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Differential physical, rheological, and biological properties of rapid in situ gelable hydrogels composed of oxidized alginate and gelatin derived from marine or porcine sources

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Abstract Marine derived gelatin is not known to associate with any communicable diseases to mammals and could be a reasonable substitute for gelatin derived from either bovine or porcine sources. The low melting point of marine gelatin (8°C) also offers greater formulation flexibility than mammalian derived gelatins. However, the suboptimal physical properties of marine gelatin generally limit the interest to further develop it for biomedical applications. This study aimed at investigating the feasibility of using oxidized alginate (Oalg) as a high activity macromolecular crosslinker of marine gelatin to formulate in situ gelable hydrogels with the goal of enhancing the latter's physical properties. The performance of Oalg/marine gelatin hydrogel was compared to Oalg/porcine gelatin hydrogel; in general, the physicomechanical properties of both hydrogels were comparable, with the hydrogels containing porcine gelatin exhibiting moderately higher mechanical strengths with shorter gelation times, smaller size pores, and higher swelling ratios. On the contrary, the biological performances of the two hydrogels were significantly difference. Cells cultured in the marine gelatin derived hydrogel grew significantly faster, with greater than 60% more cells by 7 days and they exhibited more spread-out conformations as compared those cultured in the porcine derived hydrogel. Production of ECM by cells cultured in the Oalg/marine gelatin hydrogel was up to 2.4 times greater than that of in the Oalg/porcine gelatin hydrogel. The biodegradation rate of the hydrogel formulated from marine gelatin was greater than its counterpart prepared from porcine gelatin. These differences have important implications in the biomedical applications of the two hydrogels.

Keywords Hydrogel · Oxidized alginate · Porcine gelatin · Marine gelatin · In situ

1 Introduction

Collagen is a major constituent of extracellular matrix (ECM) and it plays important roles in the morphogenesis, structure and function of both tissues and organs [1, 2]. Collagen derived gelatin has unique gelation properties attributable to the physical crosslinking of the triple-helix conformation of native collagen [3]. Gelatin retains informational signals and is degradable in vivo; its physicochemical properties can readily be modulated [4–6]. Due to the presence of a large number of functional side groups, gelatin readily undergoes chemical crosslinking. This characteristic in conjunction with its performance in cell adhesiveness and plasticity collectively define gelatin's role as a widely utilized biomaterial [7–11].

Hitherto, the most investigated gelatins are produced from either bovine or porcine sources; however, mammalian derived gelatin is known to associate with communicable diseases [12–15]. Moreover, the gelation property of mammalian gelatin (i.e., a solid at room temperature) has somewhat limited its utilities. In contrast, other than being abundant and not known to associate with any communicable diseases to mammals [5, 16], marine-derived gelatin, specifically from cold water species, has low melting temperature (typically gels below 8°C)

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[17, 18], with its aqueous solution remains a flowable liquid at room temperature. This relatively low gelation temperature greatly facilitates formulating gelatin into an in situ gelable material suitable for injection. However, a fundamental shortcoming of marine gelatin is its sub-optimal physical properties, thus limiting further development of its biomedical applications.

Theoretically, the sub-optimal physical properties of gelatin could be compensated by combining it with a crosslinker of high activity, thereby, producing a more optimal product. In this study, oxidized alginate (Oalg) was deployed as a macromolecular crosslinker to formulate hydrogels. Alginate is an anionic linear polysaccharides composed of 1, 4-linked b-D-mannuronate (M) and 1, 4-linked a-L-guluronate (G), it has been utilized as a macromolecular carrier for delivery of both drugs and proteins, and more recently, scaffold for cell growth [19, 20]. Oxidizing alginate with periodate produces multiple aldehyde and carboxylic groups capable of strong physical and/or chemical crosslinking with gelatin, which is abundant in free amino groups, to form hydrogels [21, 22].

The goal of this investigation is to compare the physicochemical and biological performance of hydrogels formulated from mammalian and marine gelatin using Oalg. We demonstrated that by employing this Oalg macromolecular crosslinker strategy, the hydrogel formulated from both mammalian and marine gelatin performed comparably, but the subtle differences of the morphological and mechanical properties of marine gelatin-derived hydrogels led to more optimal cell growth and more rapid ECM deposition.

2 Materials and methods

2.1 Synthesis and characterizations of oxidized alginate (Oalg)

Na-Alginate (5.00 g, Sigma-Aldrich, St. Louis, MO) was dissolved in 400 ml of distilled water in a shaded container, an aqueous sodium periodate (Sigma-Aldrich, St. Louis, MO) solution (100 ml, the ratios of sodium periodate to the number of repeating units of alginate was kept at 80 mol%) was added under stirring. After 24 h, an equimolar amount of diethylene glycol (Sigma-Aldrich, St. Louis, MO) was added to quench the unreacted $NaIO_4$, the reaction product was dialyzed (MWCO 3,500, Marineer, Hampton, NH) exhaustively for 3 days followed by filtration with pure Oalg obtained after lyophilization (Freezemobile 6100, Virtis, Gardiner, NY) [21]. The oxidation degree of alginate was determined by quantifying aldehyde groups formed with tert-butyl carbazate (Sigma-Aldrich, St. Louis, MO) following a previously established method [21, 22].

2.2 Preparation of precursor solutions and formation of hydrogels

Oalg solution was blended with either marine gelatin (Sigma-Aldrich, St. Louis, MO) solution or porcine gelatin (Sigma-Aldrich, St. Louis, MO) solutions, of 20% (w/v) concentration (in pH 9.4, 0.1 M borax) in various ratios, Oalg/gelatin hydrogels were formed by incubating the homogeneously mixed Oalg/gelatin solutions at 37°C.

2.3 Rheological characterization

All rheological measurements were performed with a rheometer (HAAKE RS600, Thermo-Fisher). The shear stress and viscosity of Oalg/gelatin systems were monitored as a function of time at a frequency of 1 rad/s at 37°C [23].

2.4 Hydrogel morphology

Lyophilized and fractured pieces of hydrogels were secured on an aluminum stub with copper tapes and sputtered with gold. Both surface and cross-sectional morphologies were examined by a scanning electron microscope (SFEG Leo 1550, AMO GmbH, Aachen, Germany) at 10 kV.

2.5 Swelling analysis

Swelling studies of Oalg/gelatin hydrogels were performed in 0.01 M PBS at 37°C. The weights of the lyophilized hydrogels were recorded (W_d) prior to immersion in PBS. After a stipulated duration of incubation, the hydrogels were blotted with tissue paper for removal of excess water and weighed (W_s). The swelling ratio (q) was calculated by $q = (W_s - W_d)/W_d$.

2.6 Cytotoxicity potential of Oalg/gelatin hydrogels and their degradation byproducts

Cell cytotoxicity assays were carried out in 24-well plates (initial seeding density: 5×10^3 cells/well) on both the Oalg/gelatin hydrogel formulations. Co-culture was performed at 37°C under a humidified atmosphere of 5% CO_2. Cell viability studies were performed using MTS assay to verify the non-cytotoxicity of both hydrogels and their degradation byproducts. Briefly, Oalg/gelatin hydrogel pieces, tailored to 10 mm diameter × 1 mm thick, were deposited in a 24-well culture plate with each well seeded with cells ($n = 3$ per group). Cell viability was determined at 0, 7, 14, 21, 28 days, respectively. Monolayer cultured cells were used as controls.

2.7 Cell morphology, viability, migration and distribution in Oalg/gelatin hydrogels

Briefly, fibroblasts-laden Oalg/gelatin hydrogels were stained with 200 µl of "Live/Dead™" dye solution containing 2 µm calcein AM (staining of live cells) and 4 µm EthD-1 (staining of dead cells) in PBS for 10 min at ambient temperature. Cell morphology, viability, and spatial distribution were assessed under a Laser Scanning Confocal Microscope (LSCM) (LSM510, Carl Zeiss Inc., Germany) and the images captured were digitized. Reconstruction of 3D images and measurement of cell migration depth were conducted by Zeiss LSM 510 META software.

2.8 Verification of glycosaminoglycans production

The glycosaminoglycan (GAG) contents of Oalg/gelatin hydrogels were determined by a modified dimethyl-methylene blue method [24]. Briefly, cell seeded and unseeded hydrogels were first rinsed and dehydrated by lyophilization. Samples were digested in 1 ml of a 0.5% papain/buffer solution in a 65°C water bath. An aliquot of the digest was assayed for its total GAG content by adding a 1,9-dimethyl-methylene blue dye solution (28 µM). The absorbance at 535 nm was determined and the amount of GAG was extrapolated from a previously prepared standard plot using shark chondroitin sulfate. The results were presented as the mean differences in GAG content per mg of hydrogel dry weight, reflecting the net increase of GAG in the hydrogel (GAG content in cell-seeded hydrogels minus GAG content in pristine hydrogels).

2.9 In vitro degradation of Oalg/gelatin hydrogels

After incubating for the time-spans ranging from 0 to 28 days, cell-laden Oalg/gelatin hydrogels were retrieved at various time-points; their diameters (d) and thicknesses (h) were measured with a digital caliper (Jed Pella, Inc., Redding, CA) and the samples' volumes (V) were calculated by: $V = \pi d^2 h/4$. The percentage of volume loss (VC) in each hydrogel was calculated by: $VC\ (\%) = 100\ (V_d - V_0)/V_0$

2.10 Statistical analysis

Statistical comparisons between the two groups of samples were performed by unpaired Student's *t*-test. Values were depicted as mean ± standard deviation with $P < 0.05$ defined as significant.

3 Results and discussion

Periodate oxidation specifically cleaves the vicinal glycols along the alginate structure to form their dialdehyde derivatives (Oalg) [22]. The multiple aldehyde and carbonyl groups along Oalg enables it to serve as a macromolecular crosslinker for materials abundant in free amino groups such as gelatin. The result of carbazate assays showed that alginate at an 80% theoretical oxidation degree was determined as approximately 35% in actuality [22]. Hydrogels were prepared by blending marine or porcine gelatin with Oalg. Initial crosslinking occurred via hydrogen bondings formed between the Oalg and gelatin and subsequently stabilized by Schiff base formation between the amino groups of gelatin and the free aldehydes on Oalg, thereby, enhanced the mechanical properties of the hydrogels formed.

3.1 Rheological analysis

3.1.1 The effect of reaction temperature on hydrogels' rheological properties

The rheological properties of both Oalg/porcine and Oalg/marine gelatin solutions (weight ratio 1:2) were determined at room temperature and 37°C, respectively. Both gelation times and rheological properties of the Oalg/gelatin (of marine and porcine sources) were determined and compared. Figure 1 depicted the variation of elastic (G′) and viscous modulus (G″) as well as the complex viscosity (ŋ*) versus time for 20% Oalg/porcine gelatin solutions (weight ratio 1:2) at 37°C; in general, the rheological profiles of both systems were comparable. Initially, when G′ was lower than G″, the system exhibited the typical behavior of viscous fluids. Both moduli elevated rapidly, and the buildup rate of G′ was higher than that of G″ due to crosslinking. When G′ and G″ crossed over [22], the systems progressively transitioned from viscous behavior dominated liquid-phases to elastic behavior dominated solid-phases; these transitions were defined as their gel points (t_{gel}). Both moduli of the system continued to increase and eventually leveled off, signifying formation of well-developed three-dimensional network of the system. The ŋ* also underwent a similar process, just as shown in Fig. 1, a rapid buildup at the beginning followed by level-off.

The rheological properties of two systems at room temperature and 37°C were summarized in Table 1. In general, the rheological profiles of all systems were comparable to the one shown in Fig. 1. Moreover, the t_{gel} of the Oalg/porcine gelatin system was obviously shorter than that of the Oalg/marine gelatin system; in parallel, its corresponding ŋ* and G′ were also higher. For example,

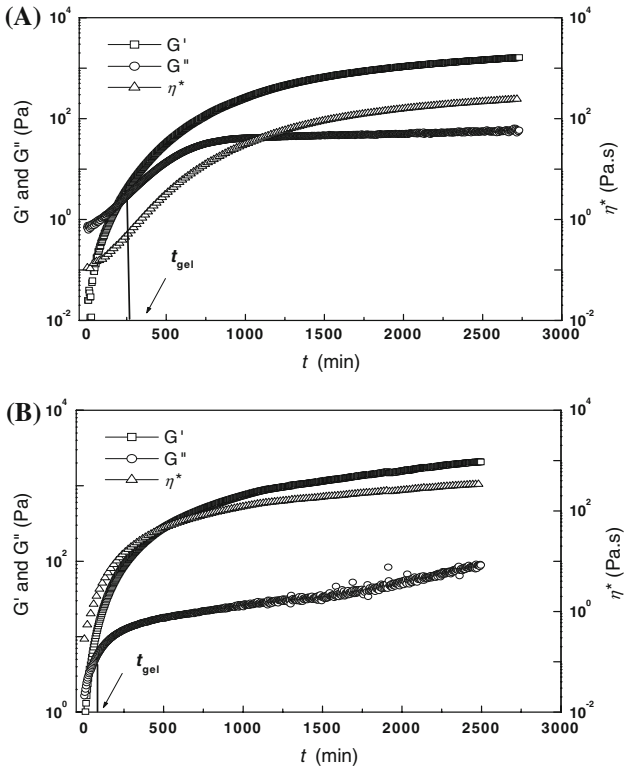


Fig. 1 The elastic modulus (G′), viscous modulus (G″) and complex viscosity (ŋ*) versus time profiles for Olag/marine (A) and Oalg/porcine (B) gelatin solutions (weight ratio 1:2) at 37°C

the t_{gel} of Oalg/marine and porcine gelatin was approximately 180 s and 100 s at 37°C, respectively, while their corresponding ŋ* was 275 and 340 Pa.s. It indicated that the porcine gelatin had higher reactivity than marine gelatin, and the overall mechanical strength (as represented by ŋ* or G′) of porcine gelatin based hydrogel was also higher than that of marine gelatin based hydrogel. However, at room temperature, the t_{gel} of Oalg/marine gelatin system was 1800 s, which was considerably longer than that of at 37°C; higher temperature would accelerate the crosslinking reaction. Due to the higher melting temperature of porcine gelatin [25], which solidifies at room temperature, it is virtually impossible to reliably test the rheological properties of Oalg/porcine gelatin system at room temperature. In general, the Oalg/porcine gelatin system has a more rapid t_{gel} with the hydrogel formed possessing greater mechanical strength; these properties are more suitable for developing pre-formed hydrogels for implantation. Conversely, being a liquid at room temperature for a relatively long time (i.e., 1,800 s) but able to rapidly congeal at 37°C (i.e., 180 s), the Oalg/marine gelatin composition is particularly suitable for formulating injectable in situ gelable materials as sufficient time is available for preparation of a precursor at room temperature but able to solidify quickly at body temperature.

3.1.2 The effects of material compositions on the rheological properties of Oalg/gelatin hydrogels

Table 2 summarized the rheological properties of Oalg/marine gelatin or Oalg/porcine gelatin of different weight ratios at 37°C. There appeared to be optimal formulation parameters to prepare hydrogels with the greatest mechanical strengths and shortest t_{gel}. When the weight ratio of the Oalg/marine gelatin was 1/5, the hydrogel obtained had the greatest strength ($ŋ^*_{max}$ = 290 Pa.s; G'_{max} = 1,920 Pa) and shortest t_{gel} (120 s); in contrast, comparable performance characteristics for Oalg/porcine

Table 1 Rheological characterization of 20% (w/v) Oalg/gelatin hydrogels at different temperature

Reaction temperature	Marine gelatin					Porcine gelatin				
	t_{gel} (s)	t_{max} (s)	G′ (Pa)	G″ (Pa)	$ŋ^*_{max}$ (Pa.s)	t_{gel} (s)	t_{max} (s)	G′ (Pa)	G″ (Pa)	$ŋ^*_{max}$ (Pa.s)
Room temperature	1800	>6000	>250	>51	>33	–	–	–	–	–
37°C	180	2700	1700	63	275	100	1500	2070	88	340

–: the parameters for porcine gelatin could not be accurately deduced at room temperature

Table 2 Rheological characterization of 20% (w/v) Oalg/gelatin hydrogels formulated by combining Oalg and gelatin at different ratios

Weight ratio of Oalg and gelatin	Marine gelatin					Porcine gelatin				
	t_{gel} (s)	t_{max} (s)	G′ (Pa)	G″ (Pa)	$ŋ^*_{max}$ (Pa.s)	t_{gel} (s)	t_{max} (s)	G′ (Pa)	G″ (Pa)	$ŋ^*_{max}$ (Pa.s)
1/1	570	3000	700	25	110	240	1600	1300	55	200
1/2	180	2700	1700	63	275	100	1500	2070	88	340
1/3	140	2600	1810	71	283	60	1200	2435	110	400
1/5	120	2400	1920	79	290	110	1400	1955	80	360
1/8	330	2700	1090	40	176	70	1500	1840	65	300

gelatin were reached at a weight ratio of 1/3, and its η^*_{max} and t_{gel} were 2,435 Pa, 400 Pa.s and 60 s, respectively. Overall, at the same weight ratio, the mechanical strengths of the hydrogels containing porcine gelatin were greater with shorter t_{gel}.

The difference in the rheological properties of the two systems was attributable to the compositions of the two types of gelatins. Gelatin derived from marine species has lower Pro and Hyp amino acid contents, and Pro and Hyp are the main points for Schiff base formation. Typical mammalian gelatin (e.g., porcine gelatin) contains approximately 90 Hyp and 140 Pro residues, respectively, per 1,000 amino acid residues; in contrast, marine gelatin contains approximately 50 and 100 Hyp and Pro, respectively, per 1,000 amino acid residues [25]. Therefore, the marine gelatin has comparatively less amino groups available for reacting with the aldehyde residues of Oalg to form Schiff base linkages. This could account for the longer gelation time, lower mechanical strength, or difference in the weight ratio of marine gelatin to achieve maximum crosslinking.

3.2 Morphological analyses of Oalg/gelatin hydrogels

All Oalg/gelatin hydrogels were yellowish in color and semi-transparent. Typical cross-sectional SEM images of lyophilized hydrogels prepared from 20% Oalg/gelatin solution at a weight ratio of 1/2 were depicted in Fig. 2. Apparently, both hydrogels had comparable highly porous interior structures with the pores interconnected, suggesting high water retention capacity; the direct implication is free diffusion of materials. By comparison, the pore sizes of porcine gelatin-based hydrogel formulation (average pore size: 100 μm) were generally smaller than those of their marine gelatin-based counterparts (average pore size: 150 μm). Evidently, the difference of the pore sizes was partially attributable to the crosslinking densities of the hydrogels formed, which was determined by the compositions of two types of gelatins. As indicated by the rheological result depicted in Table 2, under the same preparation condition, the porcine gelatin-based hydrogel was formulated with higher crosslinking density, thus, it generally exhibited smaller size pores as compared to the Oalg/marine gelatin system.

3.3 Swelling analysis

Figure 3 showed the dependence of the swelling ratio q of Oalg/gelatin hydrogel formulations of different weigh ratios. The hydrogels formulated from either marine or porcine gelatin followed comparable patterns of swelling upon immersion in water. In general, q decreased gradually with increase in the weight ratio of gelatin, reversal of the trend started at the weight ratio of 1/5 and 1/3 for marine and porcine gelatin, respectively. The q of a hydrogel was influenced predominantly by its crosslinking density; the higher the crosslinking density, the lower the swelling ratio. The gradual increase in availability of amino groups with the elevation of the weight ratio of gelatin resulted in formation of more Schiff base linkages, thereby, increase of crosslinking density and the observed rapid drop in PBS uptake. Finally, a minimum q value appeared at maximum crosslinking of both systems, which was in good agreement with the rheological results. Additionally, at the same weight ratio, the hydrogels formulated from marine gelatin have higher q than their porcine-based counterparts, which was also attributable to the differential chemical compositions of the two types of gelatins.

3.4 Cytotoxic potential of hydrogels

The potential cytotoxicity of the Oalg/gelatin hydrogels to fibroblasts was evaluated by performing MTS assay. Cells were seeded at the same initial density, with or without

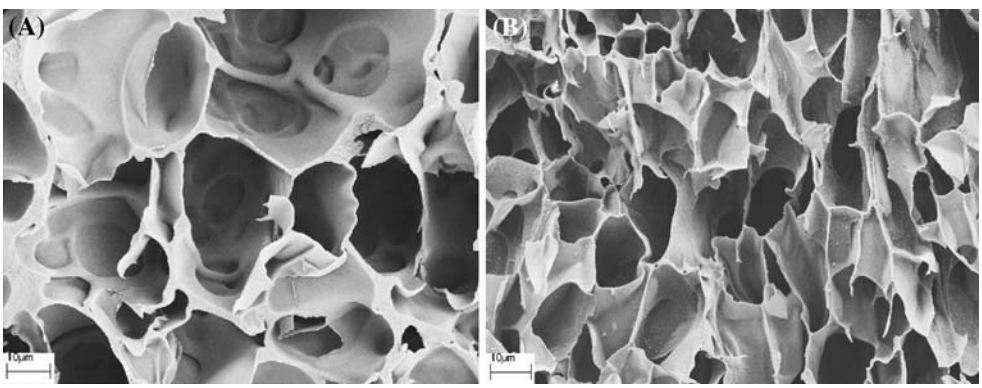


Fig. 2 Morphologies of the inner-sections of Oalg/marine gelatin hydrogel (left) and Oalg/porcine gelatin hydrogel (right) (The weight ratio of Oalg and gelatin was both 1:2)

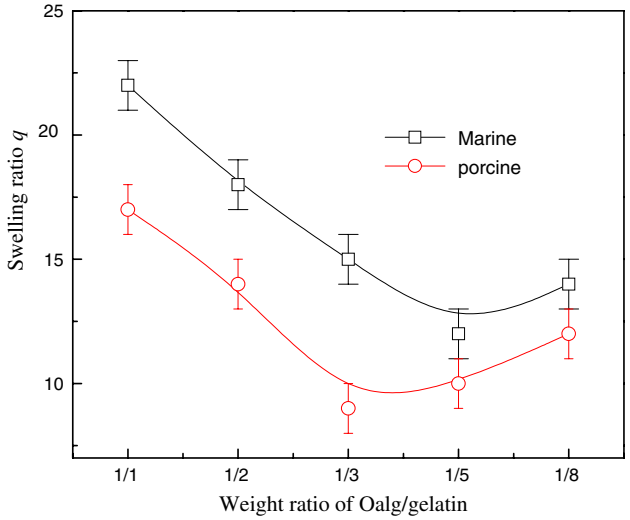


Fig. 3 Comparison of the swelling ratios of Oalg/marine gelatin and Oalg/porcine gelatin hydrogels

hydrogel; cell viabilities were examined on days 0, 7, 14, 21, and 28, respectively, and the results were depicted in Fig. 4. Cells steadily proliferated with time suggesting the hydrogels formulated from both the marine and porcine gelatins did not affect cell growth and thus, were non-cytotoxicity.

3.5 Cell growth in hydrogels

As shown in Fig. 4, there were significant differences in peak cell numbers between the cells seeded on hydrogels (3D culture) and the control (2D culture) ($P < 0.05$) after 7 days of incubation. In the hydrogels, cells were capable of migrating and interacting through their ECM in three dimensions; in contrast, in 2D culture the cells exhibited apparent contact inhibition as they neither could migrate into the support nor able to proliferate extensively [26].

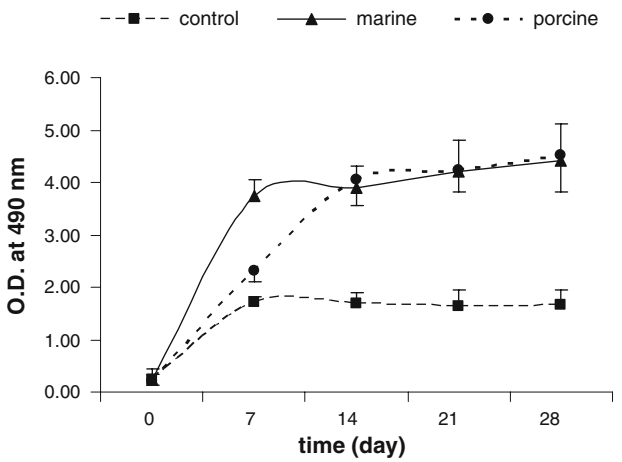


Fig. 4 Cell growth in various Oalg/gelatin hydrogels

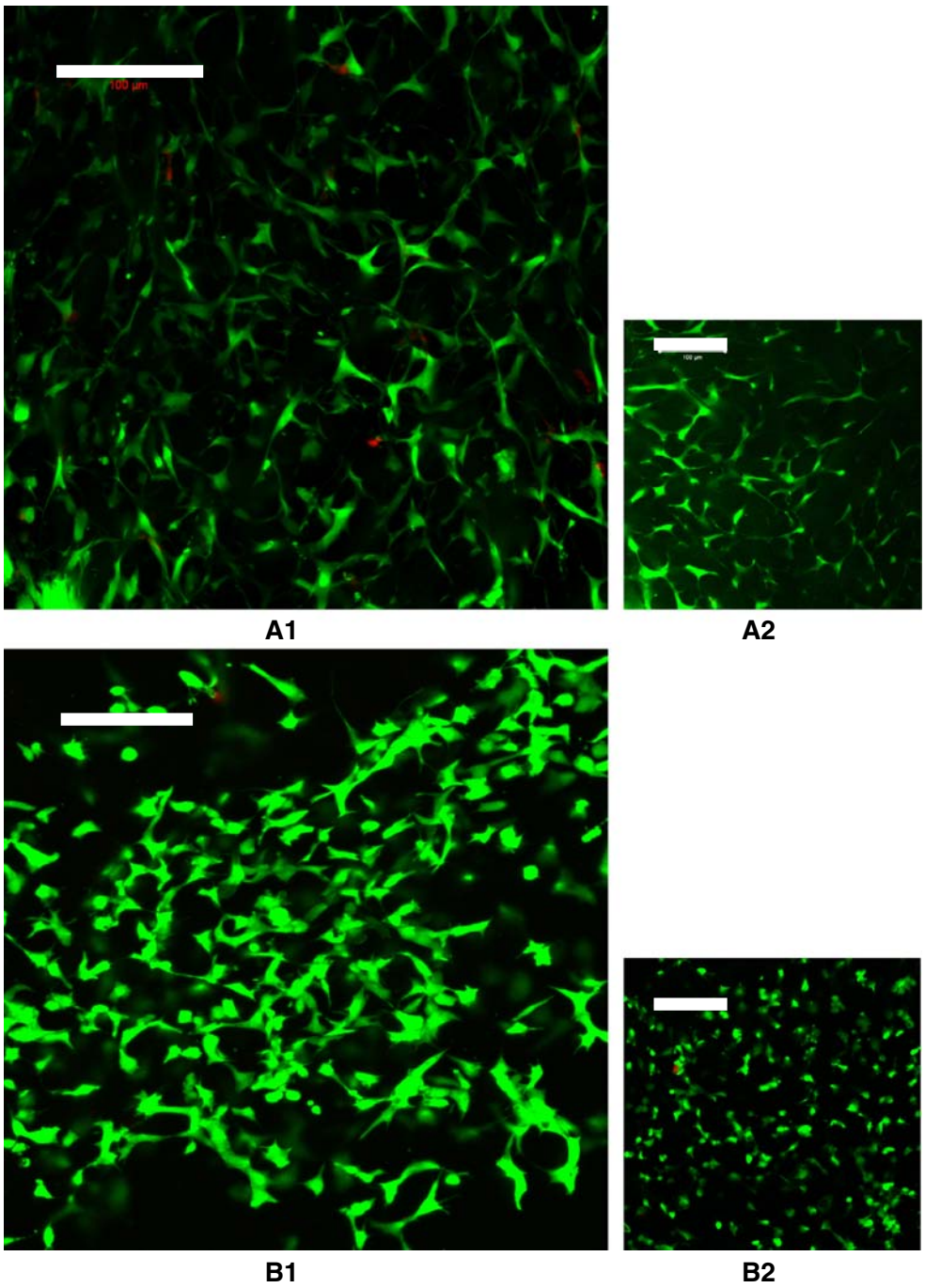
Furthermore, it was previously shown that cells in 3D culture were more resistant to apoptosis [27]. In our study, the peak cell number in hydrogels was at least 2.7-fold of its monolayer 2D counterpart.

There were also noticeable differences in cell growth patterns between the two hydrogel formulations. As shown in Fig. 4, cells cultured in the marine gelatin derived hydrogel grew faster, with 62% more cells at 7 days when compared with the porcine derived hydrogel culture. Cells residing on the hydrogel composed of porcine gelatin reached their proliferative plateau phase at 14 days, which was considerably longer than those seeded on the hydrogel composed of marine gelatin. As the mechanical strength (ŋ*) of the Oalg/marine gelatin hydrogel (Table 1 and Table 2) was lower than that of the Oalg/porcine gelatin hydrogel, in concert with the larger pore size exhibited by the former, cells need to overcome less resistance to extend and penetrate into the Oalg/marine gelatin hydrogels. This eventually would enhance the cell proliferation because of better signal communication between cells. On the contrary, the hydrogel composed of porcine gelatin had stronger mechanical property with smaller pores and were not as amenable to cell migration, spread out and proliferation as the hydrogel composed of marine gelatin at the early stage of culture.

3.6 Cell morphology and viability in hydrogels

The results of Live/Dead^{TM} staining of cells residing inside both hydrogel formulations confirmed their viabilities. The overwhelming majority (>99%) of cells were alive with the overall cell number higher than their monolayer counterparts seeded on dishes (as controls, not shown). Compared with cells seeded in the hydrogel formulated from porcine gelatin, cells migrated more readily into the hydrogel formulated from marine gelatin; they typically adopted more spread-out conformations (Fig. 5) because of the moderately weaker physical properties resulted from lower degrees of crosslinking of the marine gelatin with the Oalg. There was no noticeable difference in the cell migration depths at 21 and 28 days after seeding indicating that the initial differential capacity for cells to migrate inside the hydrogel could eventually be compensated by the degradation of Olag/gelatin hydrogels to which they were attached. These results further corroborated the differential cell growth patterns of the two hydrogels observed above. Although cells in both hydrogels showed similar mor-

Fig. 5 Cell distribution and their morphologies in various Oalg/gelatin hydrogel formulations. **a1** Oalg/marine gelatin hydrogel (3D view); **a2** Oalg/marine gelatin hydrogel (2D view); **b1** Oalg/porcine gelatin hydrogel (3D view); **b2** Oalg/porcine gelatin hydrogel (2D view). Green: live cells; red: dead cells Scale bar: 100 μm. **c** cell migration depth ▶


A1
A2
B1
B2
C
marine
porcine
migration depth (um)
200
150
100
50
0
0
7
14
21
28
time (day)

phology and behavior, cells in marine gelatin formulated hydrogels were more elongated and apparently assumed bipolar morphology. Both hydrogels' 3D cell distribution patterns showed consistent alignment of cells suggesting their direction of migration into the hydrogels; unlike 2D cultures, cells in the 3D hydrogels do not have a strong and anisotropic external cue of an artificial support (i.e., solid or permeable) and therefore, must define their orientation in a relatively isotropic environment [28]. The homogeneous cell arrangement (isotropic) epitomized by the Oalg/gelatin hydrogel is important in tissue engineering as it is capable of supporting cells, promoting their proliferation and functionality towards the formation of new structures.

3.7 Production of ECM by fibroblasts

Fibroblasts are known to secrete and deposit ECM on polymer matrices [28], however, traditional staining techniques for ECM visualization (e.g., Van Gieson's method, Sirius Red method, Masson's Trichrome method) would be inadequate for indentifying the ECM deposited inside the hydrogels as its main component gelatin would cross-react with the dyes producing false positive staining. GAGs, the main constituents of ECM, are ubiquitous polyanionic polysaccharides including chondroitin, dermatan, heparan, keratan, and heparin, [29] covalently linked to a protein core forming proteoglycans. Using GAG staining as a surrogate endpoint for ECM etection could circumvent the interference problems posed by gelatin with the conventional staining techniques. As shown in Fig. 6, the amount of ECM deposited in the hydrogel formulated from marine gelatin reached the peak value significantly faster than that of the hydrogel containing porcine gelatin (14 days vs. 21 days, $P < 0.05$). There was also a significant difference in the quantity of ECM in the first 14 days of culture ($P < 0.05$). Production of ECM by cells cultured in the Oalg/marine gelatin hydrogel was 1.5–2.4 times {calculated by (GAG_{marine}-$GAG_{porcine}$)/$GAG_{porcine}$} greater than that of the Oalg/porcine gelatin hydrogel. Apparently, ECM deposition is related but not limited by the cells proliferate inside the hydrogel. Cells secrete many proteins, including ECM components, growth factors (e.g., transforming growth factor, fibroblasts growth factor, platelet-derived growth factor, etc.) and enzymes (e.g., latent matrix metalloproteinases, etc.). Through the principle of dynamic reciprocity, cells synthesize and secret ECM; this in turn, is regulated by ECM [30]. As gelatin is a major component of ECM, it thus, interacts with the surrounding ECM further influencing ECM deposition.

3.8 Cell mediated degradation of hydrogels

Figure 7 depicted the volume loss profiles of cell-laden Oalg/marine gelatin and Oalg/porcine gelatin hydrogels. Both hydrogels showed relatively fast volume losses in the first 2 weeks, followed by slowing down in biodegradation as reflected by the subsequent moderation of the volume loss profiles. The degradation rate of the hydrogel formulated from marine gelatin was greater than its counterpart prepared from porcine gelatin, which could be attributed to the difference in both cells proliferation and the hydrogels' crosslinking densities caused by the different physicochemical properties of the two types of gelatin. As presented previously in Fig. 4, and Fig. 6, the hydrogel formulated from porcine gelatin had a higher crosslinking density than that of the hydrogel formulated from marine gelatin, which translated into a greater rate of cell proliferation of the latter. It should be note that fibroblasts residing on the hydrogels synthesized and secreted some substrate-specific enzymes for ECM remodeling. It could thus be inferred that these enzymes also targeted the Oalg/gelatin crosslinking structures contributing to the cell-mediated degradation of the hydrogel matrix leading to the higher level of ECM produced and the denser cell population in the hydrogel containing marine gelatin over the first 2 weeks, consequently, this also contributed to the

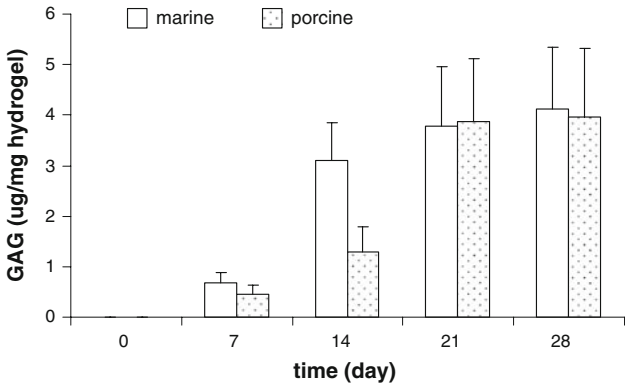


Fig. 6 GAGs production by fibroblasts in Oalg/gelatin hydrogels

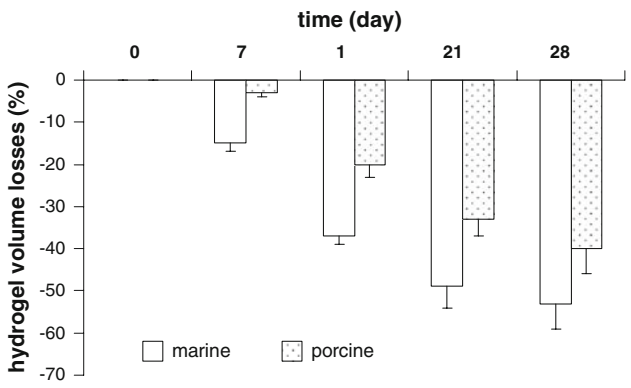


Fig. 7 Cell-mediated degradation of Oalg/gelatin hydrogels

faster overall hydrogel degradation observed during the early stage of the culture.

4 Conclusions

In situ gelable hydrogels have been formulated from marine- and porcine-derived gelatin utilizing oxidized alginate as a macromolecular crosslinker. The former can be prepared at ambient temperature, whereas, preparation of the latter has to be carried out at elevated temperature when the porcine gelatin solution is in its molten state. The hydrogel prepared from porcine gelatin is more suitable for direct implantation and its counterpart formulated from marine gelatin is ideal for preparing rapid in situ gelable materials targeting injection. The physicomechanical performances of both hydrogels were comparable suggesting this approach could largely offset the generally regarded sub-optimal physical properties of marine gelatin. On the contrary, despite being non-cytotoxic, the biological performances of the two hydrogels were considerably different; the hydrogels prepared from marine gelatin is more amenable to cell migration, cell growth, ECM deposition and undergo more rapid biodegradation.

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