International Journal of Bioprinting

RESEARCH ARTICLE

A biocompatible double-crosslinked gelatin/sodium alginate/dopamine/quaterniazed chitosan hydrogel for wound dressings based on 3D bioprinting technology

Supplementary File

S1. Supplement to the preparation of the Gel/Alg/QCS (GAQ) hybrid bioinks

Biological inks with different concentrations of QCS were characterized, as shown in **Figure S1**. GAQ0%, GAQ 0.5%, GAQ1.0%, and GAQ1.5% were all homogeneous viscous fluids at 25°C, but QCS in GAQ2% is not completely dissolved, so we set the maximum concentration of QCS as 1.5%.

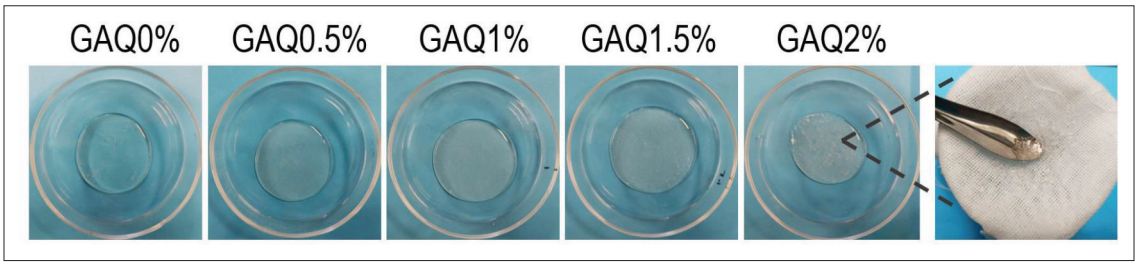

Figure S1. GAQ bioink with different concentrations of QCS.

S2. Degradation test of the GADQ hydrogels

Identical volumes of hydrogels were prepared for *in vitro* degradation experiments. Then, lysozyme solution (1.5 μg/mL lysozyme, 0.03 μg/mL collagenase, 1× PBS [pH 7.4]) was added to centrifuge tubes containing hydrogels, and cultured in an incubator at 37°C[1,2]. At the interval time point, hydrogels were taken out, washed with deionized water to remove lysozyme and salt components, and then freeze-dried and weighed[3]. The degradation of hydrogel can be calculated by the following formula:

$$\text{Degradation ratio (\%)} = (m_0\text{-}m_a)/m_0 \times 100\%$$

Where m_0 and m_a represent the initial mass of the hydrogel and the final mass after freeze-drying, respectively.

S3. Degradation analysis of the GADQ hydrogels

The stability of hydrogel dressings plays an important role in wound healing. The presence of alkaline enzyme lysozyme in the blood had a certain degree of hydrolysis of polysaccharides, so PBS containing 1.5 μg/mL lysozyme was used to simulate human blood environment to evaluate the stability of hydrogels[4]. In the degradation process of days 1, 3, 5, and 7, compared with other groups, the degradation rate of GADQ0% was the lowest and the stability was the best (**Figure S3**). The other groups showed more degradation due to the addition of QCS. Fortunately, due to the existence of double-crosslinked network, the hydrogel structure was denser, which delayed the degradation process of hydrogel. Even after 7 days, the degradation rate of GADQ1.5% was still lower than 25%, and its structure remained relatively intact. Therefore, the stability of GADQ1.5% hydrogel can be guaranteed.

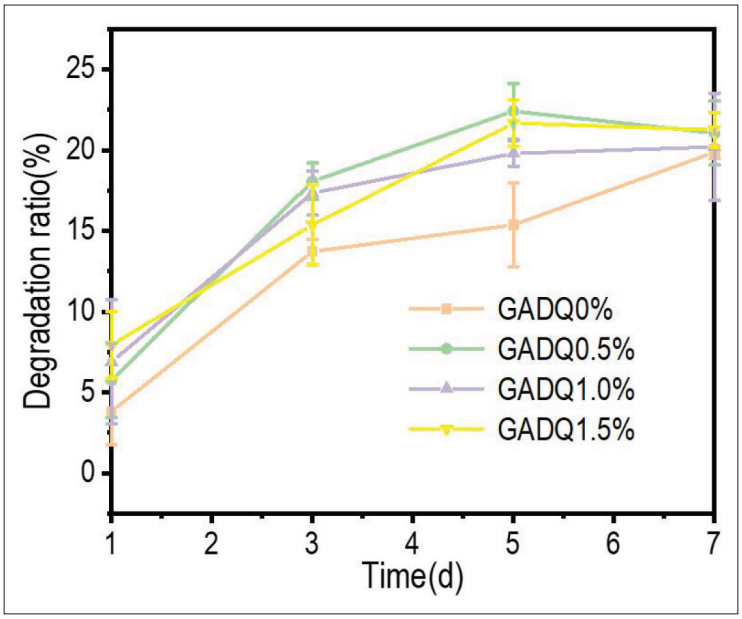

Figure S3. Degradation of four hydrogels at 37°C.

S4. Determination of bacterial concentration

The bacterial suspension was diluted to a certain concentration gradient and counted by plate coating method. At the same time, the optical density of bacterial suspensions with different concentrations was measured at the wavelength of 620 nm by using a microplate reader (INFINITE F50, Tecan, Austria)[5]. Therefore, the OD values corresponded to the bacterial concentrations were used to establish a standard curve, as shown in the **Figure S4**. The bacterial suspension concentration in the study could be calculated by measuring its OD value.

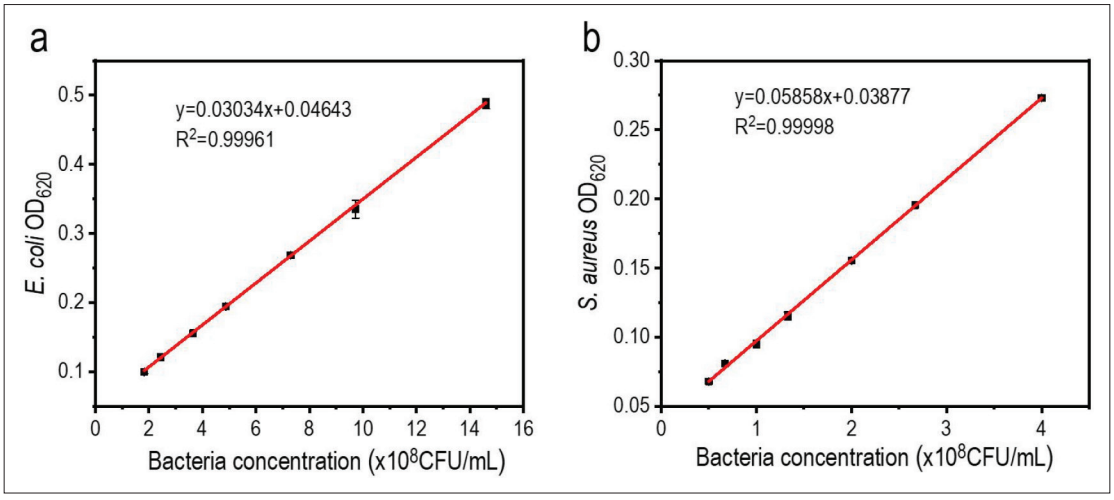

Figure S4. Standard curve of concentration and optical density value of *E. coli* and *S. aureus*.

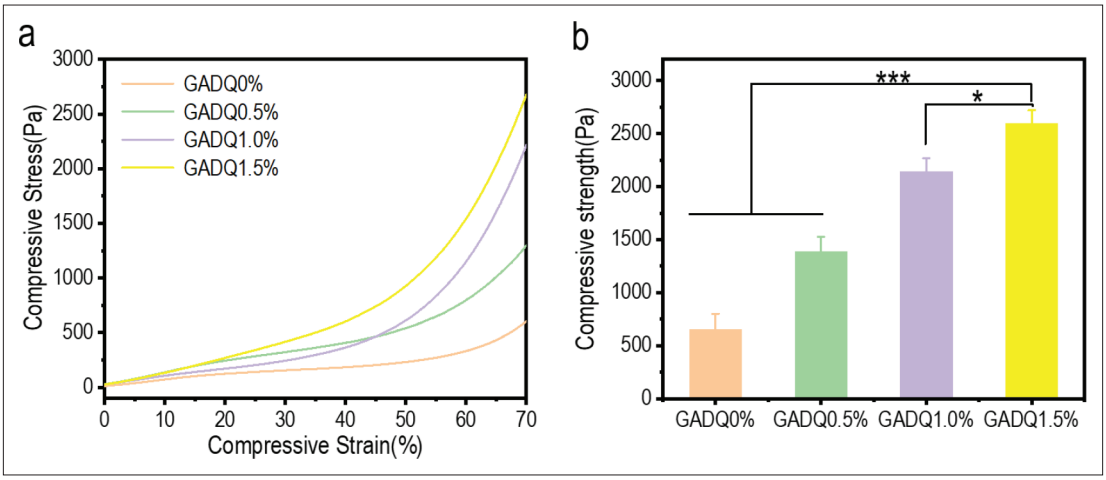

Figure S6. Compression characterization. (a) Compression stress strain curves of the GADQ hydrogels at strains from 0% to 70%. (b) Compressive strength of the GADQ hydrogels at strains of 70%.

S5. Compression test of the GADQ hydrogels

The compression mechanics of hydrogels can be tested by the universal mechanical testing machine (CMT4304). Test samples (10 mm × 10 mm × 4 mm) were immersed in deionized water until fully swollen. The compression rate was set to 1 mm/min, and the compression strength was the stress value when the compression strain reached 70%[6,7].

S6. Compression analysis of the GADQ hydrogels

In addition to the tensile tests of hydrogels, the compression properties of hydrogels were also characterized to further illustrate their mechanical properties. As shown in **Figure S6a** and **b**, the compressive strength of GADQ1.5% hydrogel at 70% strain (2595.75 ± 126.03 Pa) was much higher than that of GADQ0% (653.86 ± 145.83 pa) and GADQ0.5% (1392.56 ± 132.94 pa). At the same time, it was significantly different from that of GADQ1.0% (2146.83 ± 123.12 pa). The result showed that with the increase of QCS concentration, the compression resistance of hydrogel was improved. When more QCS participate in the construction of the hydrogel network, a denser crosslinking network was formed, and the hydrogel support performance was further enhanced[8]. Overall, GADQ1.5% had superior mechanical properties for wound dressings.

References

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