Supporting Information

Ciprofloxacin-loaded Bioadhesive Hydrogels for Ocular Applications

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S1. Materials and methods

S1.1. Characterization of CPX-loaded MCs

Particle size (PS), polydispersity index (PDI), and Zeta Potential were determined using a dynamic light scattering analyzer (Nano-ZS90 Zetasizer, Malvern Instruments, UK). Diluted samples in DPBS were used at room temperature in triplicates. Entrapment efficiency was calculated (n = 3) as the percentage of CPX loaded relative to the initial total amount of drug used for the formulation by measuring drug concentration at 277 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650 Spectrophotometer, Japan). Drug loading was calculated by dividing the entrapped amount of CPX on the total amount of MCs. Morphological characterization was performed using a scanning electron microscope (SEM) (Hitachi S-4800 scanning electron microscope). Differential scanning calorimetry (DSC) analysis (Q20, TA instrument, MA, USA) was performed to evaluate thermal properties of different nanocarriers up to 400°C at a rate of 10 °C/min under a nitrogen atmosphere (25 ml/min). 

S1.2. In vitro release study of CPX-loaded MCs

The in vitro release of free CPX and CPX-loaded MCs in DPBS was determined by using a dialysis membrane technique. A 0.05% Tween 20 solution in DPBS (pH 7.4) was used to mimic ocular surface environment pH and to maintain sink conditions during the experiments. Briefly, release studies were performed at 37.0 ± 0.5°C in triplicate. Free CPX and CPX-loaded MCs were suspended in release media at equivalent drug concentrations (2mg/mL) and transferred into donor compartments covered with dialysis membranes with molecular weight cutoff (MWCO) of 10-12 kDa. Donor compartments were immersed in 10 ml of release media (acceptor compartment). At pre-defined time intervals, 500 µl aliquots were withdrawn from the acceptor compartment. The withdrawn samples were replaced with equal volumes of fresh release media. Released CPX was quantified at 277 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650 Spectrophotometer, Japan) with a pre-validated calibration curve and reported as cumulative released amount of CPX, reported in percentage, using the following equation:

\[ C_n = C_n \text{ meas} + \frac{A}{V} \sum_{s=1}^{n-1} C_s \text{ meas} \]  

(5)
Where, \( C_n \) is the accumulated sample’s concentration, \( C_n \) meas is the estimated concentration, \( A \) is the aliquot volume, \( V \) is the released medium volume, \( n-1 \) is the overall volume of all previous aliquots, and \( C_s \) meas is the sum of concentrations of all previous samples. The release profile kinetics model was performed using a standard kinetics model as described by Costa et al. 4.

**S1.3. In vitro antimicrobial study on CPX-loaded MCs**

Minimum inhibitory concentrations (MICs) for both *Staphylococcus aureus* (ATCC® 29213™) and *Pseudomonas aeruginosa* (ATCC® 15692™) were determined by a microdilution test 5. MIC is the lowest antimicrobial concentration inhibiting microorganism growth after incubating the drug with microorganisms. 100 µl of bacterial inocula was transferred into tubes and incubated for 24 h at 37°C. In 96-well Cell Culture Clusters, different wells with various CPX and CPX-loaded MCs concentrations were prepared in triplicate for each concentration (14–0.014 µg/ml). A 150 µl solution of the microorganism suspension (1×10^6 CFU/ml; CFU: colony forming units) was added to 50 µl of drug suspension and turbidity was inspected for 12 h at 562 nm using a spectrophotometer (SpectraMax M3, Molecular Devices, Sunnyvale, CA). Controls for microorganism growth were established through preparation of samples with and without bacterial inoculation.

**S1.4. ¹H NMR analysis**

Proton nuclear magnetic resonance (¹H NMR) was used to confirm methacrylation of gelatin as described in previous studies 6,7. Briefly, gelatin and GelMA were dissolved in deuterium oxide (D₂O, 99.9 atom % D). ¹H NMR spectra were obtained using a Varian Inova-500 NMR spectrometer at 500 MHz. The peaks were identified at \( \delta=1.8 \) ppm, 2.8 ppm, 5.3 ppm, 5.7 ppm, and 5.9 ppm to confirm the conversion of gelatin to GelMA.

**S1.5. Mechanical characterization of MCs-loaded GelCORE hydrogels**

The mechanical properties of the MCs-loaded GelCORE hydrogels were measured to investigate the effect of CPX MCs loading on the bioadhesive. Both compression and tensile tests were conducted using an Instron 5542 mechanical tester as described before 8. For the tensile test, hydrogel samples with and without MCs were placed between two pieces of double-sided tape within the instrument tension grips and extended at a rate of 1 mm/min until failure. The slope of the stress-strain curves was calculated and reported as elastic modulus from the first 5-10% of the
curve. Extensibility and ultimate stress were determined from stress-strain curves by extrapolate the maximum point of the curve at y-axis for ultimate stress and for x-axis for extensibility. For compressive experiments, hydrogel samples were placed in compressive plates immersed in a DPBS bath. The compression test was conducted at a rate of 1 mm/min. The values for compressive strain (in millimeters) and load (in newtons) were plotted and the modulus was reported based at strain level. For both tests, at least 3 samples were tested per conditions.

**S1.6. Swelling and Degradation characterization of MCs-loaded GelCORE hydrogels**

Swelling ratios of the bioadhesive hydrogels were determined by incubating the hydrogels in DPBS at 37°C for 24 h. Hydrogel samples were prepared as described previously and then lyophilled to measure their dry weight. At different time points after DPBS incubation, samples were weighed after removing excess water (wet weight). The difference in dry weight and wet weight represented the swelling ratio (n=3).

To assess the degradation rate, hydrogel samples were lyophilized, weighed, and incubated in DPBS solution at 37 °C for one week. The DPBS solutions were changed every three days. At each time point, samples were lyophilized and weighed, where the weight difference from initial weight divided by initial weight represented the degradation percentage (n=3).

**S1.7. Adhesion tests on MCs-loaded GelCORE hydrogels**

**Burst pressure test.** To study the adhesive properties of hydrogels, burst pressure resistance was measured using ASTM standard F2392-04 according to a previously reported method. Briefly, collagen sheets or corneal tissue (4 × 4 cm) was placed in between two custom-built stainless-steel apparatuses including stainless steel rings with 10-mm diameter holes connected to a metallic base holder, a pressure sensor, a syringe pressure setup, and data collector. A 1-mm diameter hole was created in the collagen sheet using an 18-gauge syringe needle and sealed by injecting 30 µL of precursor solution followed by photo-crosslinking using visible light. Airflow was applied to the hydrogel until rupturing was observed and the burst pressure resistance was measured using a wireless pressure sensor (Pasco) connected to a computer (n ≥ 3).

**Burst pressure test on rabbit eyeballs.** A modified burst pressure test was conducted using defrosted New Zealand rabbit eyes obtained from Pel-Freez Biologicals. Different sized full-thickness incisions (2, 4, 6, and 8 mm) in rabbit corneas were tested after sealing with the
photocrosslinked hydrogels as explained prior. Next, the sealed eyes were connected to the burst pressure testing system, where a syringe pump applied air into the eye anterior chamber until increasing pressure caused the hydrogel to burst, while a pressure detector recorded the corresponding pressure. The burst pressure was reported as the highest recorded pressure (n=3).

**Wound closure test.** The adhesion strength of different hydrogels was measured by using a modified lap shear test according to ASTM standard F2458-05 as reported previously. Fresh porcine skin (10mm×15mm), hydrated with DPBS, was used as a model skin tissue after removing excess fat. The tissues were fixed onto the edge of two glass slides (30 mm × 60 mm) by cyanoacrylate glue. An incision was made in the middle of the tissue using a straight edge blade. 30 µL of prepolymer solution was then added onto the tissue interface followed by photocrosslinking using visible light. Maximum adhesive strength of each sample was obtained at the tearing point at a strain rate of 1 mm/min using an Instron mechanical tester (n ≥ 3).
S2. Results

Supplementary Figure 1. Development of GelCORE+MCs bioadhesives: (a) Synthesis and photocrosslinking process for the synthesis of GelCORE, (b) $^1$H-NMR-spectra in D$_2$O of gelatin and methacryloyl gelatin (GelMA) indicating addition of peaks at $\delta=5.3$ ppm, 5.7 ppm, and 5.9 ppm corresponding to C=O in the structure of methacrylate and methacrylamide groups, confirming the conversion of gelatin to GelMA. Peaks verification: 

1. Peaks corresponding to methylene unreacted lysine protons (2H) around $\delta=2.8$ ppm, Peaks correspond to acrylic protons (2H) at $\delta=5.3$ ppm and (3) for methacrylamide grafts of lysine groups at $\delta=5.7$ ppm, (4) methyl protons (3H) of methacrylamide grafts at $\delta=1.8$ ppm, (5) acrylic protons (2H) of hydroxyl groups of methacrylated grafts at $\delta=5.9$ ppm, and (6) methyl protons (3H) of hydroxyl groups of methacrylated grafts at $\delta=1.8$ ppm. Representative scanning electron microscope images of hydrogel cross-section. (c) Representative SEM imaged from GelCORE, and (d) GelCORE+MCs, showing that incorporation of MSc had no effects on the porous microstructure of the bioadhesives (scale bar =100$\mu$m).
Supplementary Figure 2. *In vitro* characterization of MCs-loaded GelCORE hydrogels. Compressive modulus of GelCORE and GelCORE+MCs.
References


