Evaluation of F127/chitosan hydrogel as a scaffold for bone repair

K. T. Vu Thai, M.-C. Yang* and W.-F. Lai**

*Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan, 106, ROC, myang@mail.ntust.edu.tw
**Center for Nano Tissue Engineering & Image Research, Taipei Medical University, Taipei, 110, Taiwan, ROC, laitw@tmu.edu.tw

ABSTRACT

An *in situ* gelled scaffold for bone tissue engineering to restore the function of bone tissues is developed. The gel is comprised of Pluronic F127 and chitosan. The gelation temperature (T_{gel}), and viscoelastic properties and mucoadhesive force of the systems were investigated by means of rheological analyses. At specific concentrations, the hydrogel exhibited a Tgel close to the body temperature due to the addition of chitosan. Mucoadhesion experiments showed a rheological synergism between F127/chitosan gels and mucin dispersion, and a change in the flow behavior. *In vitro* release results indicated that the optimized gel was able to prolong and control acyclovir release for more than 20 min. Based on cell proliferation assay, the gel exhibited biocompatibility for osteoblasts.

Keywords: F127, chitosan, viscoelastic properties, in situ forming gel, bone repair

1 INTRODUCTION

The goal of a scaffold is for the cells to attach to the scaffold, multiply, differentiate, and organize into normal, healthy bone as the scaffold degrades. The signaling molecules can be adhered to the scaffold or incorporated directly into the scaffold material [1]. In this context, the use of carriers for the release of therapeutically active principles based on polymeric solution able to gel *in situ* at body temperature, appears to be very attractive. In fact, such a preparation is liquid at room temperature and can be easily administered or injected. Once the gelation occurs, high resistance to flow and prolonged permanence of the drug at the site of administration may be obtained.

In general, the gelation of a polymeric solution can be triggered by a number of factors such as variations of temperature, as for poloxamers [2] and ethyl/hydroxyethyl cellulose [3], pH, as for cellulose acetophthalate [4] and Carbopol [5]. Among them, a promising strategy appears to be a gelation triggered by a temperature change since platforms' properties can be easily tuned as a function of therapeutic needs and administration routes.

This work is aiming to obtain an *in situ* forming gel with improved mechanical and mucoadhesive properties and improved retention time for bone tissue engineering. For this work, poloxamer Pluronic F127 and chitosan were used to prepare in situ forming gels, with the former used as a gelling agent and the latter used as a mucoadhesive agent. Concentrations from 14~20wt% and 0.5~2wt% of F127 and chitosan, respectively, were evaluated by oscillatory rheology, with the purpose of obtaining an optimal gelation temperature. The rheological and mechanical properties, as well as the mucoadhesive ability of the F127 gels as a function of chitosan concentration, were evaluated. The viscoelastic properties, gelation temperature and bioadhesive force of the gels were investigated and optimized by means of rheological analysis. Then, in order to test the feasibility of these platforms for bone tissue engineering, the optimized formulations were loaded with acyclovir and its release studied in vitro under simulated conditions in collagen solution. In addition, the biocompatibility of the gel of F127 and CS was evaluated with osteoblasts *in vitro*.

2 EXPERIMENTAL

2.1 Materials

Pluronic F127, acetic acid, mucin, bovine collagen, acyclovir, human plasma fibrinogen (HPF), Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 Ham (DMEM/F12), 3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) were purchased from Sigma, USA. Fetal bovine serum (FBS), phosphate buffered saline (PBS) were obtained from Caisson Labs, USA. Human serum albumin (HSA, MW 66 kDa) was provided by Talecris Biotherapeutics Inc, USA. Chitosan (Mw 100-300 kDa) and sodium dodecyl sulfate (SDS) were supplied by Acros Organics, USA. All chemicals were used without any further purification.

2.2 Preparation of Polymer Solutions

Chitosan solution (0.5–2 wt%) was prepared by dissolving in aqueous solution of acetic acid (0.5 wt%). The chitosan solution was then refrigerated and used as a solvent for the F127 (14-20 wt%) dispersion.

2.3 Rheological Characterization

The solid-gel transition temperature (T_{gel}) of each formulation under examination was assessed by measuring

the storage modulus (G') and loss modulus (G") using a rotational rheometer (MCR 102, Anton Paar, Austria) with a stainless steel cone and plate geometry (25 mm diameter and 1° angle and a gap of 48 μm between the cone and plate) and temperature ramp step oscillation procedure. The T_{gel} was considered as the temperature at which the two moduli were equal (the intersection of G' and G"), as reported by other researchers [1, 6, 7].

2.4 Mucoadhesion Analysis

Mucoadhesive characteristics of the polymeric systems were evaluated through a method reported in the literature [8]. This method is based on the evaluation of the ''rheological synergism" existing between the mucoadhesive polymer and mucin. In particular, it represents an additive growth of the mixture viscosity that occurs when mucoadhesive polymers are mixed with mucin dispersions, and which depends on the interactions between the macromolecular chains of the two species. Viscosity of porcine gastric mucin dispersions (15 wt%) in acetate buffer (pH 5.5 or pH 7.0) was measured in the absence (η_m) or presence (η_t) of polymeric mixtures (0.1-2.5%, w/v) in order to evaluate the mucoadhesive characteristics of the formulations in the presence of non-ionized or ionized chitosan (pH 5.5 and 7.0, respectively).

2.5 In vitro Release Study

Acyclovir was added and dispersed into the polymer platforms to form a homogeneous solution of 3 wt%. Then the loaded polymeric platform was immersed in a thermostatic bath at 37°C. Once the polymeric platform gelled, the flask was filled with bovine collagen. Then the flask was subject to continuous agitation with a magnetic stirrer. The samples were filtered through 0.45 μm syringe filters and subjected to high-performance liquid chromatography (HPLC) analysis to determine the concentration of acyclovir. Acyclovir concentration was quantified by HPLC using a silica column and a mobile phase composed of CH_3CN/H_2O (70:30 v/v). The flow rate and UV wavelength were 2 ml/min and 255 nm, respectively. The injection volume was 1 ml. The drug concentrations were determined from the calibration curve.

2.6 Cell Proliferation

The experiment was carried out in accordance with ASTM F2739-08. Human fetal osteoblasts (hFOB 1.19) were cultured in DMEM/F12 medium containing 10% FBS in 75 cm^2 cell culture flasks at 34°C under humidified atmosphere of 5% CO_2. The osteoblast culture was maintained in an incubator for 14 days and changed with fresh medium every 3 days. Then, hFOBs were harvested from their 20th passage culture by trypsin-EDTA treatment and replated. The populations of cell lines used in this experiment were those obtained from the 21st and 22nd passages. The viability of hFOB was determined with the MTT assay using an ELISA spectrophotometric plate reader at 570 nm..

A total of 1.5×10^4 cells in 1 ml of medium were seeded to each well of 24-well culture plates. Fresh medium was changed every 24 hours during incubation in a CO_2 incubator at 34°C. 1 ml of dilution for each extract using the culture medium as diluent, were incubated with cells in each well for 1, 3 and 5 days, respectively. The viability of osteoblasts was determined with the MTT assay. A solution of MTT (5 mg/ml) was prepared in PBS and sterilized by filtration. After culturing in the 24-well plate for 1, 3 and 5 days, 0.2 ml of MTT:DMEM/F12+10% FBS (1:9) were added to each well and incubated at 34°C for 4 hr. Viable cells reduce the colorless tetrazolium salt to blue formazan crystals. The blue formazan reaction product was dissolved by the addition of 0.1 ml of DMSO. Thereafter, aliquots were pipetted into 96-well plates and the samples were analyzed using an ELISA spectrophotometric plate reader at 570 nm. Three specimens for each material were evaluated [1, 9, 10].

3 RESULTS AND DISCUSSION

3.1 Rheological Characterization

Table 1 shows that T_{gel} obtained for different F127 concentrations (14%, 16%, 18% and 20 wt%) is in accordance with the results obtained in the literature [7] and confirms that the T_{gel} is dependent on polymer concentration. Hence, according to the results, the suitable solution containing 16% of F127 exhibited an adequate T_{gel} (32 ± 1 °C). Nonetheless, the main goal of the present work is to combine poloxamer with another polymer, chitosan, in order to obtain an *in situ* gel with increased mucoadhesiveness. Because the mechanism of gelation of F127 is based on micelles packing and entanglements [11], the inclusion of drugs or additives may interfere the micelle formation and, consequently, causing a T_{gel} modification [12]. Therefore, the effect of chitosan concentrations (0.5–2 wt%) on the gelation temperature of the *in situ* gel containing 16% F127 was evaluated.

SAMPLE	F127 (wt%)	CS (wt%)	T_{gel} (°C)[a]
F127-14	14	-	28 ± 0.8
F127-16	16	-	32 ± 1.0
F127-18	18	-	25 ± 0.5
F127-20	20	-	22 ± 0.3
F127-16/CS-0.5	16	0.5	32 ± 0.5
F127-16/CS-1.0	16	1.0	31 ± 1.0
F127-16/CS-1.5	16	1.5	30 ± 1.0
F127-16/CS-2.0	16	2.0	28 ± 1.0

[a] Means (±SD) of at least three replicate measurements

Table 1 Phase transition temperature for F127 formulations.

The results indicated that chitosan did not significantly affect Tgel in all the concentration ranges studied, as shown in Table 1. Chitosan is a polysaccharide with a high molecular weight (MW = 100–300 kDa), and therefore, it was expected that this could interfere the gelation. However, at the range of concentrations used here, the values of Tgel varied between 28 to 32°C. Thus, the addition of chitosan would not disqualify F127/CS formulations as injectable in situ hydrogels.

Figure 1 shows the effect of chitosan content on the dynamic shear moduli of the F127 hydrogels below (25°C) and above (37°C) the T_{gel}. All the moduli increased with the chitosan content. This may be related to the hydrogen bonds existing between chitosan and F127. Therefore, the addition of chitosan can enhance the mechanical strength of F127 gel. When the temperature was below the gelation temperature, the formulations behaved as liquid, thus G" was higher than G'. On the other hand, at temperature above the T_{gel}, the formulations gelled, thus G' was higher than G", as discussed above. In particular, at 37°C, the G' of F127-16/CS-2.0 was about 5 times of that of F127. However, higher chitosan content may not be the best among the formulation tested in this work. This will be discussed in the following sections.

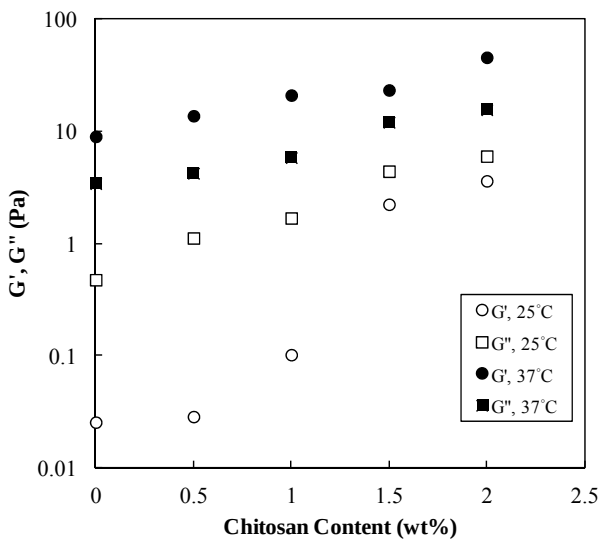

Figure 1. Effect of CS on the G' & G" of F127.

3.2 In vitro Release Study

Figure 2 shows the drug release curves of acyclovir from F127-16, F127-16/CS-0.5, F127-16/CS-1.0, F127-16/CS-1.5 and F127-16/CS-2.0. All the formulations were able to sustain acyclovir release for more than 20 min which is a time scale of interest for bone tissue engineering. In particular, the release kinetic of acyclovir from F127-16 was the fastest and, above all, poorly controlled. By adding CS from 0.5 to 2wt% into the F127 blend it was possible to achieve more reproducible drug release kinetics and so a more accurate control over drug release from the polymeric platforms. To study the application of the platforms for bone tissue engineering, the dilution of F127-16/CS-1.5 with collagen was demonstrated to not affect the gelation of the system. One of the main drawbacks that limiting the use of F127 gels as platforms for drug delivery is that they undergo a rapid dilution when exposing to large volumes of aqueous solutions [13, 14]. This causes a poor control over drug release rate which is, generally, too fast. The in vitro drug delivery experiments to assess release kinetics from a thermosensitive gel, showed that F127-16/CS-1.5 was able to prolong and control acyclovir release for more than 1 h while F127-16 exhibited a release rate too fast and not reproducible. Drug release from these thermosensitive gels takes place through a combined diffusion/dissolution mechanism and the ability of low molecular weight CS to reinforce the F127 gel structure is reflected in gel ability to better control with prolonged acyclovir release. Finally, the release kinetic of the drug from the system could be further improved in vivo by mucoadhesive interactions of the formulations with bone tissue. These encouraging results are needed for their possible use for bone tissue engineering.

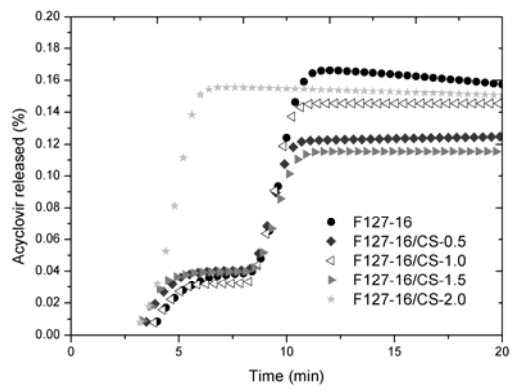

Figure 2. Acyclovir release from gel samples in collagen solution at 37°C.

3.3 Mucoadhesion analysis

Figure 3 shows the flow curves of mucin dispersion, F127-16/CS-1.5 and their mixture at pH 5.5 and 7.0 and T = 37°C. At both pH values, the presence of mucin in F127-16/CS-1.5 strongly affected its shear flow behavior. The viscosities of mucus and F127-16/CS-1.5 indeed smoothly decreased with shear rate, while their blend showed a pseudoplastic behavior, being the viscosity constant at low shear rates (Newtonian plateau) and decreasing sharply as the shear rate increased with a slope in a log/log scale of 0.04 at pH 5.5 and 0.171 at pH 7.0.

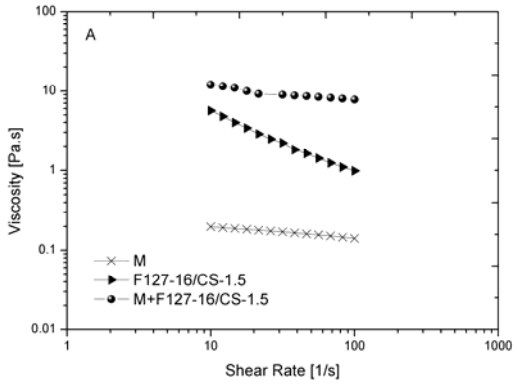

Figure 3. Flow curves of mucin dispersion at 15wt% (M), F127-16/CS-1.5 and their mixture (M + F127-16/CS-1.5) at pH 5.5 (A) and 7.0 (B); measurements were performed at 37°C.

3.4 Cell Proliferation

Figure 4 shows the viability of hFOB that were exposed to F127/CS. MTT results revealed a good cell viability between experimental groups at 24, 72 and 120 h, suggesting that F127/CS based materials exhibit good biological safety with almost non-cytotoxicity. In addition, F127/CS showed similar increases in the relative cell growth after 3 and 5 days of incubation, evidencing a positive effect of F127/CS on cell viability.

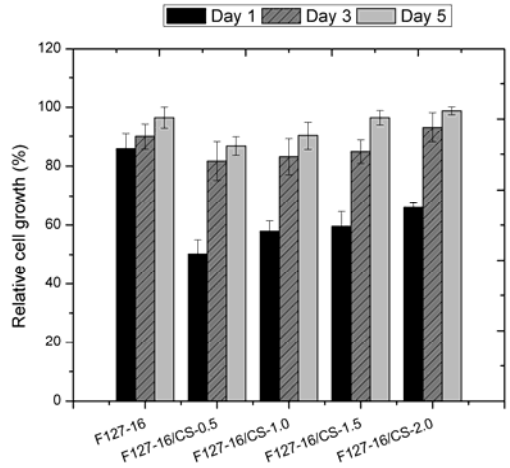

Figure 4. Proliferation of osteoblast on gel surface.

4 CONCLUSION

In this study, an *in situ* forming gel with improved mucoadhesive properties, as well as improved retention time, was obtained by the combination of F127 and chitosan. This study demonstrates that the mixing of CS with F127 can be considered as a useful method to engineer thermosensitive and mucoadhesive polymeric platforms for bone tissue engineering. In particular, the F127/CS formulation of 16/1.5 wt% showed an optimal gelation temperature (31°C) and was able to withstand low shearing forces at body temperature. The results of dissolution and release tests, on the contrary, suggest a weak effect of CS addition within F127-based gels. On the basis of their viscoelastic properties, mucoadhesive force, gelation behavior in collagen solution and *in vitro* release properties, F127/CS gels are expected to be useful for a wide range of applications in bone tissue engineering.

REFERENCES

[1] G. Dumortier, J. L. Grossiord, M. Zuber, G. Couarraze, and J. C. Chaumeil, Drug Development and Industrial Pharmacy, 17, 1255–1265, 1991.
[2] S. D. Desai and J. Blanchard, Journal of Pharmaceutical Sciences, 87, 1190-1195, 1998.
[3] K. Lindell and S. Engström, International Journal of Pharmaceutics, 95, 219-228, 1993.
[4] R. Gurny, T. Boye, and H. Ibrahim, Journal of Controlled Release, 2, 353-361, 1985.
[5] B. Srividya, R. M. Cardoza, and P. D. Amin, Journal of Controlled Release, 73, 205-211, 2001.
[6] L. Mayol, F. Quaglia, A. Borzacchiello, L. Ambrosio, and M. I. La Rotonda, European Journal of Pharmaceutics and Biopharmaceutics, 70, 199–206, 2008.
[7] T. Gratieri, G. M. Gelfuso, E. M. Rocha, V. H. Sarmento, O. de Freitas, and R. F. Lopez, European Journal of Pharmaceutics and Biopharmaceutics, 75, 186–193, 2010.
[8] E. E. Hassan, and J. M. Gallo, Pharmaceutical Research, 7, 491-495, 1990.
[9] N. Ito, N. Burri, L. Applegate, E. Federici, M. O. Montjovent, P. E. Bourban, J. A. Manson, P. F. Leyvraz, P. Y. Zambelli, and D. P. Pioletti, European Cells and Materials, 5, 81, 2003.
[10] Y. Zhang, J. R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, and C. T. Lim, Biomaterials, 29, 4314-4322, 2008.
[11] A. Cabana, A. Ait-Kadi, and J. Juhasz, Journal of colloid and interface science, 190, 307–312, 1997.
[12] A. A. Koffi, F. Agnely, G. Ponchel, and J. L. Grossiord, European Journal of Pharmaceutical Sciences, 27, 328-335, 2006.
[13] J. M. Barichello, M. Morishita, K. Takayama, and T. Nagai, International Journal of Pharmaceutics, 184, 189–198, 1999.
[14] K. W. Chun, J. B. Lee, S. H. Kim, and T. G. Park, Biomaterials, 26, 3319–3326, 2005.