Invitrogen Corporation. All the other reagents were of analytical or equivalent grade. Double distilled water was employed throughout.

2.2 Methods

2.2.1. Preparation of injectable scaffold

Cellulose were dissolved in double distilled water and oxidant was added. The reaction was stop by added ethylene glycol and exhausted dialysis. The product was lyophilization and redissolved in water. Chitosan was dissolved in water by acetic acid. The mixing of the two solutions derived the injectable scaffold.

2.2.2. Preparation of PLGA/PVA microsphere

PLGA was dissolved in dichloromethane. FITC and PVA was dissolved in water respectively. After adding the FITC solution into PLGA solution, homogenizer was applied. Then, the mixture was poured into the PVA solution for stirring and filtered. The microspheres were harvested by lyophilization.

2.2.3. Optical microscope analysis

Optical microscope image was taken using Nikon Eclipse TE2000-U. The PLGA/PVA microspheres were dispersed in water. The image was used to check the morphology of PLGA/PVA microsphere.

2.2.4. Scanning electron microscope analysis

Scanning electron microscope image was taken using JEOL JSM-5330. The image was used to check the morphology and surface of PLGA/PVA microsphere.

2.2.5. Laser confocal microscope analysis

Laser confocal microscope image was taken using Olympus FV1000. The image was used to check the FITC was actually loaded in the PLGA/PVA microspheres.

2.2.6. Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy was recorded using PerkinElmer Spectrum 100 FTIR Spectrometer. The spectrum was used to confirm the formation of Schiff's base in the injectable hydrogel.

2.2.7. In vitro release test of FITC

The in vitro FITC release was performed in PBS. The concentration of PLGA/PVA microsphere was 1 mg/ml. The FITC concentration in the chitosan-based hydrogel was 1 mg/ml. The release profile was used to demonstrate the sequential and sustained release profile of the composite.

2.2.8. In vitro cell toxicity test

The MC3T3-E1 cells were cultured in DMEM with 10% FBS and incubated at 37℃ under 5% CO_2 atmosphere. The hydrogel of different composition was kept in contact with the cells. Cell viability was assessed with MTT assay.

3 RESULTS AND DISCUSSION

An injectable composite with a chitosan-based hydrogel and PLGA/PVA microspheres was formulated and characterized using optical microscope, scanning electron microscope, laser confocal microscope, in vitro release test, Fourier transform infrared spectroscopy, and cell viability studies. Fig. 1 shows the hydrogel formation when the two solutions are mixed together in a sample bottle. The PLGA/PVA microspheres are embedded in the hydrogel.

Fig. 1: The formation of chitosan-based hydrogel embedded with PLGA/PVA microspheres.

The morphology of the PLGA/PVA microspheres were checked by using the optical microscope and scanning electron microscope showing in Fig. 2(a) and 2(b). The size of of the PLGA/PVA microspheres were about 20 μm.

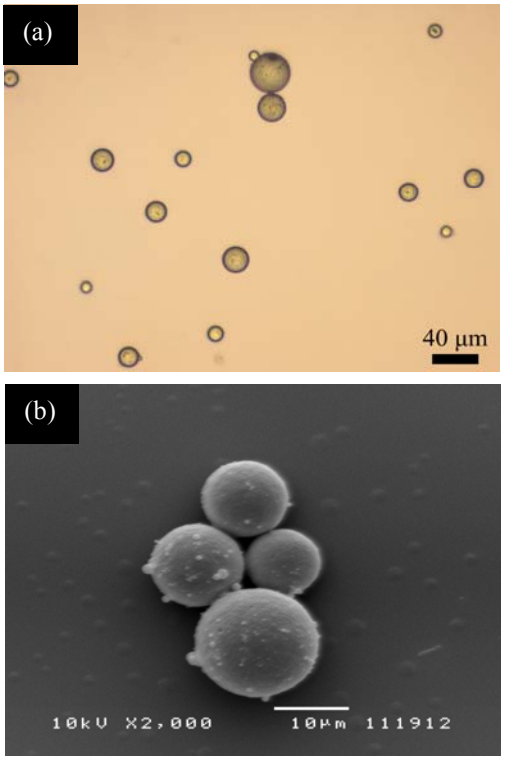

Fig. 2(a): Optical microscope image of PLGA/PVA microspheres; 2(b): Scanning electron microscope image of PLGA/PVA microspheres.

The FITC loaded in PLGA/PVA microsphere were check by laser confocal microscope. As showing in Fig. 3, the fluorescen of FITC concentrates in the microsphere, which confirms that the FITC was successfully loaded in the PLGA/PVA microspheres.

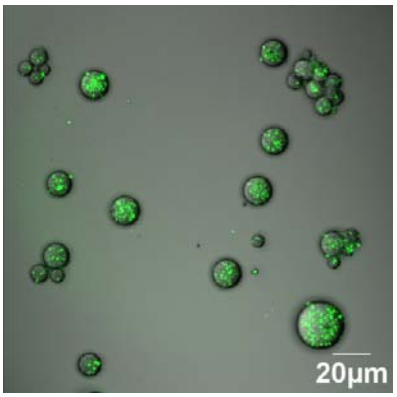

Fig. 3: Laser confocal microscope image of FITC-loaded PLGA/PVA microspheres.

The bonding in the chitosan-based hydrogel was characterized by the Fourier transform infrared spectroscopy as shown in Fig. 4. The Schiff's base formation confirm the crosslink in hydrogel.

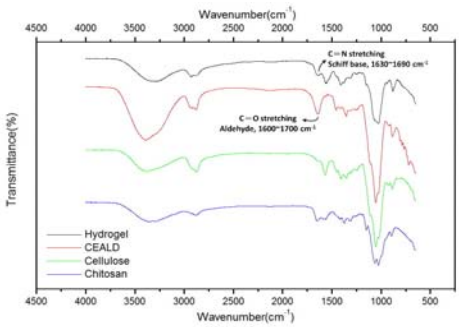

Fig. 4: The Fourier transform infrared spectroscopy of the chitosan-based hydrogel.

The in vitro release test of FITC was performed both for the PLGA/PVA microsphere and the chitosan-based hydrogel showing in Fig. 5(a) and 5(b) respectively. The release profile of the PLGA/PVA microsphere showing a sustained release profile while the chitosan-based hydrogel showing a initial burst release profile. The combination of the two release type can provide a composite with sequential release profile.

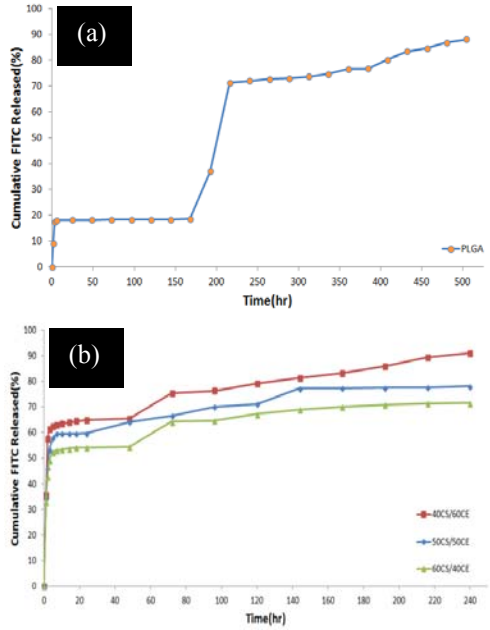

Fig. 5(a): The release profile of PLGA/PVA microspheres; 5(b): The release profile of chitosan-based hydrogel.

The in vitro cell toxicity test of the chitosan-based hydrogel was performed to find out the different composition of hydrogel giving rise to different cell viability as shown in Fig. 6.

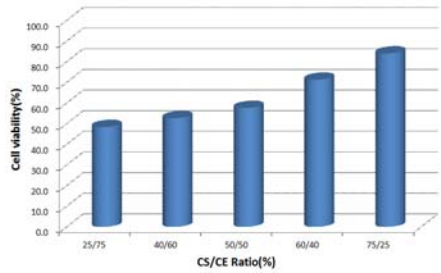

Fig. 6 The cell viability of different composition of the chitosan-based hydrogel.

4 CONCLUSION

In this study, a PLGA/PVA microsphere and a chitosan-based hydrogel loading FITC were prepared to be a composite. The encapsulation of FITC in PLGA/PVA microspheres and the bonding of crosslink in chitosan-based hydrogel were confirmed. The composite succeeded in sequential and sustained release, which can encapsulate multiple growth factors for bone repair and bone tissue engineering.

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