

Condition responsive nanoparticles for managing infection and inflammation in keratitis

Saad M. Ahsan & Ch. Mohan Rao*

*Centre for Cellular and Molecular Biology (CCMB), Council of Scientific and Industrial Research, Uppal Road,
Hyderabad – 500 007, Telangana State, India*

*Corresponding author

Email: mohan@ccmb.res.in

Address: Dr. Ch. Mohan Rao

Centre for Cellular and Molecular Biology (CCMB),

Council of Scientific and Industrial Research,

Hyderabad 500 007, India

URL: www.ccmb.res.in/staff/mohan

Ph.No.- +91-40-27195631

Fax No.- +91-40-27195633

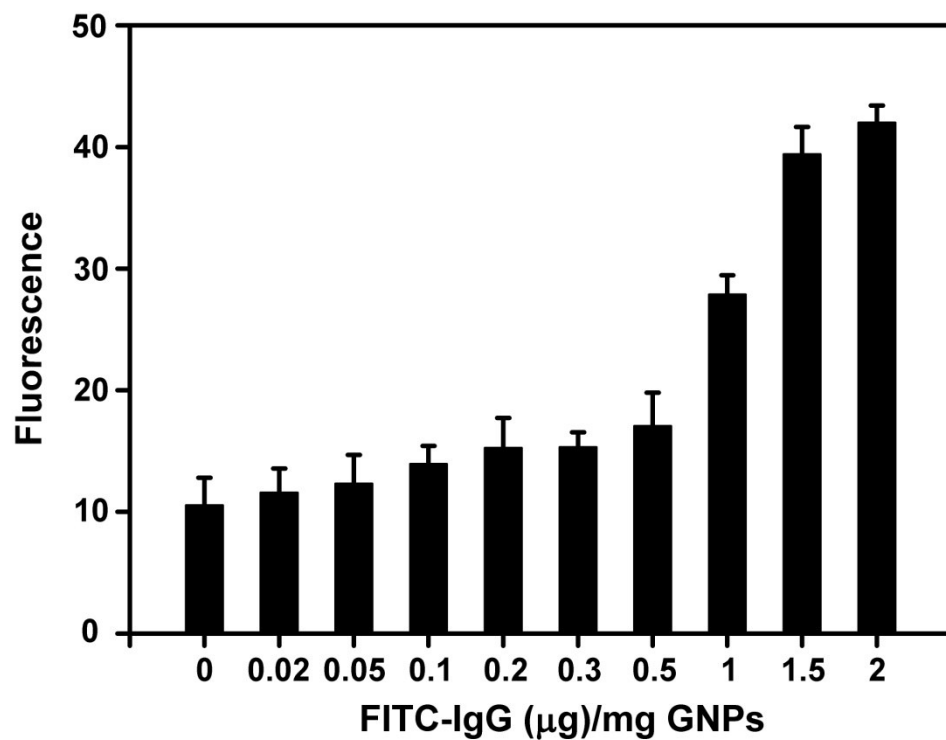


Figure S1: FITC-IgG conjugation to GNPs. EDC-activated nanoparticles were incubated with varying concentrations of FITC-IgG and incubated for 24 h at 4 °C. FITC-IgG conjugated GNPs were washed by centrifugation and resuspension in PBS and analyzed for antibody conjugation by monitoring particle associated fluorescence through flow cytometry using a BD FACSCalibur™ cell analyzer.

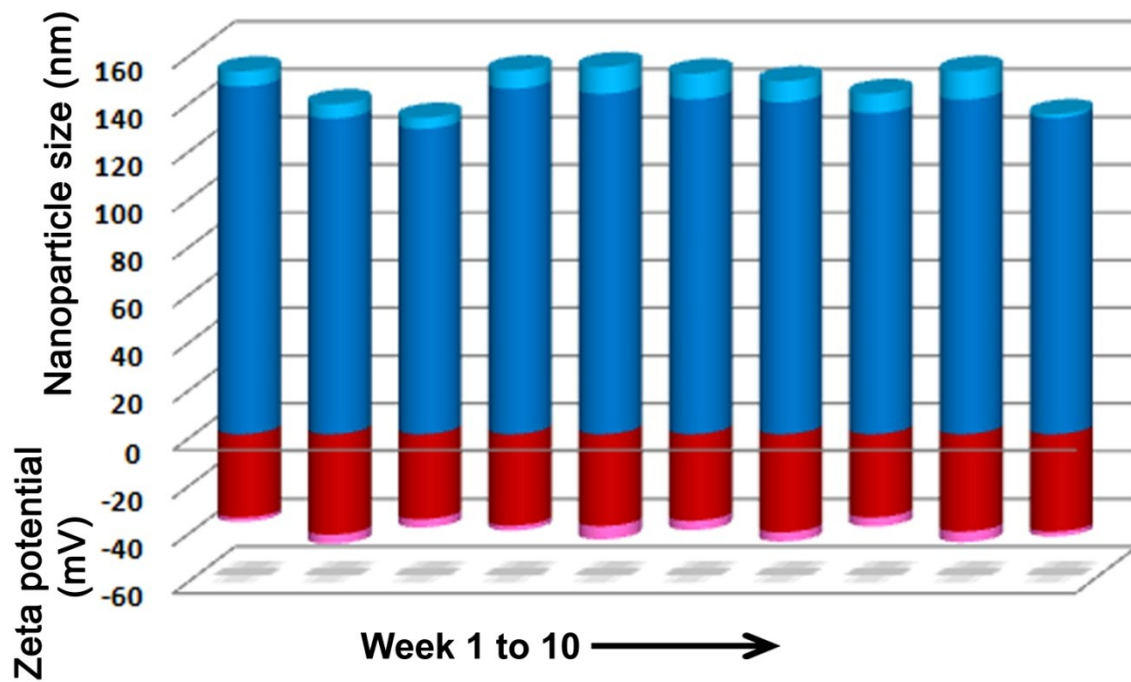


Figure S2: Stability of anti-TLR4-GNPs in PBS was tested by monitoring the size and zeta potential changes over a span of 10 weeks. All values are expressed as mean \pm SD (n = 3).

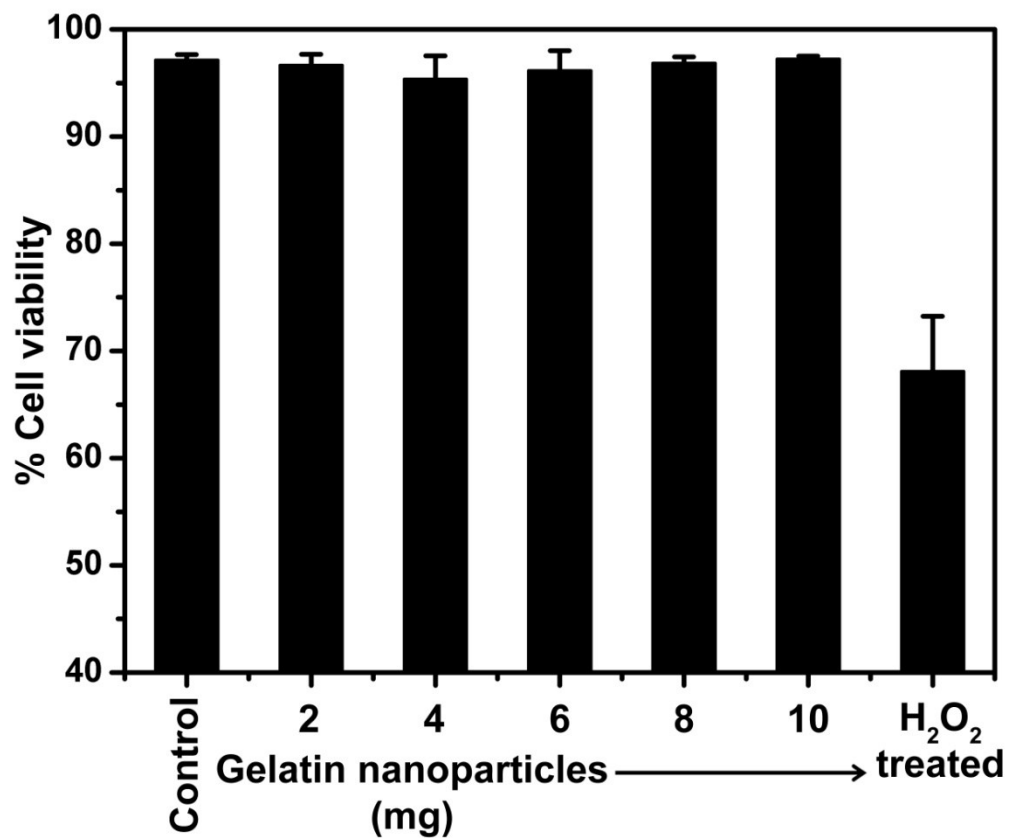


Figure S3: Biocompatibility of gelatin nanoparticles. % cell viability of cells treated with gelatin nanoparticle as determined propidium iodide (PI) uptake followed by flow cytometry. Untreated cells served as negative control while hydrogen peroxide (H₂O₂) treated cells served as positive control.

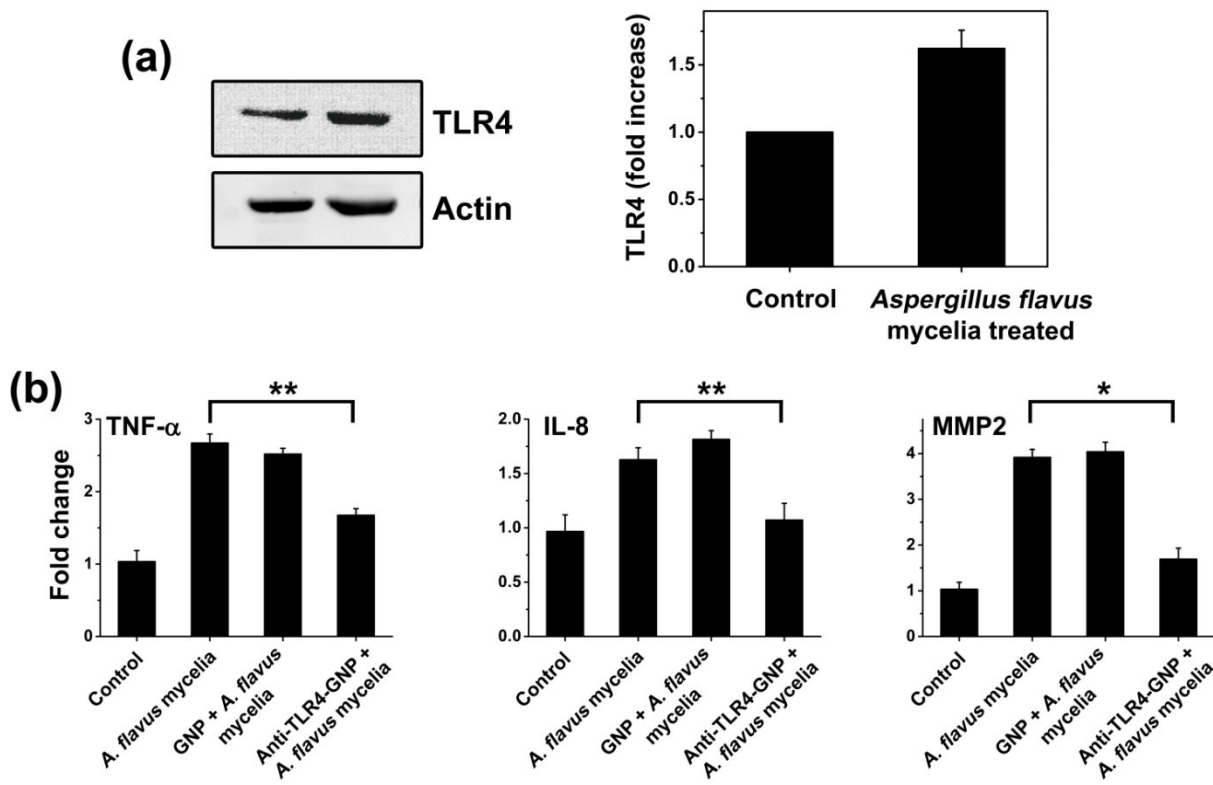


Figure S4: *Aspergillus flavus* induced TLR4 expression in human corneal epithelial (HCE) cells and anti-TLR4-GNP mediated suppression of inflammatory cytokines. (a) Western blot analysis of TLR4 over-expression in Human Corneal Epithelial (HCE) cells upon treatment with *Aspergillus flavus* mycelia ($0.2 \mu\text{g mL}^{-1}$). Histogram representing the fold increase in TLR4 expression upon treatment with *Aspergillus flavus* mycelia. (Actin was used as an endogenous control). (b) Fold change in mRNA levels of TNF- α , IL-8 and MMP2 in Human Corneal Epithelial (HCE) cells treated with *Aspergillus flavus* mycelia, GNPs + *Aspergillus flavus* mycelia and anti-TLR4-GNPs + *Aspergillus flavus* mycelia. Untreated cells served as control. Bars represent mean \pm SD (n=3) (*p<0.05, **p<0.01).