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Biodegradable and injectable polymer–liposome hydrogel: a promising cell carrier

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Biodegradable and injectable hydrogels are widely used in many important applications such as cell culturing, tissue engineering, drug delivery, and cell therapy. Here we developed an inexpensive and facile method to prepare a biodegradable and injectable polymer–liposome hydrogel using aldehyde modified xanthan gum (ALD-XA) as a matrix material and phosphatidylethanolamine (PE) liposomes as cross-linkers. To the best of our knowledge, this is the first time for hydrogels containing crosslinked lipid liposomes to be prepared. The hydrogel was formed through dynamic Schiff base linkages between aldehyde groups of ALD-XA and amino groups of PE liposomes. After mixing ALD-XA and PE liposome solutions, the xanthan gum-based liposome hydrogel could be rapidly prepared within 5 min at room temperature. Owing to the dynamic equilibrium of the Schiff base bonds, the hydrogel responds to various stimuli, including physical stimulus (heat), chemical stimulus (pH variation), and biological stimulus (histidine exposure). Additionally, the hydrogel could easily be biodegraded by papain as a result of the digestion of xanthan gum backbones by enzymes. The hydrogel also exhibits excellent self-healing capability. Cells encapsulated in the prepared hydrogel are viable for a long period of time, indicating that it is an excellent carrier material suitable for three-dimensional (3D) cell culturing. These advantages suggest that the xanthan gum-based liposome hydrogel is a promising candidate for smart cell carrier for cell therapy and excellent cell culturing scaffold for tissue engineering.

Introduction

Hydrogels prepared from hydrophilic polymers with 3D network structures are promising materials for many biomedical applications.[1] A hydrogel material retains a large amount of water, and meanwhile adopts a stable structure by physical and/or chemical crosslinking.[2] Therefore hydrogels can be feasibly tailored and engineered via appropriate structural design to achieve desired properties for different applications in biological and medical enginering,[3] for examples, as carriers for controlled cell culturing and therapy[4–9] or drug delivery[10–16], as scaffolds for tissue engineering,[17-19] as antimicrobial or/and antifouling biomaterials,[20-22] as bio-tissue with good mechanical properties,[23-29] as chiral recognition materials,[30] or as model materials for cell studies,[31,32] etc.

A well-known natural hydrogel material is the human tissue composed of polysaccharides, proteins, and other biological molecules,[33] in which the biological functions (i.e. cell culturing, molecule delivery and biosignaling) can be performed in a metabolic balanced state. Extensive research has been performed to develop functional hydrogel materials under mild gelation conditions based on a wide range of natural materials such as chitosan,[4,11,18,34,35] hyaluronic acid,[34,36] alginate,[32,37] agar,[7,38] xanthan gum,[39] elastin,[40] heparin,[41] soy protein,[42] etc. There are many advantages to use these natural materials which can provide biological cues for cell adhesion, differentiation, proliferation and tissue regeneration.[43] However, these natural polymers themselves may lack tunable structures which limit their direct utilizations as hydrogels.[43,44] It is therefore necessary to tailor and re-engineer these natural materials in combination with the use of other natural/synthetic materials to obtain the needed structures to achieve the desired properties for end biomedical applications.

Recently, hydrogels crosslinked via dynamic covalent bonds attracted extensive attention due to their "smart property" originated from the kinetically controlled structures.[45] Dynamic covalent chemistry is related to covalent bonds that can be formed, broken, and re-formed reversibly under controlled equilibrium conditions,[46] leading to robust structure, shear-thinning character and self-healing property.[47] Here we were inspired to prepare the biodegradable hydrogel based on the dynamic covalent bonding method using polysaccharides and phospholipids.

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† Electronic Supplementary Information (ESI) available: Chemical formulas of PE, XA, and ALD-XA; DLS data of PE liposomes prepared by extrusion and released from the xanthan gum-based liposome hydrogel degraded by papain; and gelation experiment of ALD-XA via reaction with carboxylated chitosan confirming the existence of the aldehyde groups in ALD-XA. See DOI: 10.1039/x0xx00000x

In this work, we developed a new method to create hydrogels with ALD-XA as a matrix material and PE liposomes as cross-linkers. This method is based on the dynamic covalent chemical reactions—Schiff base linkages. We thoroughly characterized the prepared hydrogel material and demonstrate that the material is self-healable, biodegradable, biocompatible, and multi-responsive, which has a great potential to be used in biomedical applications.

Experimental

Materials

Phosphatidylethanolamine (PE, 98%) was purchased from Shanghai Yantuo biotechnology (China). Papain (> 3500 U/mg) and dialysis membranes (MWCO 5K) were ordered from Sangon Biotech. Xanthan Gum (XA, USP grade), sodium periodate ($NaIO_4$, 99.8%), ethylene glycol (≥ 99%), L-Histidine (99%) were bought from Aladdin Industrial Inc. All chemicals were used as received. Ultrapure water (18.2 MΩ·cm) was purified by a Millipore water purification system and used in this study.

Preparation of Liposome Solution

PE liposomes were prepared by sonication and extrusion. Firstly the lipids were dissolved in PBS (phosphate-buffered saline, 0.1 M, pH = 6.25) and hydrated upon 3–5 freeze/thaw cycles, followed by ultrasonication in a bath sonicator for several minutes at ~ 70 ^{o}C. The lipid suspension was then extruded at this temperature through a 100 nm polycarbonate membrane with 21 passes using a Mini-Extruder purchased from Avanti Polar Lipids. All the PE liposome solutions were cooled down to room temperature before the next-step experiment.

Aldehyde Modification of XA

XA was dissolved at 80 ^{o}C to reach a solution concentration of 0.5% (w/v). Then an aqueous solution of $NaIO_4$ (0.5 M) was added dropwise under stirring until at a molar ratio of 1:4 XA/$NaIO_4$. The reaction proceeded for 4h in a dark environment at room temperature. 1% (v/v) ethylene glycol was added and the solution was stirred continually for 1h at the ambient temperature. In order to obtain a uniformly aldehyde modified Xanthan Gum (ALD-XA), the solution was purified by dialysis against ultrapure water for 3 days and the final ALD-XA product was obtained by freeze-drying (LGJ-12, Beijing Songyuan Huaxing Technology, China). The average yield of ALD-XA was about 82%.

Fabrication of Xanthan Gum-based Liposome Hydrogel

ALD-XA was dissolved in PBS (0.1 M, pH = 6.25) at 80 ^{o}C and then mixed under vortex with the same volume of PE liposome solution at the concentration of 15, 20, or 30 mg/mL. The sample was then shaken at room temperature to reach a steady and homogeneous state, as shown in Fig. 1A.

Morphology

Morphology of Xanthan gum-based liposome hydrogel was examined with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The hydrogel samples were freeze-dried and then sputter-coated (JS-1600, Beijing HTCY, China) with gold for 15 s. The cross-sectional morphologies were imaged using a Carl Zeiss Ultra Plus Field Emission SEM (Jena, Germany) operated at 1.20 kV accelerating voltage. TEM images of a negative stained hydrogel sample using 1% aqueous phosphotungstic acid were captured on a JEM-2100 TEM system (JEOL, Japan).

Fourier Transform Infrared (FTIR) Spectrometry

FTIR spectra of PE, XA, ALD-XA, and as prepared hydrogel samples were measured to identify the expected functional groups. Spectra were recorded with a resolution of 2 cm^{-1} and averaged over 32 scans between 4000 and 800 cm^{-1}. Various samples, freezing-dried and ground into powder, were characterized with a FTIR spectrometer (Nicolet iS50, USA) against a blank potassium bromide (KBr) pellet background.

Nuclear Magnetic Resonance (NMR) Spectrometry

XA and hydrogel samples were dissolved in D_2O (Cambridge Isotope Laboratories, USA) and transferred into 5 mm NMR tubes (NORELL, USA). The samples were characterized with a Bruker Avance 300 NMR spectrometer. The ^{1}H NMR spectra were collected at a proton frequency of 300 MHz with 64 scans at 23 ^{o}C.

Dynamic Light Scattering (DLS) Study

The average particle size of liposomes in the solution was measured using a Zetasizer Nano ZS particle size and zeta potential analyzer (Malvern Instruments, UK) in the size measurement mode at 23 ^{o}C. The light source was He–Ne laser (4 mW) at a wavelength of 633 nm. The scattering angle was 173^{o}. The mean particle size, represented by z-average, was obtained by averaging three measurement results.

Rheology Analysis

The viscoelastic properties of the xanthan gum-based liposome hydrogel were characterized via dynamic shear oscillation measurements at a small strain. The rheology measurements were carried out with a HAAKE RheoStress 6000 Rotational Rheometer (Thermo Fisher Scientific) using a parallel-plate of 35 mm diameter with plate-to-plate distance of about 1 mm. Frequency sweep experiments, from 0.1 to 10 rad/s in the linear viscoelastic region of the samples, were carried out with a fixed strain of 1% at 23 ^{o}C.

Injection Experiment

The injection experiments were performed using a 1 mL syringe with a 25 G needle (0.5 mm × 20 mm) to evaluate the injectability of the xanthan gum-based liposome hydrogel *in vitro*. The hydrogel samples were delivered onto the surfaces

of a petri dish and a glass slide respectively at the ambient temperature.

Self-healing Analysis

Two different experiments were conducted to confirm the self-healing capability of the prepared xanthan gum-based liposome hydrogel. A hydrogel sample was extruded into a water-containing vial using the above-described method in the first experiment. After 10 min, several photos were taken. The second experiment was carried out on a glass slide. A hydrogel disc was cut into two patches. Then they were placed together and their integrity was examined in terms of the contact time.

Multi-responsiveness Analysis

Hydrogel samples were prepared by mixing ALD-XA and PE liposome solutions as discussed above (typically using 20 mg/mL ALD-XA and 20 mg/mL PE liposome). The responses of the hydrogel samples to the variation of the temperature and pH value were monitored. For the temperature responsive test, the integrity of a hydrogel sample in a vial was investigated after heating the sample to 80 ^{o}C in water bath and then cooling down it to room temperature. For the pH responsive test, hydrochloric acid (HCl) aqueous solution (1.0 M, 50 µL) was added to another hydrogel sample under vortex. Afterwards, sodium hydroxide (NaOH) aqueous solution (1.0 M, 50 µL) was added under vortex. The integrity of the hydrogel sample was then studied. Various pictures were taken during the experiments to test the sample integrity.

Biodegradable Test

Pure water (100 µL), histidine aqueous solution (100 mg/mL, 100 µL), papain aqueous solution (100 mg/mL, 100 µL) were added to the hydrogel samples (20 mg/mL ALD-XA and 20 mg/mL PE liposome) under the same condition with vortex. The mixture was left for observation at room temperature over time.

Cytotoxicity evaluation of hydrogels

MTT assay was performed on MCF7 (breast adenocarcinoma, human) cells, purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), for cytotoxicity evaluation of the hydrogel using the extract from the hydrogel.[48] MCF7 cells were cultured in the complete growth medium containing RPMI-1640, 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37 ^{o}C in 5% CO_2 humidified atmosphere. The cells were seeded in a 96-well microplate at a density of 5 × 10^4 cells/mL in the 100 µL complete growth medium and then incubated for 24 h. The culture medium was removed and 100 µL extraction medium of xanthan gum-based liposome hydrogels was added to each well. After 24 h the extraction medium was removed and cells were washed three times with PBS. The 100 µL cell culture medium containing 50 µg MTT (Methylthiazolyldiphenyl-tetrazolium bromide, 98%, Sigma) was added to each well under incubation for another 4 h. Finally the culture medium in each well was replaced by 150 µL DMSO (Dimethyl sulfoxide, Sigma), followed by measuring the visible light absorbance with a Multiskan FC Microplate Photometer (Thermo Fisher Scientific) at 492.7 nm.

3D cell culture

MCF7 cells were harvested by PBS containing 0.25 % (w/v) trypsin and 0.53 mM EDTA, centrifuged from the RPMI-1640 medium and resuspended in the PE liposome solution. Then the MCF7/PE liposome solution was pipetted into a vial with ALD-XA dissolved in PBS, followed by gentle vortex to form the hydrogel. All 3D cell culture experiments were carried out in hydrogel formed by 20 mg/mL PE liposome solution mixed with 20 mg/mL ALD-XA solution (~ 5 × 10^6 cells/mL). The hydrogel was transferred from the vial to a petri-dish containing the culture medium and then incubated for 48 h. After that, the hydrogel in the petri-dish was rinsed with PBS and stained with fluorescein diacetate (FDA). The fluorescence images were collected using a confocal laser scanning microscope (CLSM, Leica TCS-SP8). The hydrogel samples were excited at 488 nm and the z-stack images were obtained over a hydrogel depth of about 300 µm to confirm the 3D distribution and viability of the MCF7 cells. Viability is represented by the ratio of FDA stained cells to the total cells encapsulated in the hydrogel.

Results and discussion

Preparation of Hydrogel

ALD-XA and PE liposomes serve as the matrix material and cross-linkers respectively for this two-component hydrogel. XA was chosen because of the following three reasons: Firstly, XA is a polysaccharide with broad applications in food industry because it is safe, biocompatible, and biodegradable.[39] Secondly, this high-molecule-weight polymer is stable and has a large number of crosslinking sites which can be engineered into different biomaterials.[39,45,49] Thirdly, the sugar ring in XA with vicinal hydroxyl groups (Fig. S1A†) can be easily oxidized into aldehydes under suitable conditions for further reaction. PE was used because it is generally found in all living cells and constitutes the inner leaflet of a biological membrane. Its hydrophilic amino head group can react to form a Schiff base bond (Fig. S1B†). Liposomes are microscopic spherical hollow vesicles self-assembled by amphiphiles like PE. They have been widely used in drug delivery, as gene carrier, and for functionalization of biomaterials.[50,51] However, the dispersed liposomes have three main drawbacks, greatly limiting their clinical applications. Firstly, liposomes may not be stable under a shear force or when the temperature, solvent, solution pH value, or solution concentration is varied.[52] Secondly, it is difficult to functionalize liposomes to be highly sensitive and responsive to external stimuli, which hinders their extended applications in pharmaceutics.[52,53] Thirdly, controlled release of the molecules encapsulated inside the dispersed liposomes to a specific site *in vivo* is challenging.[54] The method developed in this research to embed liposomes inside ALD-XA via Schiff

base linkages could overcome the above-mentioned disadvantages, providing an effective means to prepare hydrogel materials for the controlled release of drugs via injection.

The PE liposome solution containing small unilamellar vesicles was easily prepared using the extrusion method, as revealed by the DLS analysis (Fig. S2†). The obtained PE liposomes were well-dispersed in the solution with an average diameter of ~ 120 nm (polydispersity index (PDI) = 0.08).

Functionalization of xanthan gum was achieved through the oxidation of the raw material with sodium periodate, followed by dialysis and lyophilization. XA Oxidation led to sugar ring opening and dialdehyde derivatives formation (Fig. S1C†) from vicinal hydroxyl groups. The Fourier transform infrared (FTIR) spectra of XA (Fig. 2B) and ALD-XA (Fig. 2C) show the carbonyl stretching modes for ketones of XA and aldehydes of ALD-XA which are too similar to be differentiated in the range from 1700 to 1750 cm^{-1}. However, the weak signal at 894 cm^{-1} (Fig. 2C) is slightly enhanced, which may indicate the formation of hemiacetal groups confirming the existence of the aldehyde groups after oxidation since the hemiacetal groups can only come from the reaction between the aldehyde and hydroxyl groups in ALD-XA.[34] Further evidence on the formation of the Schiff base crosslinking can be observed in ^{1}H NMR spectra of a hydrogel sample and a XA sample

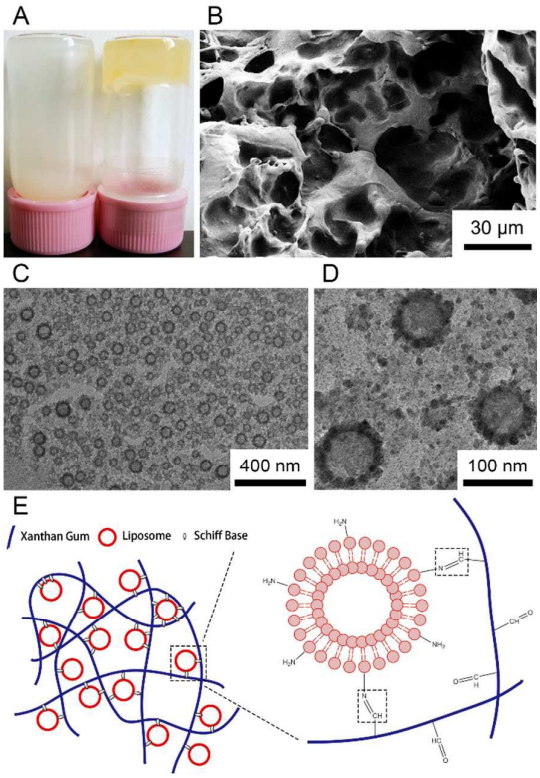

Fig. 1 (A) The prepared hydrogel (right, by mixing 20 mg/mL ALD-XA and 20 mg/mL PE) and the control sample (left, by mixing 20 mg/mL XA and 20 mg/mL PE). (B) SEM image of freeze-dried hydrogel sample. (C) A TEM image and (D) an enlarged TEM image of a hydrogel sample. (E) Schematic showing the xanthan gum-based liposome hydrogel crosslinked by Schiff base bonds.

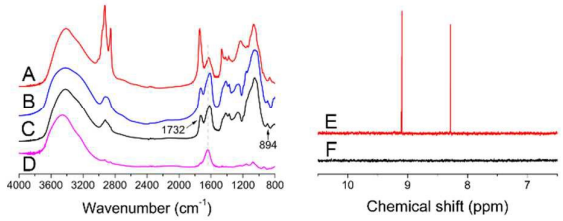

Fig. 2 FTIR spectra of PE (A), XA (B), ALD-XA (C), and xanthan gum-based liposome hydrogel (D) and ^{1}H NMR spectra of hydrogel (E) and XA (F).

respectively, as shown in Fig. 2E and Fig. 2F. In comparison to the ^{1}H NMR spectrum of XA (Fig. 2F), two new peaks appear at ~ 8.29 ppm and ~ 9.10 ppm (Fig. 2E), which can be assigned sequentially to the Schiff base protons and the unreacted aldehyde protons (–CHO).[55,56] Considering the dynamic chemical bonding feature of the Schiff base, this ^{1}H NMR characterization provides solid evidence that XA had been aldehyde functionalized to ALD-XA. More importantly, the gelation experiment of ALD-XA and carboxylated chitosan in the appropriate pH, as shown in Fig. S3†, confirms the presence of the aldehyde groups in ALD-XA since only ALD-XA can react with carboxylated chitosan to form the hydrogel.

When the ALD-XA solution and the PE liposome solution (each at 20 mg/mL concentration) were mixed with the same volume, the xanthan gum-based liposome hydrogel was formed within 5 min at room temperature, which can hold its own weight without any support, as shown in Fig. 1A (right). It was found that the concentration of the ALD-XA solution and the PE liposome solution did not significantly influence the gelation time. The hydrogel was constructed by the reaction between the aldehyde groups of ALD-XA and the amino groups of PE liposomes through Schiff base reaction, allowing for a dynamic crosslinking balance in the hydrogel network. (Later in this paper, we will provide evidence to show the liposomes still exist in the hydrogel.) XA solution was used as control to mix with the PE liposome solution under the same condition, resulting in no hydrogel formation as shown in Fig. 1A (left). We also found, when the PE concentration was lower than 10 mg/mL, the hydrogel was difficult to form because of the lack of sufficient linkages. When the PE concentration was higher than 30 mg/mL, the PE liposomes were difficult to form using the extrusion method. Therefore the best PE concentration used for the hydrogel formation is around 20 mg/mL.

Structural Characterization

The SEM image, as shown in Fig. 1B, illustrates the porous structure of a freeze-dried hydrogel sample. The pore diameters are in the range of 10–100 μm which have been suggested to be dependent on the length scale of ice crystal during freeze-drying.[34] The TEM image of the hydrogel sample (Fig. 1C) clearly shows the presence of the liposomes after the hydrogel formation. These liposomes are still spherical shaped and hold their membrane integrity, as revealed by the enlarged TEM image (Fig. 1D). In addition to this direct observation of the hydrogel network, we also collected FTIR spectra of the hydrogel and the control samples to identify the

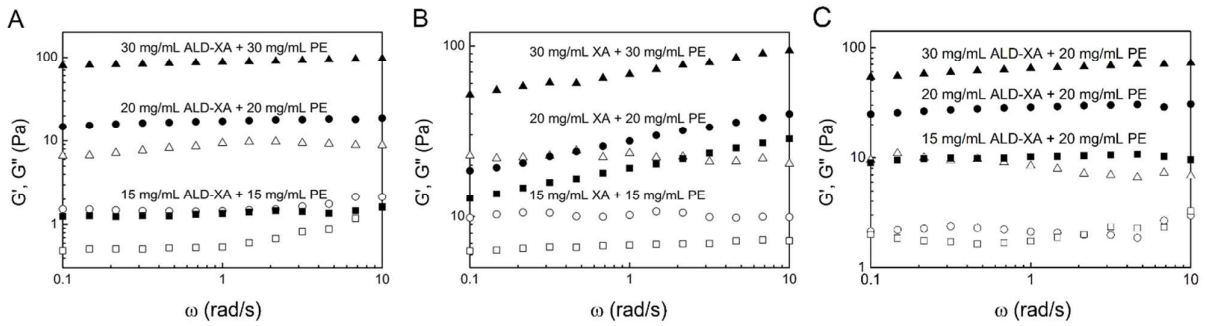

Fig. 3 Measured storage modulus *G'* (filled dots) and loss modulus *G"* (open dots) of the prepared hydrogel samples (A and C: ALD-XA + PE) and the control samples (B: XA + PE) at different reactant concentrations. Sweeps were performed with a fixed strain of 1% at 23 °C.

Schiff base crosslinkages in the hydrogel. The FTIR spectrum collected from the hydrogel sample shown in Fig. 2D does not have the peak from the aldehyde groups (ν(C=O), 1732 cm^{-1}) observed from the spectrum collected from ALD-XA (Fig. 2C). Instead, it has the vibrational signal from the imide bonds (ν(C=N), 1645 cm^{-1}) indicating the Schiff base formation. Corresponding to the FTIR spectra, ^{1}H NMR spectrum of the hydrogel sample mentioned above (Fig. 2E) has a new peak at ~ 8.29 ppm (–CH=N–)[55,56], suggesting the conjugation of xanthan gum-based liposomes via Schiff base bonds as well. As shown in the scheme of the hydrogel (Fig. 1E), the crosslinking was formed via the reaction between an aldehyde group in ALD-XA and an amino group distributed around the outer shell of a PE liposome. Once the Schiff base bonds were formed, the hydrogel network was established with the PE liposomes as "macroscopic" crosslinking points in the ALD-XA matrix.

Furthermore, the storage modulus *G'* and the loss modulus *G"* versus frequency were measured to characterize the viscoelastic property of the hydrogel materials. Three hydrogel samples were prepared with varied ALD-XA and PE liposome solution concentrations: 15 mg/mL ALD-XA + 15 mg/mL PE, 20 mg/mL ALD-XA + 20 mg/mL PE, and 30 mg/mL ALD-XA + 30 mg/mL PE. As expected, the storage modulus *G'* and the loss modulus *G"* level off during the sweeps from 0.1 to 10 rad/s, as shown in Fig. 3A, exhibiting typical elastic behaviour. In contrast, the storage modulus *G'* measured for the control samples (Fig. 3B) prepared by mixing XA and PE liposome solutions increases with increased frequency, showing the typical feature of a colloidal solution. This further confirms that the coupling reaction which occurred between the ALD-XA and PE liposomes in the solution is the key to generate hydrogel network. In addition, Fig. 3C shows, with a fixed typical PE concentration of 20 mg/mL, *G'* and *G"* values of the hydrogel samples increase as the concentration of ALD-XA increases, as a result of the higher crosslinking density.

Responsiveness Characterization

Owing to the Schiff base linkages, the hydrogel should show significant responses (sol-gel phase transition) to either pH variation or temperature change. As shown in Fig. 4A, when a HCl aqueous solution was added, the hydrogel sample (prepared by mixing 20 mg/mL ALD-XA and 20 mg/mL PE) was decomposed, changing from a gel to a sol state. After a NaOH aqueous solution was added, the hydrogel was regenerated. This must be attributed to the dynamic transition between the Schiff bases and the amino/aldehyde groups in the sample network when the pH value was varied.

Likewise, the hydrogel was liquefied when heated to 80 °C in a water bath and solidified again after cooled down to room temperature (Fig. 4B). Several cycles of the decomposition and regeneration process of a hydrogel sample upon the temperature change were monitored to check its durability. It was found that the hydrogel could tolerate the cyclic process for at least 10 times.

Moreover, Fig. 4C presents the results of hydrogel's responses to several biological molecules. Amino acids have amino groups that can react with the aldehyde groups to substitute the original Schiff base linkages.[11] Hence, histidine as a model amino acid was added to a hydrogel sample. The hydrogel material then degraded quickly. This indicates that the histidine amino groups compete with those in PE liposomes to react with the aldehyde groups in ALD-XA. We also know that certain enzymes, such as papain, are able to digest XA macromolecules into small oligomers. When a

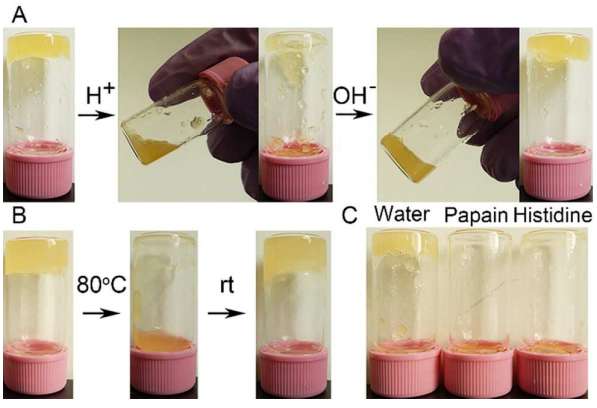

Fig. 4 Sol–gel phase transitions of hydrogel samples via different stimuli. (A) Response to pH variation of the hydrogel (left: original hydrogel; middle: hydrogel decomposition after addition of a HCl aqueous solution; right: hydrogel regeneration after addition of a NaOH aqueous solution). (B) Thermal response of a hydrogel sample (left: original hydrogel; middle: hydrogel decomposition after heated to 80 °C; right: hydrogel regeneration after cooled down to room temperature (rt)). (C) Response of the hydrogel to bio-stimuli under different conditions (left: water; middle: papain solution; right: histidine solution).

papain solution was added to a hydrogel sample, the hydrogel degraded substantially, indicating that the ALD-XA was digested by papain. The DLS data (Fig. S2B†) shows that the average hydrodynamic diameter of the liposomes released from the hydrogel was ~ 137 nm. This indicates that the lipid still exist in the liposome form in the hydrogel. If the liposomes collapsed in the hydrogel, the released lipid aggregation should have a much smaller average size (the DLS data do show some small quantity of smaller sized particles, perhaps indicating that some liposomes are destroyed, but the effect is minor). The average liposome size was slightly larger than that of the original liposomes (~ 121 nm), which may be contributed by the small amount of the digested ALD-XA residue coated on the liposomes. As a control experiment, a water sample with the same volume as the histidine/papain solution used above was added to a hydrogel sample. We did not observe any hydrogel degrading. Instead, the hydrogel swelled.

Self-healing Property

Fig. 5A clearly shows that the xanthan gum-based liposome hydrogel is syringe-injectable. When it was extruded onto a vertically positioned glass microscope slide, the hydrogel did not fall off from the slide surface (Fig. 5B), demonstrating the good integrity of the hydrogel, which can still hold its network structure. Additional experiments were carried out to verify the self-healing property of the prepared hydrogel. A hydrogel sample was injected into a water-containing vial and within 10 minutes a homogeneous hydrogel (similar to the original one) was regenerated (Fig. 5C). Besides, a hydrogel sample with a disc shape was cut into two pieces on a glass slide and separated. Then the two pieces were placed together along the cutting line at the ambient condition without any external stimulus. In 15 minutes, the two pieces gradually merged together and the cutting line became almost invisible at the end (Fig. 5D). This must be attributed to dynamic Schiff base bonds which can be formed, broken, and re-formed reversibly. When the two individual pieces were put into contact, dynamic Schiff base bonds were quickly formed in between, leading to the mergence of the two pieces. Therefore, the hydrogel not only is injectable but also has the self-healing property. This enables the possibility to use this hydrogel as a cell carrier material *in vivo*.[57,58]

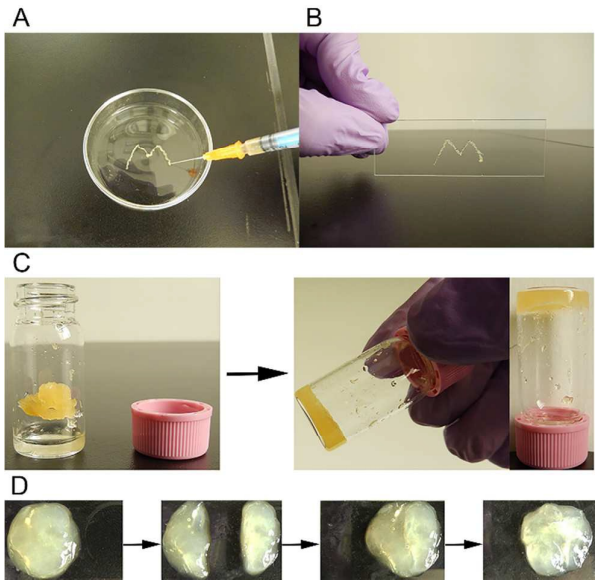

Fig. 5 Injectable and Self-healing property of the xanthan gum-based liposome hydrogels. A hydrogel sample delivered by a syringe onto a horizontally placed polystyrene petri dish (A) and a vertically placed glass plane (B). (C) Regeneration of hydrogel after injection into a vial containing water. (D) Two pieces of the hydrogel merged together into a homogenous sample.

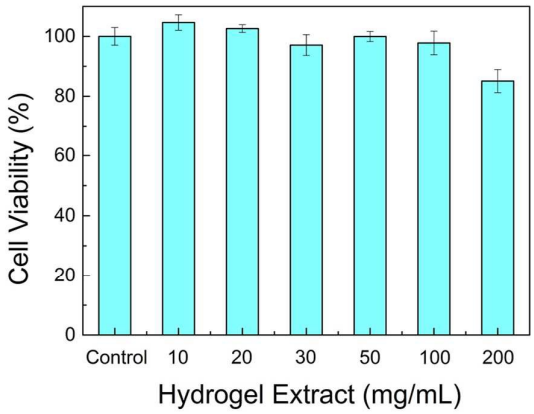

Fig. 6 Cytotoxicity evaluation of the hydrogel extract from the xanthan gum-based liposome hydrogels by MTT assay with MCF7 cells.

Carrier for 3D Cell Culturing

A material used for cell carriers should be biocompatible. Here, MCF7 cells were used to evaluate the cytotoxicity of the xanthan gum-based liposome hydrogel. The MTT assay result (Fig. 6) indicates that the cytotoxicity of the hydrogel starts to appear when the hydrogel extract reaches the concentration of 200 mg/mL, which is much higher than the concentrations needed (15 to 30 mg/mL) for the hydrogel preparation in this study. In other words, the cell viability remains great for the hydrogel prepared at normal concentrations. So our hydrogel shows the excellent biocompatible, forecasting its potential biomedical application.

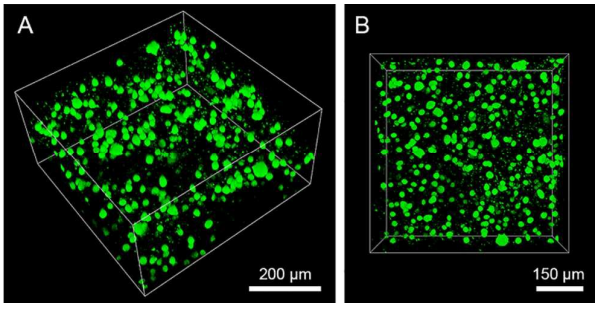

Fig. 7 3D (A) and z-axis maximum projection (B) views of confocal images of the FDA stained MCF7 cells encapsulated in the xanthan gum-based liposome hydrogel and incubated at 37 °C in 5% CO_2 for 48h.

To prove the hydrogel can be applied as a cell carrier material, MCF7 cells were suspended in the PE liposome solution and then mixed gently with ALD-XA dissolved in the PBS solution to induce the hydrogel formation. Fig. 7 is the confocal microscopic images of MCF7 cells in a hydrogel sample stained with the FDA reagent, elucidating the excellent viability of the cells encapsulated in the hydrogel. Almost all the cells were stained with FDA after they were cultured in the hydrogel for 48h. The survival of the encapsulated cells in such a long time is crucial for the hydrogel to serve as a cell carrier material which can be applied in the cell therapy and tissue engineering.

To the best of knowledge, it is the first time to crosslink liposomes to construct a hydrogel material, which presents injectable, biodegradable, biocompatible, self-healing and multi-responsive abilities. It is well known that liposomes prepared from phospholipids by themselves are excellent carriers/vehicles in biomedical engineering and pharmaceutics. As demonstrated in this research, the successful preparation of a hydrogel involving lipid liposomes may lead to more versatile applications of liposomes in the biomedical field. We will continue to study such hydrogel materials, developing their applications as excellent cell carrier and scaffold materials.

Conclusions

A method to prepare a novel xanthan gum-based liposome hydrogel was developed in an inexpensive and facile way by mixing ALD-XA and PE liposome solutions under the ambient condition. The hydrogel network was constructed via the reaction between the aldehyde groups of ALD-XA and the amino groups of PE liposomes. The formed dynamic Schiff base crosslinkages endowed the hydrogel with distinct multi-responsive and self-healing properties. Various stimuli, including physical stimulus (heat), chemical stimulus (pH variation), and biological stimulus (histidine exposure), could trigger the decomposition or/and regeneration of the hydrogel. Additionally, papain could biodegrade the hydrogel by cleaving the xanthan gum backbones. Syringe-injection and the cutting/merging experiments prove that the hydrogel is self-healable. Furthermore, MCF7 cells could be viable for a prolonged time in the hydrogel, suggesting that this novel xanthan gum-based liposome hydrogel can be applied as a cell carrier for the 3D culturing purpose. The biodegradability, injectability, multi-responsiveness, self-healing property, and biocompatibility have rendered this xanthan gum-based liposome hydrogel a broad range of applications in biomedical engineering, for examples as cell carrier/culturing scaffold. For the first time this research develops a new hydrogel which contains lipid liposomes. In the future we will carry out *in vivo* experiments using this promising hydrogel material.

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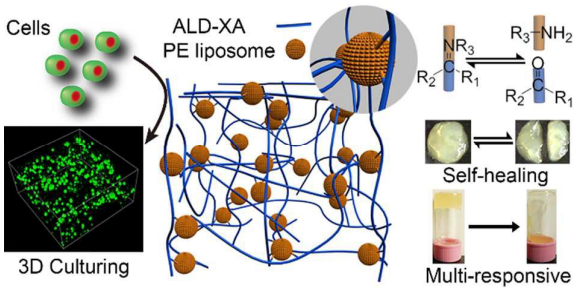
Graphical abstract



A biodegradable and injectable polymer–liposome hydrogel crosslinked via dynamic Schiff base bonding with distinct multi-responsive and self-healing properties can be applied as a promising cell carrier material.