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Biological self-assembly of injectable hydrogel as cell scaffold *via* specific nucleobase pairing†

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A biological hydrogel was self-assembled *via* Watson–Crick base pairing of thymine and adenine from functionalized star poly(ethylene glycol). Our work should provide a novel methodology to generate robust injectable scaffolds with tailorable properties for biomedical applications.

Biodegradable hydrogels have served as highly functional cell scaffolds for tissue engineering and in delivery of cell growth factors (GFs).[1] Conventionally, biodegradable hydrogels have been synthesized by chemical crosslinking methods using crosslinking agents, photopolymerization, click chemistry and covalent conjugation.[2] While the versatility of crosslinking has been broadly exploited, chemical hydrogels are limited by irreversible modification and aggregation of the entrapped proteins and the potential toxicities of chemically reactive moieties. A major issue in the use of hydrogels and tissue reconstruction procedures in general is that the establishment of a biomimic environment into the engineered tissue is necessary for engineered tissue survival.[3] A goal is the synthesis of a biological hydrogel *via* self-assembly that might therefore permit manipulation of inductive properties for cells proliferation. Recently, self-assembly conjugation in polymeric materials has become increasingly prominent in the production of bioactive surfaces and matrices.[4] A great variety of self-assembly methods have been reported for conjugation of peptides, proteins, DNA, oligonucleotides and nanoparticles, which are based on electrostatic interactions, inclusion complex, ionic interactions, hydrophobic interactions, hydrogen bonding, crystallinity and affinity interactions.[5]

Here, we present a flexible way to self-assemble a polymeric hydrogel *via* specific pairing of functionalized nucleobases. The biological self-assembly of a poly(ethylene glycol) (PEG) hydrogel system is established by Watson–Crick base pairing between thymine (T) and adenine (A) *via* hydrogen bonding (Fig. 1). Compared with linear PEG, multi-arm star-shaped PEG has the tendency to induce stereo-complex formation, which shows promise in biomedically-relevant hydrogel systems.[6] Four-arm PEG was herein chosen for self-assembly instead of linear PEG in this study. Firstly, thiol thymine (T-SH) and thiol adenine (A-SH) were synthesized. Maleimide terminated four-arm PEG (PEG-Mal) was functionalized with either T-SH or A-SH functionalities as self-assembly precursors (referred as PEG-T and PEG-A) *via* Michael-type addition (Fig. 1a and b). After dissolution and mixing of precursors in an aqueous environment, a stereo-PEG hydrogel network was self-assembled due to the formation of pairing complexes (Fig. 1c).

The storage moduli G' of self-assembled hydrogels (PEG 20 kDa, 20 wt%) were monitored as a function of molar ratio of PEG-T/PEG-A at 37 °C in PBS (Fig. 2a). When PEG-T/PEG-A was mixed with different molar ratios, storage moduli were

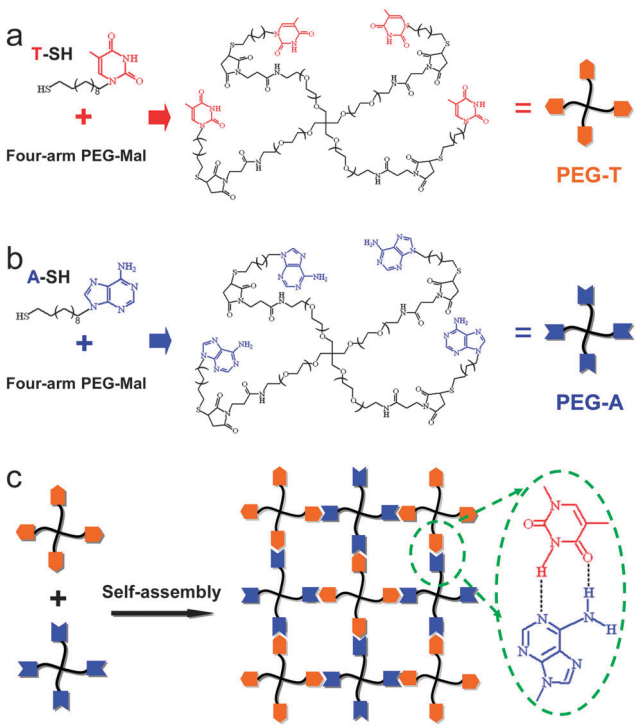

Fig. 1 (a, b) Four-arm poly(ethylene glycol)-maleimide (PEG-Mal) was functionalized with thiol thymine (T-SH) and thiol adenine (A-SH), respectively, as self-assembly precursors *via* Michael-type addition. (c) Schematic of biological self-assembly of ideal four-arm PEG hydrogel network *via* intermolecular hydrogen bonding of Watson–Crick base pairs between thymine and adenine functionalities.

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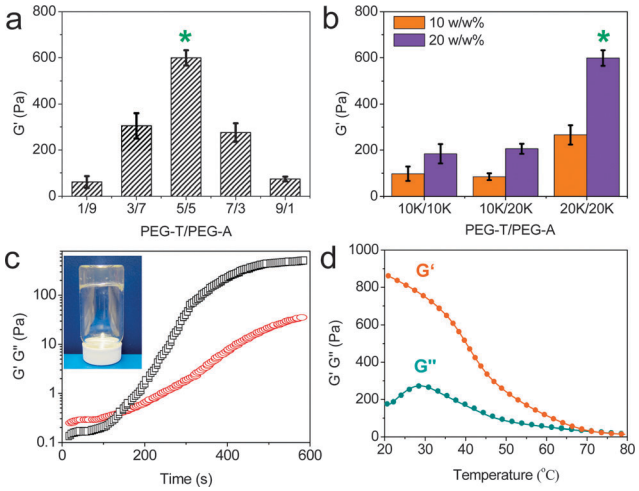

Fig. 2 (a) Storage modulus of self-assembled hydrogels as a function of PEG-T/PEG-A molar ratio. (b) Storage modulus of self-assembled hydrogels with different molecular weights and concentrations. (c) Rheological characterization of self-assembled hydrogel networks. The inset image illustrates the formed PEG-20K/20K hydrogel in PBS. (d) Rheological properties of the self-assembled hydrogel as a function of temperature.

measured between 63 and 600 Pa. Results indicated that the storage modulus of the 5/5 hydrogel with equimolar amounts of PEG-T/PEG-A was the highest (599.4 Pa), which showed a significantly higher G' than the others ($p < 0.05$). Less PEG-T or PEG-A in mixtures may decrease the number of ligand units available for hydrogen bonding, which leads to imperfect networks and lower storage moduli.

Two different molecular weights (10 and 20 kDa) of four-arm PEG were studied for self-assembly in aqueous solution at 37 °C (Fig. 2b). Three self-assembly hydrogels consisting of PEG-T/PEG-A with molecular weights of 10 or 20 kDa, *e.g.* PEG-10K/10K, PEG-10K/20K and PEG-20K/20K were prepared. The PEG-20K/20K hydrogel showed the highest storage modulus, followed by PEG-10K/20K and PEG-10K/10K hydrogels. Fig. 2b also showed that G' significantly increased with increasing the polymer concentrations from 10 to 20 wt/wt%. This indicated that higher concentrations of polymer created a higher effective cross-link density and therefore led to a tighter network. Since PEG-20K/20K (20 wt/wt%) comprised more polymer chains and gave the strongest storage modulus, a concentration of 20 wt/wt% and molecular weight of 20 kDa were used for subsequent studies, and further discussion refers only to this hydrogel.

Dynamic time sweep rheological experiments were conducted to monitor the network evolution and evaluate the viscoelastic behavior of the hydrogel (Fig. 2c). Upon mixing of homogeneous PEG-T and PEG-A solutions (20 wt/wt%) at 37 °C, the storage modulus G' was significantly increased and larger than the loss modulus G'' after 150 s. The data indicate a final storage modulus G' value of ~600 Pa after 500 s, in excess of the loss moduli $G''(\omega)$, which resulted in a hydrogel with a typical viscoelastic property. The inset in Fig. 2c illustrates the viscoelastic hydrogel after self-assembly in PBS.

Fig. 2d shows the temperature-dependent rheological characteristics of the hydrogel. With increasing temperature, G' gradually decreased, which was associated with a concomitant decrease of G''. On heating the hydrogel with $G' > G''$, a transition from gel-to-sol occurred at 72 °C, its gel transition temperature. Above 72 °C, G'' starts to exceed G', indicating that the mixtures behave as weak viscous liquids.

As shown in Fig. 3a, the incubation temperature has a significant influence on weight loss of the self-assembled hydrogels in PBS. At lower temperatures, the hydrogel showed a slower weight loss rate than that at higher temperatures. At 50 °C, the hydrogel showed a significantly faster weight loss, due to destruction of hydrogen bonding, and totally dissolved in 9 days. Compared to the result at 50 °C, the hydrogels lost weight steadily up to 18 and 24 days at 37 and 20 °C, respectively. The swelling kinetics of the hydrogel were also investigated in PBS at different temperature as a function of incubation time (Fig. 3b). The hydrogel at a lower temperature showed a lower swelling ratio rate than that at higher temperature, which was consistent with the weight loss data shown in Fig. 3a. Compared to the 20 and 37 °C data, the swelling ratio of the hydrogel significantly increased after 24 h incubation at 50 °C. At 37 °C, the swelling ratio of the hydrogel significantly increased from 17.3 to 26.8 after incubation for 14 days.

GFs that induce adipose-derived stem cells (ASCs) proliferation include insulin, dexamethasone (Dex) and vascular endothelial growth factor (VEGF).[7] Fig. 3c shows the initial concentration of insulin, dexamethasone and VEGF entrapped within the hydrogel. The hydrogel showed the highest efficiency loading of VEGF, and about 96% of VEGF could be entrapped within the hydrogel, but there was no significant difference compared with dexamethasone and insulin ($p > 0.05$). The *in vitro* cumulative amount of GFs released from the hydrogel at specific time points was quantified. As observed in Fig. 3d, the GFs incubated in PBS demonstrated a total cumulative release of approximately 68–89% over the 14-day time period, with total release in 18–20 days. The release of dexamethasone showed a significantly faster rate than both insulin and VEGF ($p < 0.05$).

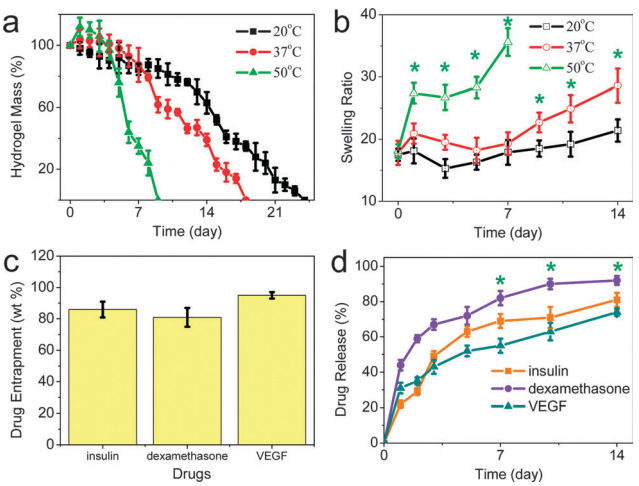

Fig. 3 (a) Degradation of the hydrogel at different temperatures in PBS. (b) Swelling kinetics of the hydrogel at different temperatures. (c) Entrapped GFs in hydrogel in PBS. (d) Cumulative release profiles of GFs from hydrogel.

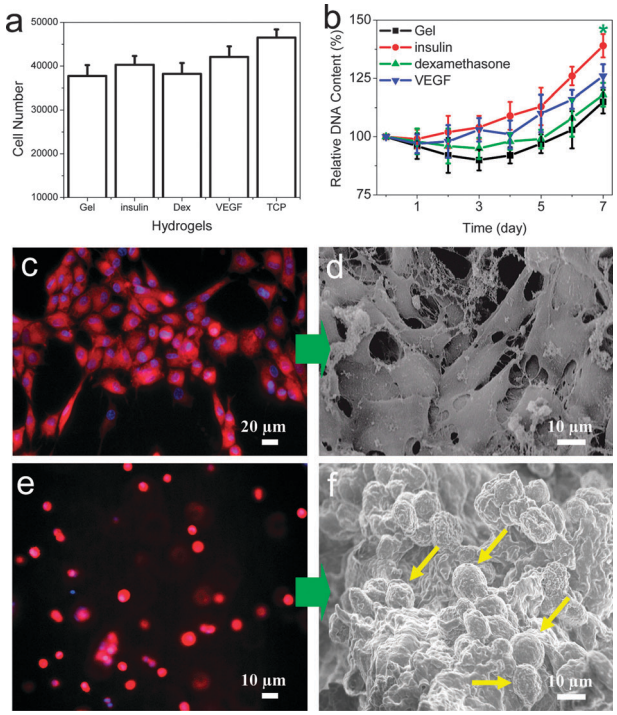

Fig. 4 (a) Number of adhered ASCs to the surface of hydrogels. (b) Proliferation of ASCs cultured in hydrogels. (c, d) Confocal laser scanning microscopy (CLSM) and SEM images of ASCs on hydrogel after 7 days incubation. (e, f) CLSM and SEM images to show ASCs cultured in hydrogel after 7 days incubation.

The adhesion of ASCs to the top surface of the control and GFs entrapped hydrogels after culture for 24 h was characterized (Fig. 4a). All hydrogel samples showed slightly less attached cells than the control tissue culture plate (TCP). Cell numbers on the GFs entrapped hydrogel surfaces was greater than that of the control hydrogel, but no significant difference was found ($p > 0.05$). The proliferation of ASCs in the presence of the biological hydrogel was monitored over time *via* DNA content. As shown in Fig. 4b, all hydrogels caused a significant increase in cell proliferation *in vitro* after 7 days incubation ($p < 0.05$), while the insulin loaded hydrogel showed a significant increase in cell proliferation over the control hydrogel ($p < 0.05$).

Confocal microscopy images illustrate overlaid live/dead fluorescent staining of the adhesion of ASCs after 7 day incubation. The attached cells were present in the superficial area of the biological hydrogel and maintained their polygonal morphologies with size ~20 μm (Fig. 4c), which was consistent with the SEM observation shown in Fig. 4d. The encapsulated cells in the hydrogel for 3D culture were also observed. Viable cells were observed after 7 days of culture (Fig. 4e), and more than 98% of the ASCs survived. Elliptical or round shaped cells were uniformly distributed in the hydrogel, which is an indicator of phenotype retention of ASCs and is essential for matrix formation. An SEM image of the hydrogel encapsulated with ASCs is shown in Fig. 4f. The cells were encapsulated within the hydrogel and possessed normal spherical morphologies with cell size ~10 μm. Spherical shaped ASCs were distributed in the scaffold, indicative of the biological nature of the hydrogel for cell survival. These results demonstrated that the biological hydrogel was able to preserve the proliferation of the encapsulated ASCs. Coupled with the cell attachment data, our results suggested a novel potential mechanism for targeted proliferation of stem cells *via* biological self-assembly.

As presented, we have developed a novel methodology to self-assemble a biological four-arm star-shaped PEG hydrogel as an injectable scaffold that specifically allows for targeted GFs delivery and cell encapsulation. The formation of the self-assembled hydrogel is attributed to Watson–Crick base pairing *via* intermolecular hydrogen bonding between thymine and adenine. The potential application of this biological hydrogel as a cell scaffold in soft tissue engineering was confirmed by encapsulation behavior of human ASCs. These unique characteristics of this biological hydrogel make it a promising candidate as an injectable scaffold for pharmaceutical and biomedical applications.

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