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Supporting Information

Mussel-inspired Dopamine Oligomer Intercalated Tough and Resilient Gelatin methacryloyl (GelMA) Hydrogels for Cartilage Regeneration

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1. Experimental

Rheological test

Rheomrtric scientific HAAK (MARS, Germany) strain-controlled rheometer was used for dynamic rheological test. The hydrogels (D= 16 mm, H= 1.5 mm) were loaded into the plates (D= 20 mm) of the rheometer and allowed to relax until the normal force was 0. The linear viscoelasticity region was determined by strain amplitude sweeps (0.01-100%). To characterize the temperature dependence of the storage and loss modulus of GelMA hydrogel, GelMA hydrogels with different contents of ODMA and gelatin hydrogel were tested by oscillatory shear deformation with temperature scanning ranging from 25 to 40 °C (heating rate 2 °C/min) at constant frequency (1 Hz) and constant shear strain (1 %). The dynamic frequency sweeps with the angular velocities ranging from 0.01 to 10 Hz at 1 % strain amplitude (liner region) were also performed.

Cell culture

Bone marrow stem cells (BMSCs) were extracted from 7-day-old SD rats' femurs. BMSCs were seeded in culture flasks at 10⁶ cells/cm² and incubated in cell incubator (37 °C, 5% CO₂), and the medium (α -MEM + 10% FBS + 1% Penicillin-Streptomycin Solution) were exchanged every 2-3 days. Then, the BMSCs were seeded on the GelMA hydrogels, ODMA-GelMA hydrogels, CS (0.05g/per sample) loaded ODMA-GelMA hydrogels, and TGF- β_3 loaded ODMA-GelMA hydrogels with the density of 5 × 104 cells/ml. After 3 and 7 days. The cell proliferation was measured with MTT assay. And the cell immediately stained with Calcein AM and Ethidium homodimer in PBS solution and incubated for 30 minutes. Next, they were washed 3 times with PBS solution to remove the background fluorescence, and then visualized under a fluorescent microscope (CLSM TCSSP5, Leica, Germany).

Chondrogenic differentiation

BMSCs (2.5×10^5 cells) were added to the hydrogels allowed to adhere to the hydrogel in 24well cell culture cluster for 4 h. Then 1.5 ml of serum free chondrogenic medium (highglucose DMEM, 1% ITS+Premix, 50 µg/ml ascorbic acid, 100 µg/ml sodium pyruvate, 100 nM dexamethasone, 40 µg/ml L-proline) were added into the cell culture plates. And half of the medium were replenished 2 to 3 times a week. These cells were harvested after 14 days of chondrogenic culture and the GAG content and the protein expression content of type-II collagen were evaluated by a GAG and type-II collagen Elisa kit (MEIMIAN, Jiangsu, china) per the manufacturer's instruction.

¹H NMR analysis of DMA and GelMA



Fig. S1. ¹H NMR of (a) DMA and (b) GelMA

¹H NMR was used to characterize the chemical structure of the ODMA-GELMA hydrogel. As shown in Figure S1a, the NMR spectrum exhibits the characteristic resonance signals of catechol hydroxyl groups (8.85 ppm), phenyl protons (6.7 and 6.5 ppm), methylene protons (3.4 and 2.6 ppm), and alkenyl hydrogens (5.7 and 5.3 ppm), all of which are consistent with the corresponding chemical shifts of the DMA groups. The number of methacrylamide grafts on the GelMA was around 5.5 ppm (figure S1b). This result indicates that the methacrylamide groups were successfully grafted onto the GelMA and dopamine. Thus, chemical bonds can form during the polymerization process.

Electrospray ionization mass spectrometry (ESI-MS) analysis of ODMA

The ESI-MS experiments were performed using an Agilent 7890B-5977X gas chromatograph equipped with a mass spectrometry detector. This instrument was equipped with Z-spray source alignment operating at a capillary voltage of 3.5 kV, a desolvation temperature of 150 °C, and a sample flow rate of 10 μ l·min⁻¹. The mass spectra for all the samples were recorded at mass-to-charge ratios (m/z) of 100–1000.

The ESI-MS spectra of Tris-DMA exhibit strong peaks at an m/z value of 220.1, which is attributed to the DMA monomer. The mass peak at an m/z value of 441.2 represents an oxidized form of the ODMA dimer complexes. The ESI-MS results confirmed the oxidization of DMA into low-molecular-weight oligomers under a Tris-HCl solution.



Fig.S2 ESI-MS spectra of Tris-DMA

Density of functional theory (DFT) study

a density of functional theory (DFT) study was carried out in an attempt to gain further insight into the presence of non-covalent bonds between the ODMA and GelMA. The simulation was performed using the DMol3 DFT program in Material Studio (Accelrys, San Diego, CA)^{1, 2}. The physical wave functions were expanded in terms of numerical basis sets (DNP version 3.5), which were comparable with 6–31G** basis sets. The core electrons were treated with DFT semi-core pseudo potentials³. The exchange-correlation energy was calculated from the Perdew-Burke-Ernzerhof (PBE) generalized gradient approximation (GGA)⁴. A Fermi smearing of 0.005 Ha (1 Ha = 27.211 eV) and a global orbital cutoff of 5.2 Å were employed. The convergence criteria for the geometric optimization and energy calculation were set as follows: (a) a self-consistent field tolerance of 1.0×10^{-6} Ha/atom; (b) an energy tolerance of 1.0×10^{-5} Ha/atom; (c) a maximum force tolerance of 0.002 Ha/Å; (d) a maximum displacement tolerance of 0.005 Å.

Interaction energy calculation

The interaction energy (E_{int}) , indicating the intensity of the interaction between the components in the system, was derived from the following equation:

$$E_{int} = E_{total} - \sum E_{component}$$
(S1)

where E_{total} and $E_{component}$ represent the total energy of the system and the energy of each component in the system, respectively. A negative E_{int} value indicates a stable attraction between the components. A more negative E_{int} value indicates a stronger interaction in the system.

DMA monomers and the RGD residue in the GelMA chain were chosen as targets to study the interactions between GelMA and DMA, as shown in Figure S3 and Table S1. An analysis of the interaction energy suggested that ODMA had a stronger interaction (hydrogen bonding) with GelMA (-3.9386 eV, -0.5522 eV) than GelMA (-0.1659 eV) (Table S1). This is attributed to the presence of a catechol group in the DMA, which can form stable hydrogen bonds with the GelMA. As shown in Fig. R4, the DMA interacts with the GelMA through the catechol groups of DMA and the -NH2, -COOH groups of the GelMA chains by hydrogen bonding.



Fig. S3 (a) RGD-RGD interaction is though -NH2, -COOH of RGD, (b) ODMA-RGD, -OH interact with the -NH2 of RGD (c) ODMA-RGD, -OH interact with the -COOH of RGD.

Model	Interaction energy (eV)
RGD-RGD (-NH2, -COOH)	-0.1659
ODMA-RGD (-OH, -NH2)	-3.9386
ODMA-RGD (-OH, -COOH)	-0.5522

Table S1. Interaction energy between ODMA and RGD



Fig. S4 (a) The GelMA hydrogel and PDA incorporated GelMA hydrogel were broken after compression. (b) GelMA was fractured after twisting. (c) GelMA was broken after bending.



Fig. S5 Rheological properties of the hydrogels. (a) The temperature dependence of the storage and loss modulus of $ODMA_{0.05}$ -GelMA hydrogel. (b) GelMA hydrogel. (c) Gel hydrogel. (d) The frequency dependence of the hydrogels.



Figure S6. Compression stress-strain curve (a) and compression modulus (b) of hydrogels.



Fig. S7 The swelling ratio of ODMA-GelMA hydrogel with different concentration of ODMA.



Fig. S8 Degradation of the ODMA-GelMA hydrogel with different concentration of ODMA.



Fig. S9 ALP release from the ODMA-GelMA hydrogel with different concentration of ODMA.

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