Supporting Information

Oxidized chondroitin sulfate-crosslinked and CuCDs-loaded decellularized bovine pericardium with improved anti-coagulation, pro-endothelialization and anti-calcification properties for BHVs

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Methods

1. Decellularization of bovine pericardium

Bovine pericardium (BP) was obtained at the slaughterhouse, immediately stored in sterile PBS, and transported to the laboratory at 4 ℃. The decellularization protocol of the fresh BP used in this study was carried out according to the previously reported method. Briefly, the pericardia were incubated on an orbital shaker with 1% (w/v) sodium dodecyl sulfate (SDS) at room temperature for 6 h, which was followed by 1% (v/v) Triton X-100 incubation for 30 min. After being thoroughly washed with phosphate-buffered solution (PBS), the samples were then treated with 2 U mL^{-1} deoxyribonuclease (DNase grade II) and 20 mg/mL ribonuclease (RNase) overnight at 37 °C with gentle shaking. The decellularized BP (denoted as DBP) was stored in glycerin at -20 ℃ for future experiments.

2. Stability characterization of CuCDs

The stability of CuCDs was evaluated at different pH conditions (pH=4-10). Solutions of CuCDs with different pH were prepared and the UV absorption spectra of various solutions were determined by UV-vis spectrophotometer and the fluorescence emission spectra by fluorescence spectrometer.

3. Determination of the amine content

The free amine content of the cross-linked BP samples was determined using the ninhydrin (NHN) assay. Briefly, the cross-linked BP samples (0.5 × 0.5 cm^2) were dropped into 1 mL of ninhydrin solution, heated to 100 °C, and maintained for 20 min. Subsequently, the absorbance of various sample solutions at 570 nm was measured using a spectrophotometer. And the amount of free amino groups in samples is proportional to the optical absorbance of the solution.

4. Determination of the carboxyl group content

-COOH group content was determined by the toluidine blue-O (TBO) method. Briefly, 5 × 10^{-3} M TBO (Sigma-Aldrich) was dissolved in NaOH solution (0.1 mM, pH = 10). BP samples (1 × 1 cm^2) were immersed in 24-well plates containing 1 ml of TBO solution and incubated for 12 h at room temperature. The samples were washed several times with deionized water after removing the supernatant from the sample surface. Subsequently, 1 ml of 50 wt% acetic acid was added to elute the TBO dye from the samples. Finally, 150 μl of TBO eluate from each well was transferred to a 96-well plate and the absorbance of eluate was measured at 630 nm using a microplate reader. And the amount of the carboxyl group in samples is proportional to the optical absorbance of eluate.

Results and Discussion

Stability characterization of CuCDs

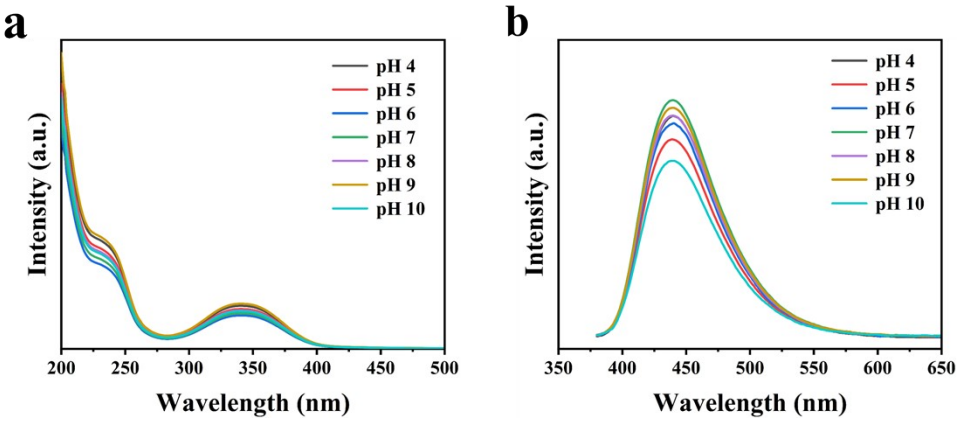

Figure S1 CuCDs stability a) UV-vis of CuCDs at different pH conditions; b) fluorescence emission spectra of CuCDs at different pH conditions

The stability of CuCDs solutions was tested at different pH conditions. The UV-vis results (**Figure S1a**) indicated that the carbon dots showed consistent structural stability in various pH solutions, and the materials had distinct absorption bands around 229 nm and 342 nm. Scanning with an excitation wavelength of 365 nm, the maximum emission wavelength of CuCDs was observed to be about 438 nm under different pH conditions (**Figure S1b**). The above structures indicate that our prepared CuCDs have great stability in structural and optical properties.

Quantum yield (QY) of CuCDs

Table S1 QY of the CuCDs dispersed in distilled water.

Samples	Integrated emission intensity (I)	Abs. (A)	Refractive index of solvent (n)	QY(φ)
Quinine sulfate	1261277591	0.0826	1.334	0.53
CuCDs	274123658	0.0425	1.334	0.41

Determination of the amine content

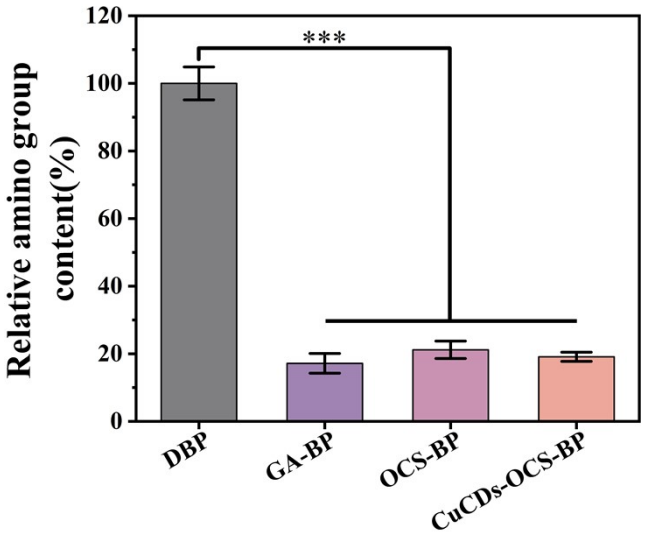

Figure S2 Relative amino group content remaining within the cross-linked BP samples

The amino group of pericardium reacted with the aldehyde group on OCS to form a cross-linking network, thereby depleting the amino group. The result of residual relative amine content of different BP samples was shown in **Figure S2**, and the residual amine content of OCS-BP was slightly higher than that of GA-BP, which was due to the larger molecular weight and steric hindrance of OCS. The addition of CuCDs had no significant effect on the residual amine content. DBP indicated the decellularized BP and GA-BP indicated the glutaraldehyde-fixed BP.

Determination of the carboxyl group content

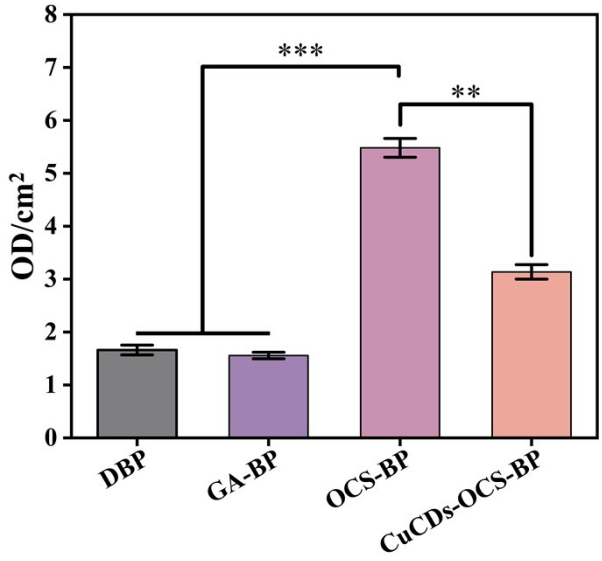

Figure S3 The carboxyl group content in the cross-linked BP samples

Toluidine blue-O (TBO) was used to determine the carboxyl group content in the samples, and the higher OD value indicated the higher carboxyl group content. As shown in **Figure S3**, the OD value of the OCS-BP group was obviously increased, which was due to the presence of unreacted carboxyl and sulphonic groups on OCS. However, the carboxyl group content of CuCDs-OCS-BP group was significantly lower compared with that of OCS-BP group, indicating that the carboxyl group of OCS-BP was activated by EDS/NHS, and the CuCDs were successfully coupled to OCS-BP, which consumed the carboxyl group.

FTIR spectra of BP samples

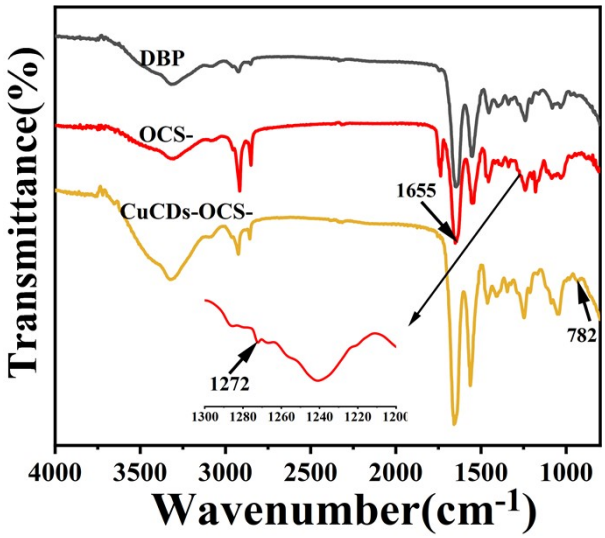

Figure S4 FTIR spectra of BP samples

Figure S4 showed the FTIR spectra of various BP samples, on the spectra of OCS-fixed BP, a new peak at 1655 cm^{-1} indicated the formation of imine bonds. Additionally, a new peak emerged at 1272 cm^{-1}, which was attributed to the stretching vibration of the S=O bond in OCS. Furthermore, a weak new peak at 782 cm^{-1} was observed in the CuCDs-OCS-BP spectrum, corresponding to the N-Cu-N bond in CuCDs, confirming the successful load of CuCDs on the sample surface.

DSC curve of BP samples

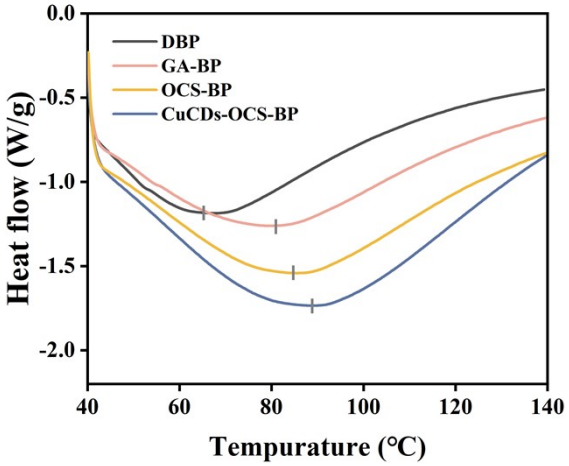

Figure S5 DSC curve of BP samples

Quantitative analysis of protein adhesion

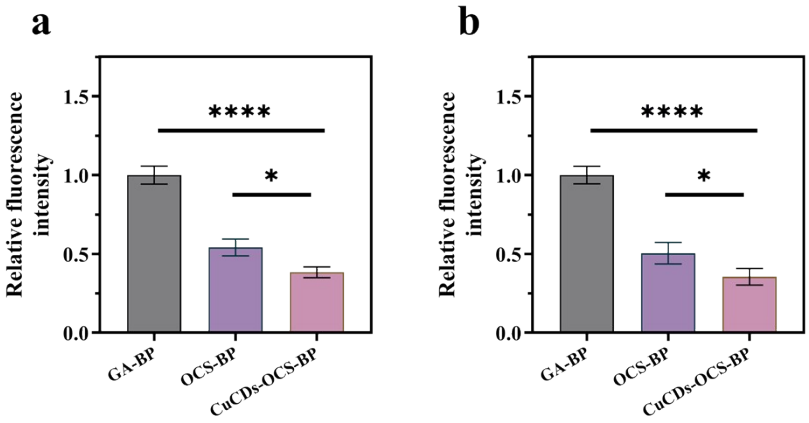

Figure S6 Quantitative analysis of relative fluorescence intensity reflected by proteins adsorbed on different samples: a) BSA-FITC and b) FBG-FITC adsorbed on different BP samples.

Quantitative analysis of fluorescence intensity revealed (**Figure S6**) that CuCDs-OCS-BP presented the lowest fluorescence intensity, indicating the strongest resistance of CuCDs-OCS-BP to protein adsorption.

In vivo immunohistochemical analysis

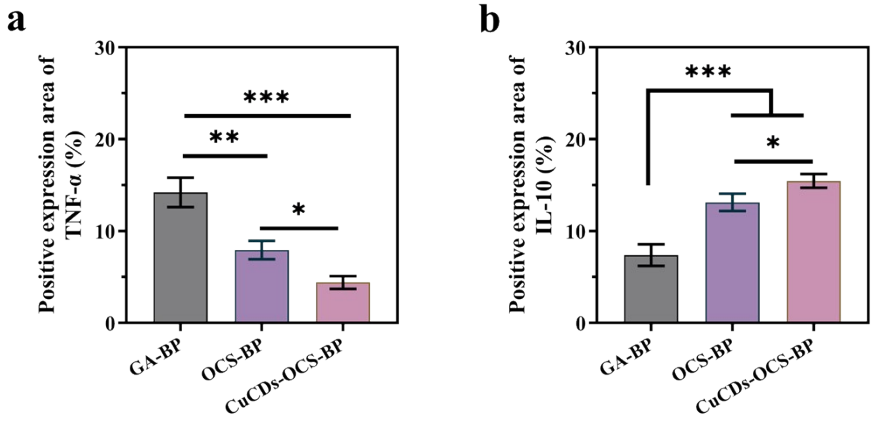

Figure S7 The positive expression area of TNF-α and IL-10 in samples at 28 days.

Positive expression analysis of TNF-α and IL-10 *in vivo* at 28 days after implantation (**Figure S7**) showed that TNF-α was most prominently positively expressed in GA-BP and most weakly expressed in CuCDs-OCS-BP; conversely, IL-10 was most weakly expressed in GA-BP and most strongly positively expressed in CuCDs-OCS-BP at 28 days. This suggested that the strongest inflammatory response was triggered by GA-BP, while the inflammatory response triggered by CuCDs-OCS-BP was the mildest.