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NOTE

Evaluation of gelatin hydrogel as a potential carrier for cell transportation

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We prepared uncleaved gelatin composed mainly of collagen α-, β-, and γ-chains. Gelation and melting of uncleaved gelatin occurred rapidly with moderate decrease and increase in temperature (23°C–37°C). The viability of cells encapsulated in the gelatin gel was greater than 96% after 7 d at 23°C.

[**Key words:** Collagen; Gelatin; Gel; Transportation; Cell carrier; Renaturation]

For both research and clinical application in regenerative medicine, it is a common practice to transport cells among sites of collection, processing, and clinical administration. Cryopreservation is a conventional technology for preservation of stem cells (1), also applied for cell transportation. However, the process of cryopreservation requires multiple steps with a special container and liquid nitrogen to preserve ultra-low temperatures. Furthermore, a rapid decline in cell viability after thawing is a serious problem in cryopreservation (1). Transporting live cells cultured in a complete medium is an alternative method (2); however, cells suffer inevitable shear stress generated by turbulence of the containers during transportation. This mechanical stimulation affects stem cell fates. Mesenchymal stem cells undergo strain-mediated apoptosis (3), and differentiation of stem cells toward both endothelial and bone-producing cell phenotypes is induced by shear stress (4).

Gel transportation systems in which live cells are encapsulated have recently been investigated (5,6). We speculated that gelatin is a potential carrier for a cell transportation system able to overcome the limitations of cryo- and liquid systems. Gelatin is a biocompatible polymer obtained by thermal denaturation of collagen. Numerous studies have shown that gelatin is a biocompatible and safe material for biomedical use (7–9). The gelation solution is stable at 37°C and forms a gel on decreasing temperature. A gelling temperature (*Tg*) lower than the biological temperature and excellent biocompatibility are critical properties for a temperature-responsive cell transportation carrier. However, to date, cell transportation using a gelatin carrier has not been investigated.

To assess the feasibility of a gelatin carrier for cell transportation, we prepared uncleaved gelatin (UCG) composed mainly of collagen α-, β-, and γ-chains and used as a cell encapsulation carrier. The temperature-responsiveness of gelation of UCG and mechanical properties of the gel were evaluated by dynamic viscoelastic measurements. Cell encapsulation and dispersion were performed using the temperature-responsive sol–gel transition of gelatin, and cell viability was examined *in vitro*. This study demonstrated that gelatin gel is a potential carrier for cell transportation.

Collagen in acidic solution was gently heat-denatured under conditions that minimized cleavage. In brief, 500 g of 0.56% collagen solution (pH 3, collagen BM, biomedical grade from pig skin, Nitta Gelatin, Japan) was warmed under constant stirring in a 500-mL glass beaker placed in a water bath at 60°C. The temperature of the collagen solution was monitored with a digital thermometer. Ten minutes after the temperature exceeded 50°C, the pH was adjusted to 7.0 by addition of sodium hydrochloride solution. The heat-denatured collagen solution was condensed to over 10% of collagen concentration in a rotary evaporator at 55°C. The final concentration was adjusted to 10% by addition of pure water. Electrophoretic analysis (described below in this section) confirmed that this treatment did not cleave collagen α-chains. The uncleaved gelatin from pig skin is abbreviated here as PS-UCG. Aliquots (3 g) of 10% PS-UCG were transferred to 15-mL biological tubes and subjected to the measurements described below. Commercial type A gelatin from pig skin (MP Biomedicals, USA), type A gelatin from bovine bone (Wako Pure Chemical Industries, Japan), and type B gelatin from pig skin (beMatrix LS-H, Nitta Gelatin, Japan) (abbreviated here as PSA-G, BB-G, and PSB-G, respectively) were used as references.

The gelatin solutions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 5% slab gel containing 0.1% SDS (PAGEL, Atto, Japan) and using a running buffer at pH 8.5. Sample solutions (4–50 μg per gel lane) were applied to the gel and separated under 20 mA constant current to determine the dominant molecular weights within each solution. A molecular-weight marker (Spectra Multicolor High Range Protein Ladder, Thermo Fisher Scientific, USA) was used as the standard. The gel was stained with Quick-CBB (Wako Pure Chemical Industries) to visualize the collagen bands.

The sol–gel transition of gelatin solutions was monitored by dynamic viscoelastic measurements using a rheometer (HAAKE MARS III, Thermo Fisher Scientific) equipped with a Peltier plate for temperature control. A double-cone sensor DC60/1Ti (diameter of 60 mm, cone angle of 1°) was used. Three measurement procedures were used to measure *Tg* and melting temperature (*Tm*) (procedure

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A), sol–gel transitions using temperature profiles simulating practical cell transportation processes (procedures B-1 and B-2), and gelling at around room temperature (procedure C). For measurements of *Tg* and *Tm* (procedure A), the sample solution was oscillated at 1 Hz at a constant shear stress of 0.1 Pa under a linear temperature gradient. A 4-mL aliquot of 5% PS-UCG or 5% PSA-G solution in PBS was warmed in a water bath (60°C) for 12 min with occasional vortex mixing and placed in the temperature-controlled rheometer with the sensor prewarmed to 37°C. After 2 min, one of four measurement programs was initiated: decreasing temperature from 37°C to 4°C (at -0.6°C min^{-1}), constant temperature of 4°C for 90 min, and increasing temperature from 4°C to 45°C (at 0.6°C min^{-1}). The change in storage modulus (G′) and loss modulus (G″) were recorded, and *Tg* and *Tm* determined from the point at which G′ and G″ intersected with the linear temperature gradient. For measurement of sol–gel transitions using temperature profiles simulating practical cell transportation process (procedure B-1), the PS-UCG solutions in PBS were prepared as above and samples placed in the rheometer. After 2 min, the measurement program using controlled stress mode at a frequency of 1 Hz was initiated: 37°C for 30 min (shear stress of 0.1 Pa), 10°C for 30 min (shear stress of 1 Pa), 23°C for 90 min (shear stress of 1 Pa), where decreasing and increasing temperatures were applied at rates of -6°C min^{-1} and 6°C min^{-1}, respectively. Procedure B-2: 37°C for 30 min (shear stress of 0.1 Pa), 23°C for 90 min (shear stress of 1 Pa), 37°C for 30 min (shear stress of 0.1 Pa). Gelling at near room temperature was measured using procedure C: at a constant stress of 0.1 Pa at setting temperatures of 20°C, 23°C, or 25°C.

A cell viability test was conducted using a simulated cell transportation process: mixing of medium and gelatin at 37°C; covering of live cells; gelling of the mixture at 10°C or 23°C; melting of the gel at 37°C, and dispersion of cells with trypsin. NIH-3T3 mouse embryonic fibroblast cells were used. The 10% PS-UCG solution was mixed with condensed Dulbecco's modified Eagles medium (DMEM) supplemented with fetal bovine serum (FBS) at 37°C. The final PS-UCG concentrations were 5%; final DMEM was 1×, final FBS content was 10%. The NIH-3T3 cells were seeded at 5.4×10^4 cells cm^{-2} on 24-well cells culture plates and cultured until confluent. The plates were then placed in a refrigerator at 10°C or an incubator at 23°C without CO_2 tension to form gels. After 30 min the plates at 10°C were moved to the incubator at 23°C. The plates were preserved for 2 or 7 d, simulating domestic cell transportation in atmosphere. The PS-UCG gels with covered NIH-3T3 cells were then incubated at 37°C under 5% CO_2 for 1 h to melt gels and the cells were dispersed with trypsin. The cell covered with liquid DMEM were collected by the same procedure, and used as a control. Cell viability after dispersion was determined by conventional trypan blue dye exclusion. In brief, after removal of the melted gels, the cells were washed thrice with PBS and dispersed with 0.25% trypsin. The dispersed cells were suspended in the medium and a 20-μL aliquot was mixed with an equal volume of 0.4% trypan blue solution. Stained and unstained cells were counted with a hemocytometer, and cell viability was calculated as follows:

$$\text{Cell viability}(\%) = Nu/(Nu + Ns) \times 100 \quad (1)$$

where *Nu* and *Ns* are the number of unstained and stained cells, respectively. Cell viability data were expressed as means ± SD ($n = 4$) and were evaluated by one-way ANOVA. Statistical significance among groups was then determined by Tukey's test ($p < 0.05$ was considered significant).

Electrophoresis of PS-UCG solutions showed distinct protein bands at about 120 and 250 kDa, corresponding to the α- and β-chains of collagen and indicating that collagen molecules were not cleaved by the preparation process. In contrast, electrophoresis of PSA-G and BB-G solutions produced a smeared staining pattern with no sharp bands. The broad band of <100 kDa in these gels indicated substantial cleavage of collagen molecules. PSB-G solutions showed an intermediate electrophoretic pattern, in which the protein bands at 120 and 250 kDa were clear but weak compared with those of PS-UCG. Fig. S1 depicts the SDS-PAGE patterns of the gelatin samples.

The viscoelastic measurements of PS-UCG solutions in PBS or DMEM showed that there were no significant differences in *Tg* and *Tm* between PBS and DMEM: *Tg* in PBS (23.6 ± 0.1°C, $n = 3$), *Tg* in DMEM (23.7 ± 0.3°C), *Tm* in PBS (37.5 ± 0.2°C), and *Tm* in DMEM (37.3 ± 0.2°C). *Tg* and *Tm* of the PS-UCG solutions in PBS were significantly higher than those of the PSA-G solution in PBS (19.7 ± 0.1°C and 33.5 ± 0.1°C, respectively).

Fig. 1 shows the gelling and melting of PS-UCG on two temperature profiles B-1 and B-2 that simulated practical cell transportation. The solution maintained fluidity (G′ < G″) at 37°C for 30 min. Rapid gelation was observed under decreasing temperature from 37°C to 10°C or 23°C, with the increase in G′ at 10°C faster and larger than that at 23°C. The increase in temperature from 10°C to 23°C rapidly decreased G′ to the half maximum value (Fig. 1A), but then G′ gradually increased. A subsequent increase in temperature to 37°C caused rapid melting of the gels.

Fig. 2 shows the temperature dependence of gelatin gelation at near room temperature (20°C, 23°C, and 25°C). The PS-UCG solutions reached a gel point within 4 min and showed active gelation even at 25°C, although gelling was suppressed as the setting temperature increased from 20°C to 25°C (Fig. 2A). The gelling point of PSA-G, BB-G, and PSB-G at 25°C were much longer (22 min, 30 min, and 15 min, respectively) than that of PS-UCG, and the subsequent gelling was slower (Fig. 2B). The G″ at their gel points were 1.8 Pa (PS-UCG), 1.2 Pa (PSA-G), 0.7 Pa (BB-G), and 1.5 Pa (PSB-G), indicating the gelatin solutions were sufficiently fluidic before they reached gel points.

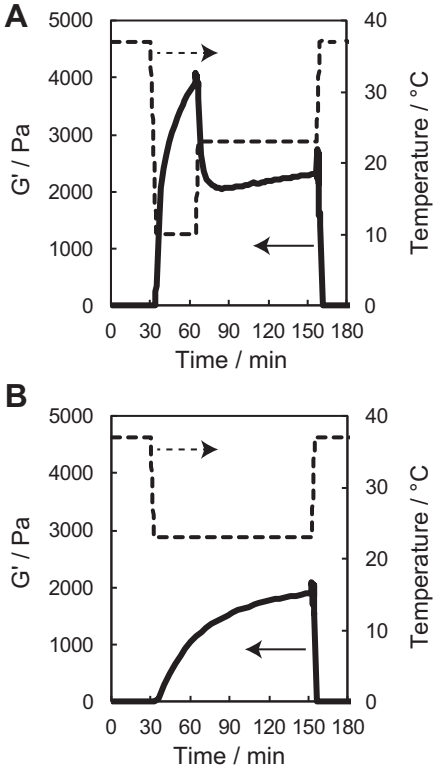

FIG. 1. Representative gelling and melting curves of PS-UCG solution using temperature profiles simulating practical cell transplantation: simulating (A) the use of a refrigerator for gelation (10°C), (B) gelation at room temperature (23°C).