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In vitro properties of gellan gum sponge as the dental filling to maintain alveolar space

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ABSTRACT

The insertion of dental filling into the dental cavity is a common method of maintaining the space and shape of dental cavities after dental extractions. To develop novel dental fillings meeting clinical requirements, gellan gum (GG) is selected in this study to prepare fillings with specific characteristics by modulating gellan gum concentrations (denoted as GG-DF). The results indicate that the microstructure, porosity, and compression modulus of 1.5% and 1.75% GG-DF are similar to a commercially product (Teruplug®) and with good blood absorption capacity. The degradation ratio of Teruplug® in an amylase/phosphate buffer solution after 4 weeks is 80% whereas that of 1.5% and 1.75% GG-DF is 65%. Cell migration studies confirmed that the migration ability of fibroblast was significantly inhibited by gellan gum. These results demonstrate that 1.5% and 1.75% GG-DF are potentially viable for developing into dental fillings.

1. Introduction

There are risks involved in the decay and resorption of alveolar bony tissue upon exodontias, tooth extraction, traumatic injuries or endodontics because of a lack of on-site physiological treatment. In general, the alveolar bone may atrophy 40–60% in two years after a tooth is removed without appropriate therapy (Cawood & Howell, 1998; Devlin & Sloan, 2002). Furthermore, damage or degeneration of the jaw bones may cause loosening or even the loss of other teeth. When a patient is considering having a tooth implantation, a proper protective procedure to reserve space for ossification may be required (Bodic, Hamel, Lerouxel, Baslé, & Chappard, 2005; Szabo, Suba, & Barabas, 1997). Without treatment, problems occur because the gingival tissues heal faster than the alveolar bony tissues. To protect the original vacancy and to prevent excessive growth of gingival tissues from occupying the space for the alveolar bone after tooth extraction, some products that function as dental fillings or dental plugs have been utilized to provide protection and retain the space for alveolar bone cells to gradually grow into (Accorinte et al., 2008; Serino & Biancu, 2003; Wand & Tsao, 2007).

The ideal materials used to prepare a dental filling should have three characteristics. First, the filling is required to have proper compressibility in order to allow proper control during the void-filling procedure. An ideal resorptive dental protective filling should behave like a sponge (meaning an elastic, porous mass) with good compressibility and easy fill into the dental cavity. Second, proper resorption time is required, which is typically one year for osteoblasts to mature and of the total ossification of a wound. In clinical practice, a minimum of at least 4 weeks of retaining proper protective capability is required for a dental filling. If the resorption time of the filling is too short, the gingival tissues will occupy the space reserved for the alveolar bony tissues to grow into. Finally, the filling must be capable of protecting the wound and promoting hemostasis. Clinically, exodontias procedures usually induce massive bleeding and salivating. A dental filling should adsorb blood and stop bleeding readily while consolidating alveolar bone after a tooth extraction. Currently commercial dental filling (Teruplug®) is made predominately from collagen. Collagen, although an excellent biocompatible material that promotes tissue regeneration, is disadvantageous because it is mechanically weak, quickly degrades and extremely expensive. In this study, we aimed to find an alternative material that meets the requirements of being clinically friendly, readily adsorptive and yet inexpensive for dental applications.

Gellan gum (GG), which is also known as polysaccharide S-60, is produced by a non-pathogenic strain of Sphingomonas elodea. Its main chain consists of four repeating carbohydrates, including two D-glucose, one L-rhamnose, and one D-glucuronic acid. Currently, Gellan gum (a biodegradable and food additive approved by the FDA) is extensively used in the food industry (Karim & Bhat, 2008), but it has rarely been investigated for biomedical applications except for use in drug delivery (Babu, Sathigari, Kumar, & Pandit, 2010). Because gellan gum is an anionic polysaccharide, it

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could act to prevent cell adhesion. Our previous studies have shown that gellan gum film could effectively act as a barrier, inhibiting the growth of fibroblasts at wound sites (Lee, Chen, & Tsao, 2010). In this study, we prepared water-insoluble gellan gum sponges and evaluated the potential applications in dentistry. We explored its basic properties, including molecular structure, water content, porosity, compression modulus, blood absorption, degradation behavior and wound closure, and compared them to commercial product.

2. Methods

2.1. Fabrication of the gellan gum dental filling

Various weights of gellan gum (GG) were dissolved in 100 ml of deionized water and heated at 85–90 °C until they became transparent solutions. The concentration (wt/vol) of gellan gum solutions were 0.75%, 1%, 1.25%, 1.5% and 1.75%. Then, 2 ml of gellan gum solution were poured onto petri dishes (diameter 1.1 cm), frozen at −20 °C overnight and lyophilized to obtain a 3D sponge (denoted as 0.75% GG-DF, 1% GG-DF, 1.25% GG-DF, 1.5% GG-DF, 1.75% GG-DF). The resultant porous GG-DF sponges were then cross-linked by immersing them into the deionized water containing 15 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide for 24 h at room temperature. The treated GG-DF sponges were washed with distilled water three times, frozen at −20 °C and lyophilized again.

2.2. Morphology characterization

Scanning electron microscopy (SEM) (FEI Quanta 400 F) was employed to examine the morphology of the GG-DF and commercial product (Teruplug®) with an emphasis on the porous characteristics. Prior to SEM, the samples were sputter-coated with gold.

2.3. Water content

The water content of the GG-DF and the Teruplug® was determined by swelling the plugs in phosphate buffered saline (PBS) at pH 7.4 for 2 h at room temperature. The wet weight (W_{wet}) of the swollen sponge was measured immediately after gently blotting with filter paper to remove surface liquid and then lyophilizing and reweighing (W_{dry}) the sponge. The water content of the plug was calculated using the formula (Lee, Hung, Cheng, & Wang, 2005):

$$\mathrm{WC} = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100\%$$

2.4. Determination of porosity

The porosity of the GG-DF and Teruplug® was determined using Archimedes' principle (Wan, Wu, Cao, & Dalai, 2008). The exterior volume (V_s) of the plug (1 cm × 1 cm) was measured using a Vernier caliper. The sample was then immersed in a pycnometer containing 99% ethanol solution. The actual volume (V_a) of the sample was calculated using the formula:

$$V_a = \frac{(W_w - W_o) - (W_t - W_p)}{0.789\,\mathrm{g/cm^3}}$$

where W_w is the weight of the ethanol and the pycnometer, W_o is the dry weight of the pycnometer, W_t is the combined weight of the ethanol, the pycnometer and the plug sample, W_p is the combined weight of the dry pycnometer and dry plug sample, and 0.789 g/cm^3 is the density of 99% ethanol solution. The porosity of the plug was then determined using the following formula:

$$\mathrm{Porosity}(\%) = \frac{V_s - V_a}{V_s} \times 100\%$$

Porosity values were expressed as means ± standard deviations ($n = 3$).

2.5. Mechanical properties measurement

To assess the effect of various gellan gum concentrations on the mechanical properties of the dental fillings, compression tests were performed on an Instron (MODEL: JTM-S230) mechanical tester. The GG-DF and Teruplug® sponges (1 cm thick, 8 mm in diameter, $n = 5$) were compressed in the direction normal to the circular face of the cylindrical samples at a rate of 2 mm per minute until the sponge failed. The Young's modulus was defined as the slope of the linear region of the stress–strain curve in the 5–15% of the strain range. Ultimate stress and ultimate strain values were taken as the point where the sponge failed.

2.6. In vitro degradation test

To simulate the oral environment, in vitro degradation tests of the GG-DF and Teruplug® were conducted by incubating the fillings in 10 ml of amylase (20 U, from human saliva)/phosphate buffer solution on a shaker set at 40 rpm and 37 °C. At predetermined time intervals, the filling was taken out of the incubation medium, washed with distilled water and dried, and the weight of the filling was measured. Another 10 ml of amylase (20 U)/phosphate buffer solution were added into the vial, and the test resumed. The degradation profiles were expressed as the accumulated weight losses of the fillings:

$$\mathrm{Degradation\ percentage}\,(\%) = 100 - \frac{W_o - W_t}{W_o} \times 100\%$$

where W_o is the sample original weight and W_t is the sample dry weight after degradation.

2.7. Absorption and blood clotting testing

The absorption rate of the GG-DF sponges was determined using human whole blood. The latter was obtained from the Taiwan Blood Services Foundation (TBSF, Taipei, Taiwan). GG-DF sponges were cut into 1 cm × 1 cm squares and placed into glass bottles. Then, 0.4 ml of human whole blood was dispensed onto the dressing. The absorption rate was defined as the time required for the dispensed fluid to be completely absorbed by the sponge (Kang et al., 2011).

The blood clotting test was modified from Ong, Wu, Moochhala, Tan, and Lu (2008). Sponges were cut into 1 cm × 1 cm squares and placed into glass bottles. Next, 0.25 ml of human whole blood (containing the anticoagulant citrate dextrose at a 1:6 ratio) was slowly dispensed onto the surface of the dressings. The bottles containing the samples were then incubated at 37 °C. After a predetermined amount of time (30 and 60 s), 20 ml of distilled water was carefully added by dripping water down the inside wall of the bottles, to prevent disrupting the clotted blood. Red blood cells that were not entrapped in the clot were hemolyzed with distilled water, and the absorbance of the resultant hemoglobin solution was measured at 540 nm (UV–VIS spectrophotometer Agilent 8453, Santa Clara, CA, USA). The absorbance of 0.25 ml of whole blood in 20 ml of distilled water was used as a reference value. We also observed the morphology of blood coagulation by SEM. A sample of 0.5 ml of human whole blood was added to the GG-DF or Teruplug®. After incubating at 37 °C for a predetermined amount of time, the sponges were fixed, dried and sputter-coated with gold for SEM studies.

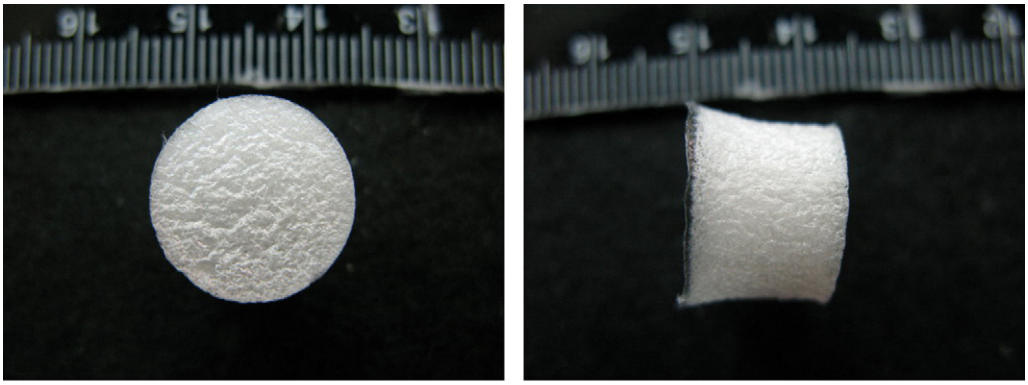

Fig. 1. Morphology of the 1.5% GG-DF: (a) front view and (b) side view.

2.8. Wound closure assay

The wound closure assay was a modification of the Platypus Technologies Oris Cell Migration Assay (Abingdon, UK) (Carragher & Frame, 2011). Each well of an Oris 96-well plate was prepared as follows: the center of the well was coated with 1 mg/ml of gellan gum or 1 mg/ml of collagen and allowed to dry for 24 h. Then, a stopper was placed in the well to cover the material coated zone. L929 fibroblasts (cell density 5×10^4/ml) were seeded onto the plating medium in this outer area. On day 2, the plugs were removed. After 12 and 48 h, cells were stained with Giemsa stain (Sigma–Aldrich, Dublin, Ireland). The Oris 'detection mask' was used to visualize the wound area, which was photographed.

3. Results and discussion

3.1. Morphologies and porosity of the GG-DF sponges

Morphology and SEM images of GG-DF sponges are shown in Figs. 1 and 2. The sponge samples have a pore size between 200 and 300 μm^{-1} and vary with the gellan gum solution concentration. The porous diameter decreases with a decreasing concentration of gellan gum solution. This relationship occurs because higher gellan gum solution concentrations have higher viscosity (Hsieh, Chang, & Lin, 2007); therefore, it is easy to maintain smaller pore diameter after lyophilizing. Porosity was calculated from the equation in Section 2.4. As shown in the Table 1, porosity decreases with an

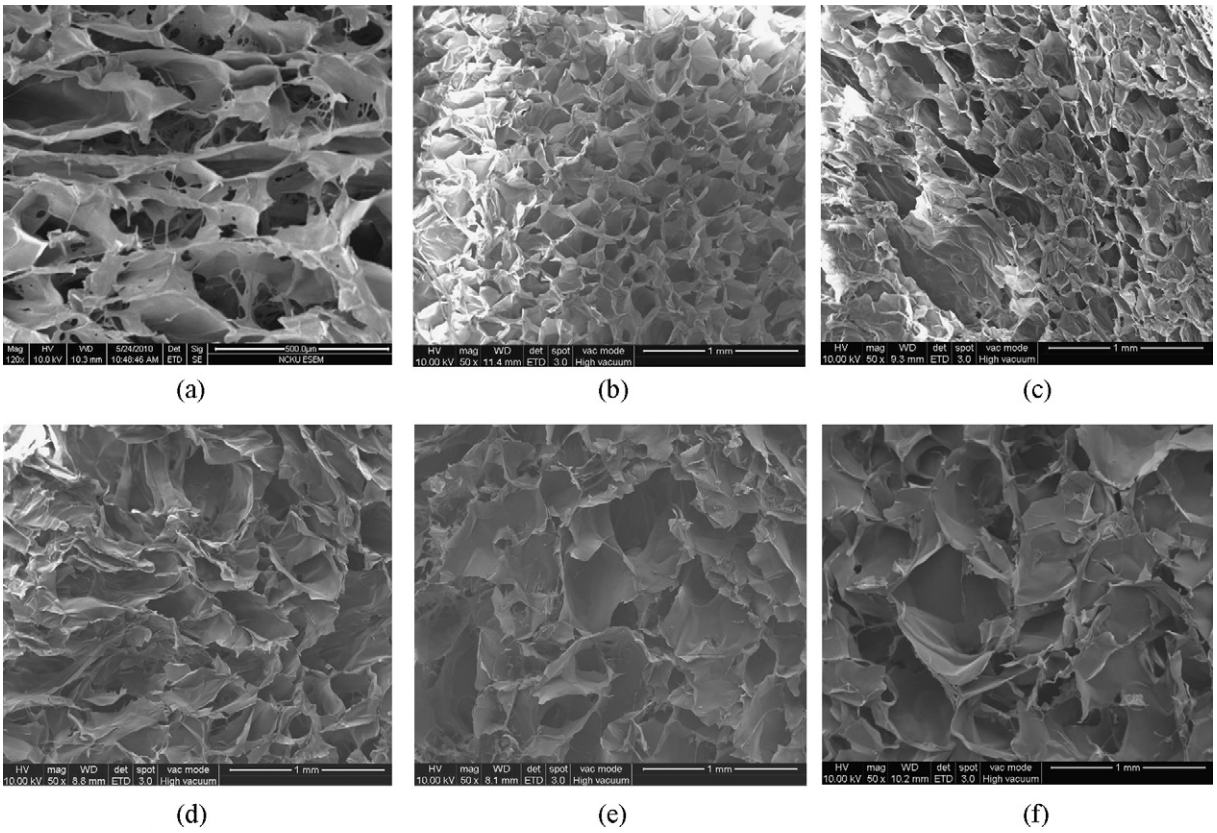

Fig. 2. SEM micrographs of the cross-section of the dental fillings: (a) Teruplug®, (b) 0.75% GG-DF, (c) 1.0% GG-DF, (d) 1.25% GG-DF, (e) 1.5% GG-DF and (f) 1.75% GG-DF.

Table 1
Porosity and water absorption capacity of gellan gum dental fillings (GG-DF).

Designation	Porosity (%)	Water absorption (%)
0.75% GG-DF	95.54 ± 11.26%	96.9 ± 0.19%
1% GG-DF	85.4 ± 21.17%	96.9 ± 0.19%
1.25% GG-DF	66.27 ± 8.03%	97 ± 0.54%
1.5% GG-DF	28.98 ± 4.53%	96.2 ± 0.64%
1.75% GG-DF	20.61 ± 2.9%	92 ± 0.10%
Teruplug®	38.9 ± 1.5%	92.2 ± 4.7%

Table 2
Mechanical properties of gellan gum dental fillings (GG-DF).

Designation	τ_{max} (MPa)	Compressive modulus (MPa)
0.75% GG-DF	0.31 ± 0.02	3.4
1% GG-DF	0.594 ± 0.07	4.2
1.25% GG-DF	1.04 ± 0.07	8.6
1.5% GG-DF	1.75 ± 0.15	15.9
1.75% GG-DF	2.11 ± 0.1	21.1
Teruplug®	1.48 ± 0.24	18.5

increasing gellan gum solution concentration. The 95–24% porosity could be obtained by controlling the concentration of gellan gum. From these results it is evident that 1.5% GG-DF, 1.75% GG-DF and Teruplug® have similar morphologies and porosity profiles.

3.2. Water absorption

To maintain the GG-DF shape for the socket filler, the degree of water absorption is important. A sponge with high water adsorption may absorb excess water and expand, causing deformation. Table 1 shows water absorption ratio of sponges produced under various concentrations of gellan gum. With an increase in the gellan gum solution concentration, the capacity of water absorption is reduced. The capacity of water absorption of gellan gum sponge is closely related to the porosity. Some studies have indicated that scaffolds with higher porosity have increased water storage space and would thus have a higher capacity of water absorption (Davidenko, Campbell, Thian, Watson, & Cameron, 2010). Other studies have suggested that smaller pore sizes cause increased capillary phenomenon leading to increased water absorption. In this study, we could see similar results from other papers. The most appropriate water absorption ratio for a sponge as a socket filler is not yet known. Compared with the commercial product, we noted that the 1.75% GG-DF and Teruplug® had similar water absorption capacity and about 92%.

3.3. Mechanical properties

An ideal resorptive dental protective material should have compressibility in order for the clinician to easy fill into the dental cavity. The slopes of the compressive stress–strain curves from 0% to 5% deformation were used to calculate the compressive modulus. As shown in Table 2. The results indicate that with increasing gellan gum solution concentration, the Young's modulus and maximal shear stress (τ_{max}) both increase. The compressive moduli of the sponges of 0.75%, 1%, 1.25%, 1.5% and1.75% GG-DF are 3.4, 4.2, 8.6, 15.9 and 21.1 MPa, respectively. It is known that compressive strength and Young's modulus increase with decreasing porosity

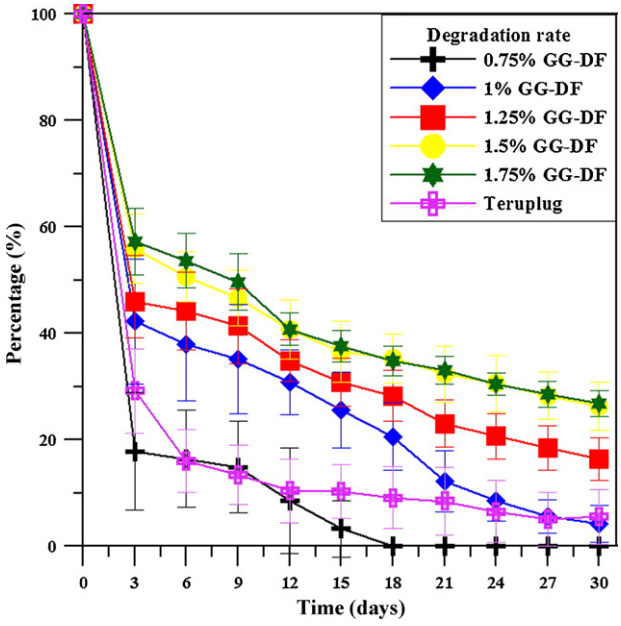

Fig. 3. Degradation of Teruplug® and GG-DF in amylase//phosphate buffer solution.

(Jang & Matsubara, 2005). In addition, previous studies have indicated that the Young's modulus of the porous sample significantly increases as the amount of material in the network increases. Therefore, the gellan gum sponge's mechanical properties are primarily determined by the concentration of gellan gum. From the results, we also find that the mechanical properties of 1.5% and 1.75% GG-DF are similar to Teruplug®.

3.4. In vitro degradation

An intrinsic characteristic of natural polysaccharides is their ability to degrade through the actions of naturally occurring enzymes that are present in the serum and saliva. Therefore, it

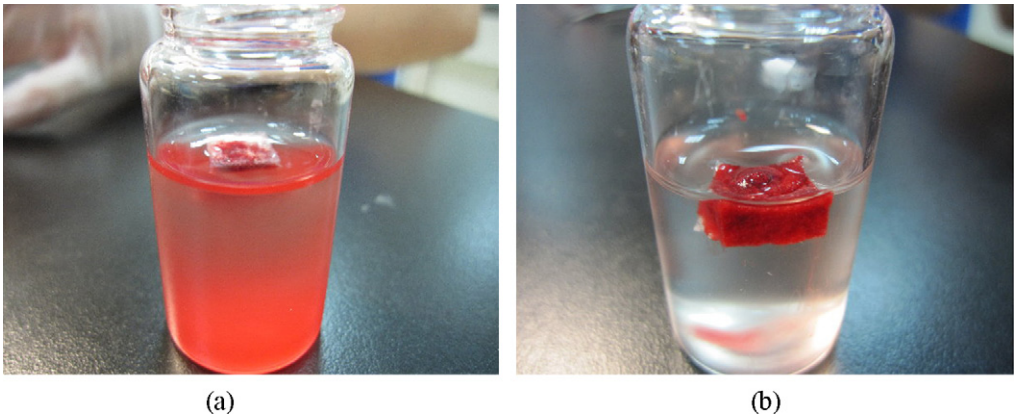

Fig. 4. Photographs showing that more hemoglobin leaked from the Teruplug® (left) than from the 1.5% GG-DF (right).

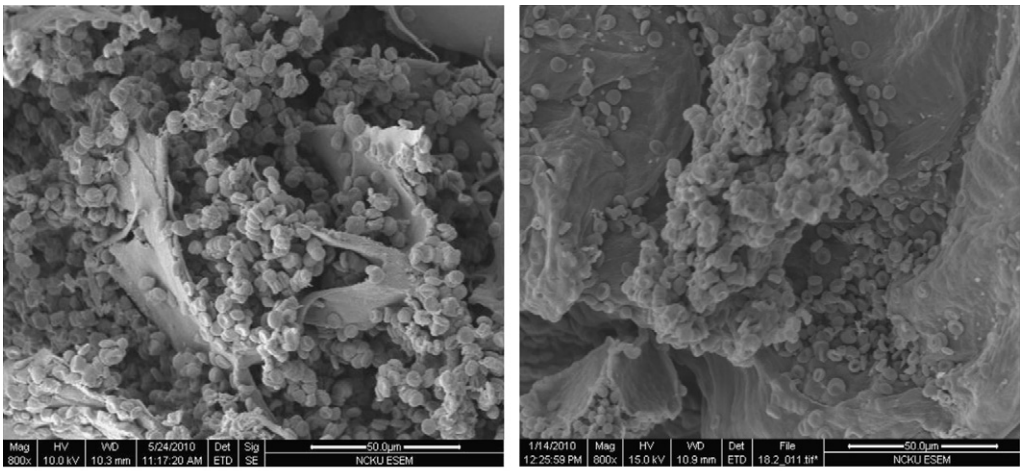

Fig. 5. SEM micrographs of human whole blood on the: (a) Teruplug® and (b) 1.5% GG-DF after 60 s of absorption.

is of particular interest to study the enzymatic degradation of GG-DF sponges prepared by different gellan gum solution concentrations. Gellan gum is a linear polysaccharide that is composed of glucose-rhamnose-glucuronic acid units bonded by α(1–4) glycosidic links. Fig. 3 shows the degradation rate of the GG-DF sponges and Teruplug® at a pH of 7.4 in the presence of α-amylase. The control sample (Teruplug®) and 0.75% GG-DP each with a significant weight loss during the first week, but other samples degraded at a slower rate. The degradation ratio of Teruplug® in the amylase/phosphate buffer solution after 4 weeks was 80%, and that of 1.5% and 1.75% GG-DF was 65%. We also incubated the GG-DF sponges and Teruplug® in 10 ml of phosphate buffer solution without amylase at 37 °C. The degradation ratio of Teruplug® after 4 weeks was 78%, and that of 1.5% and 1.75% GG-DF was 40%. Degradation profiles of those fillings in phosphate buffer solution with and without amylase were similarly. Teruplug® also having a much higher degradation rate than 1.5% and 1.75% GG-DF. To maintain the alveolar space, a minimum of about 4 weeks duration is required. The results indicate that with increasing gellan gum concentration, the degradation rate decreased. Therefore, the degradation rate of the GG-DF sponge can be controlled for clinical requirements by varying the concentration of gellan gum.

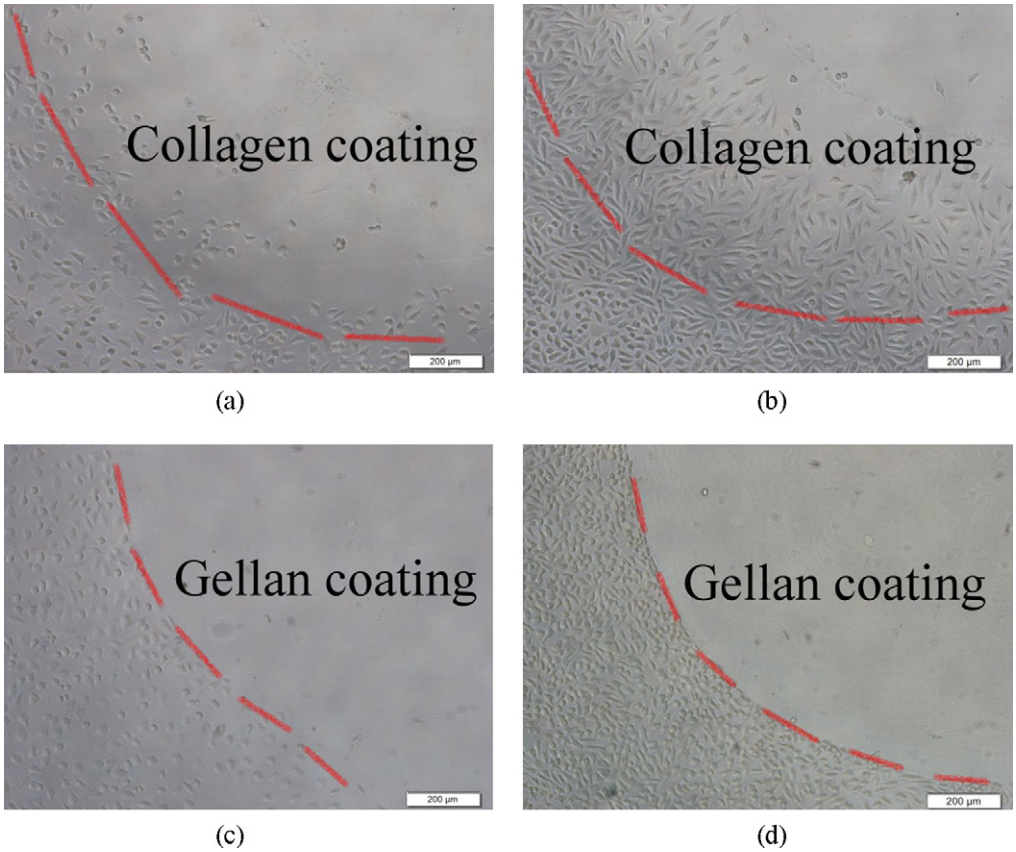

Fig. 6. Cell migration as measured by microscopy. The analytic zone into which cells have migrated is separated by the red dotted line. (a and b) fibroblasts migrated after 12 and 48 h (collagen coating), (c and d) fibroblasts migrated after 12 and 48 h (gellan gum coating).

3.5. Blood absorption

The GG-DF demonstrated higher blood absorption rates compared with Teruplug®. When 1.5% GG-DF sponges were placed into 0.25 ml of human whole blood for 60 s, the absorbance of the remaining hemoglobin not absorbed into the sponge was reduced from 1.8 (at $t = 0$) to 0.8 OD. This result indicates that about 66% of RBC was absorbed by the 1.5% GG-DF sponge. In contrast, about 55% RBC were absorbed by the Teruplug®. The blood absorption capacity of the 1.5% GG-DF sponge may be attributed to its extensive porous structure. To assess blood coagulation, the sponges with absorbed blood were placed in 0.9% saline solution. As shown in Fig. 4, the Teruplug® swelled significantly in saline, and blood leakage from the sponge was observed. This result was not observed with the 1.5% GG-DF sponge, which indicates that the blood was well confined inside the 1.5% GG-DF. The SEM evaluation of blood clot formation on the 1.5% GG-DF sponge and Teruplug® revealed that red blood cells formed aggregates (Fig. 5a and b). More specifically, the red blood cells coalesced into an erythrocyte clot in both the 1.5% GG-DF sponge and Teruplug® (Kang et al., 2011).

3.6. Wound healing

An ideal resorptive dental protective material should prevent gingival fibroblast growth and migration into the alveolar space (Chang et al., 2010; Chung, Nam, & Kim, 2009). The effect of inhibiting fibroblast migration with gellan gum was investigated by a wound healing assay. The wound healing migration assay is an established and widely used procedure that allows monitoring cell migration at different intervals of time in response to an artificial wound produced on the cell monolayer. In addition, this method allows the observation of changes in cell morphology occurring during this process. As the photos show, fibroblasts migrated to the central barren spot after 12 h incubation and nearly filled it after 48 h incubation (Fig. 6). When the center of the well was coated with 1 mg/ml collagen, fibroblasts also migrated to the central barren spot after 12 h of incubation. However, if the center of the well was coated with 1 mg/ml gellan gum, fibroblast migration was significantly inhibited. The mechanism by which the gellan gum caused the significant reduction in cell migration is not clear; however, the reduction is likely due to the negative charge of gellan gum.

4. Conclusion

The maintenance of dental cavities after a dental extraction (also termed alveolar ridge preservation) is crucial for the success of dental implants and prosthetics. Currently, Teruplug® is the most common product used for preserving dental cavities. Teruplug® consists of collagen fibers and with plasticity, hemostatic function and good biocompatibility. However, controlling the degradation characteristics of Teruplug® can be challenging, and its high cost limits its clinical applications. In this study, we used gellan gum as the substrate to prepare dental fillings (GG-DF) and demonstrated that the microstructure, porosity, and compression modulus of 1.5% and 1.75% GG-DF are similar to those of Teruplug®. In vitro degradation tests, 1.5% and 1.75% GG-DF can endure for an extended period of time (more than 4 weeks), while Teruplug® can only endure for 2 weeks. Furthermore, 1.5% GG-DF had better blood absorption capability than Teruplug®. Another key to preserve the shape of dental cavities successful is the prevention of epithelial cells and fibroblasts from migrating into dental cavities. Through cell migration studies, gellan gum was found to have superior inhibitory activities on cell migration. At present, no standards regarding dental filling quality are available for clinical evaluation. To evaluate the feasibility of the novel gellan gum dental filling in this study, a commercially available product was chosen as a control. On the basis of the results observed, we conclude that of 1.5% GG-DF prepared in this study appeared to be of great potential for development into dental fillings.

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