Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B This journal is © The Royal Society of Chemistry 2013

J. Zhang, et al. "Nanocomposite Contact Lens for Protein Drug Delivery"

Supporting Information

1. The light transmission of gelatin NPs-grating p(HEMA-co-AMEA) were measured using a spectrophotometer with three wavelengths.

Wavelength		White light	450 nm	550 nm
Samples		_		
p(HEMA-co-AEMA)		93.2 ±1.0 (%)	93.2 ±1.0 (%)	93.2±1.0 (%)
Gelatin NPs	1:200	85.3 ±2.7 (%)	85.3 ±2.6 (%)	85.2 ±2.8 (%)
vs. p(HEMA-	1:1000	90.5 ±1.3 (%)	90.4 ±1.3 (%)	90.5 ±1.3 (%)
co-AEMA)	1:5000	93.2 ±1.0 (%)	93.1 ±1.0 (%)	93.2 ±1.0 (%)

Tuble of a Light Transmission of the nanocomposite oused tens	Table S-1	Light Tr	ansmission	of the	nanocom	posite-based	lens
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To maintain suitable light transmission, we focus on the study of the nanocomposite-based lens materials when the weight ratio of gelatin NPs to p(HEMA-co-AEMA) is 1:1000.

2. Calibration curve



Figure S-1. (a) UV-absorption spectra of different concentrations of BSA. (b) Standard curve of BSA vs. intensity of UV absorption.

3. The released BSA from gelatin NPs-grafting p(HEMA-co-AEMA) gels with different weight ratio: 1:200, 1:1000, 1:5000 were monitored for 12 days (supporting information). There is a slight difference in release kinetics among the release systems of the gelatin NPs-grafting p(HEMA-co-AEMA) with different ratios.

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Figure S-2. Release profile of nanocomposite-based lens materials with different loading rate of gelatin NPs.

4. Bioactivity study of released globular protein from gelatin NPs and gelatin NPs-grafted p(HEMA-co-AEMA)

In addition to determine the activity of the peptide bonds of BSA through a protein assay described in the manuscript, we also studied the bioactivity of another different globular protein, basic Fibroblast Growth Factors (bFGF), released from the gelatin NPs and the gelatin NPs- grafted p(HEMA-co-AEMA) using the same process. The release profiles of bFGF from gelatin NPs and gelatin grafted-p(HEMA-co-AEMA) were studied, respectively. Here, we would like to show that the released bFGF reserve the bioactivity in the MAPkinase assay.

MAPKinase or Mitogen Activated protein kinase pathway is activated by extracellular binding of bFGF. The cascade reactions of the pathway in a cell, due to the stimulation of bFGF, lead to to phophorylation of a MAP kinase enzyme ERK [1]. The key elements, ERK and phophorylated ERK proteins (pERK) of this cascade mechanism can be exploited to study the bioactivity of bFGF in the living system.

Our designed assay involves the exposure of endothelial cell lines to released bFGF from the gelatin NPs and gelatin NPs-grafted p(HEMA-co-AEMA) when the release time (t) is 5 days and 10 days, respectively. The positive control samples are the cells treated with free bFGF.

Confluent Human Umbilical Vein Endothelial (HUVEC) cells on 30mm cell culture plates were washed with *Phosphate Buffered Saline* (PBS) and starved in culture media (M 131, 2.5% MVGS, 0.01% penicillin-streptomycin). After 20 hrs' starving, the medium of M 131 supplemented with 0.01% penicillin-streptomycin and samples, i.e. control sample (free bFGF) and released bFGF from gelatin NPs-grafted p(HEMA-co-AEMA) when released time (t) is 5 days and 10 days, respectively, were introduced to the cell culture. The plates were incubated at room temperature for 15 min and rinsed wit PBS and lysed with NP-40 lysis buffer. The protein concentrations were determined using the Pierce® BCA Protein Assay, and separated by electrophoresis on 10% SDS-polyacrylamide gel electrophoresis. After separation, the proteins were transferred to the high-quality polyvinylidene difluoride (PVDF) membranes. Western blotting was then used to detect the phophorylated ERK proteins (pERK).

Figure S-3 shows the images of protein bands (ERK ans pERK) extracted from cells exposed to standard bFGF and released bFGF when t = 5 days, and 10 days respectively. Comparing to the control sample (standard free bFGF), the released bFGF collected at t = 5 days and 10 days give the same ERK and pERK bands at 42 kD and 44 kDa, respectively. Thus, the released bFGF reserved the bioactivity.

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Figure S-3. Mapkinase activity (triplicate measures) to free bFGF and released bFGF when t is 5 and 10 days, respectively.

References

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