Gelatin-based hydrogels blended with gellan as an injectable wound dressing

Yueyuan Zheng\textsuperscript{a,†}, Yuqing Liang\textsuperscript{a,†}, Depan Zhang\textsuperscript{a}, Xiaoyi Sun\textsuperscript{a}, Li Liang\textsuperscript{b}, Juan Li\textsuperscript{a,*}, You-Nian Liu\textsuperscript{a}

\textsuperscript{a} College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, P.R. China.

\textsuperscript{b} State Key Lab of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, P. R. China

Corresponding authors. Fax: +86-731-88879616; Tel.: +86-731-88879616. E-mail: juanli@csu.edu.cn (J. Li)

\textsuperscript{†}Both authors contributed equally to this work.
Experimental

In vitro release study.

In vitro release study was carried out using Franz diffusion cell (TK-12B, Shanghai KAIKAI Technology Co.) with a receptor compartment (7 mL PBS) through piglet skin (donated by Laboratory of Professor Wenhu Zhou, School of Pharmaceutical Sciences, Central South University). The full-thickness abdominal skin from piglets was excised and fat adhering to the dermis side was cleaned using a blunt scalpel, followed by repeatedly washed with saline. Care was taken to avoid damaging skin. The skins were sealed in aluminum foil paper at -20 °C and used within 2 weeks. A predetermined amount of TA-loaded hydrogel (0.5 g) or TA solution (0.5 mL) was placed on the donor side. The receptor medium was continuously stirred at 100 rpm and maintained at 37 ± 0.5°C with a water jacket. At predetermined time intervals, 2 mL samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh buffer. Collected samples were analyzed by UV spectrophotometer at 276 nm.

Cell cytotoxicity assay.

L-929 mouse fibroblast cell line was obtained from the cell bank of Xiangya Central Laboratory of Central South University (Changsha, China). Cells were cultured in DMEM medium containing 10% FBS and 1% antibiotics (100 units mL\(^{-1}\) penicillin and 100 μg mL\(^{-1}\) streptomycin) under 5% CO2 atmosphere. Cells were seeded into a 96-well plate at a density of 5 × 10\(^4\) cells per milliliter, respectively. After 24 h of culture, the cell culture medium was replaced with fresh medium containing hydrogel sample (50 μL per well). After 24 h of incubation, the supernatants were removed and the fresh medium contained CCK-8 solution was added into each well. After another 1 h of incubation, the absorbance at 450 nm was measured in a microplate reader (Bio-Tek ELx800, USA). Cell viability (%) was calculated from the ratio of the absorbance of the treated cells to the untreated cell.

Cell migration assay.
L929 cells were cultured to ~80% confluence in 24 well plates in DMEM medium, washed with PBS. Subsequently, a mock wound (scratch) was induced across the cell sheet using a 200 μL pipet tip on the bottom of each well and rinsed with medium to remove cell debris. Separately, hydrogel precursor mixture (50 μL) was injected onto the denuded area at 37 °C. Then DMEM (1 mL) were added to each well. Scratched cell sheets treated with culture medium (1 mL) served as a control. Images of the treated and untreated cells were taken after 0, 12, and 24 h to monitor the cell migration propensity.

**Histopathological examination.**

On the 12th day of post wounding, three mice from each group was sacrificed and the skin tissue was removed by a scissor. Skin specimens were fixed in 10% buffered formalin, processed, embedded in paraffin, and then sectioned perpendicular to the wound surface into 4 mm sections by standard procedures. Sections were stained with hematoxyline-eosin (H&E) and Masson’s trichrome. All sections were analyzed using light microscopy by two pathologists in a blinded manner. Samples were scored concerning microscopic appearance in a blinded fashion, and the score was adapted from the literature, as shown in Table 1. The collagen content was analyzed using the software of Image J.

**Table 1. Parameters for microscopic assessment of skin generation.**

| Microscopic parameter                      | Score  |
|-------------------------------------------|--------|
| Epidermal integrity                      | 0      |
| Epidermal-dermal junction                 | 1      |
| Collagen organization                     | 2      |
| Apoptotic keratinocytes & visualization   | 0      |

|                | Destroyed | Partial | Normal |
|----------------|-----------|---------|--------|
| Epidermal integrity | 0         | 1       | 2      |
| Epidermal-dermal junction | 0         | 1       | 2      |
| Collagen organization   | 0         | 1       | 2      |
| Apoptotic keratinocytes & visualization | 0         | 1       | 2      |
Figure S1. Photographs showing the synergistic gel formation of gelatin/gellan mixtures at 37 °C. (A) 0.3% gellan, (B) 10 % gelatin, (C) blend hydrogels, and (D) TA-loaded hydrogels.

Figure S2. The storage and loss moduli of gellan as a function of temperature dependence at a frequency of 1.0 Hz and a strain of 0.1%, a heating rate of 1.0 °C/min.
Figure S3. The hydrogels are mouldable to macroscale shapes after injection.

Figure S4. Rheological measurements of blend hydrogel prepared at pH 4.
Figure S5. (A) Storage moduli of TA-loaded blend hydrogels as a function of strain at 37 °C; (B) Viscosity of TA-loaded blend hydrogel at a function of shear rate at 25 °C.
**Figure S6.** Photos of mixtures of gelatin/TA, and gellan/TA with different TA concentrations. The concentration of gelatin is 10% gelatin, and the concentration of gellan is 0.3%.

**Figure S7.** The release profiles of TA-loaded hydrogel.
Figure S8. Wound closure of mice treated with blend hydrogel.

Figure S9. Cell cytotoxicity of blend hydrogel.

References

(1) Knapik, A.; Kornmann, K.; Kerl, K.; Calcagni, M.; Contaldo, C.; Vollmar, B.; Giovanoli, P.; Lindenblatt, N. Practice of split-thickness skin graft storage and histological assessment of tissue quality. Journal of Plastic, Reconstructive & Aesthetic Surgery 2013, 66 (6), 827-834.