Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Material

3D microscaffolds with triple-marker sensitive nanoprobes for studying fatty liver disease *in vitro*

Simran Kaur Rainu[a] and Neetu Singh[* a,b]

a. Centre for Biomedical Engineering, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

b. Biomedical Engineering Unit, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

**Corresponding author: sneetu@cbme.iitd.ac.in*

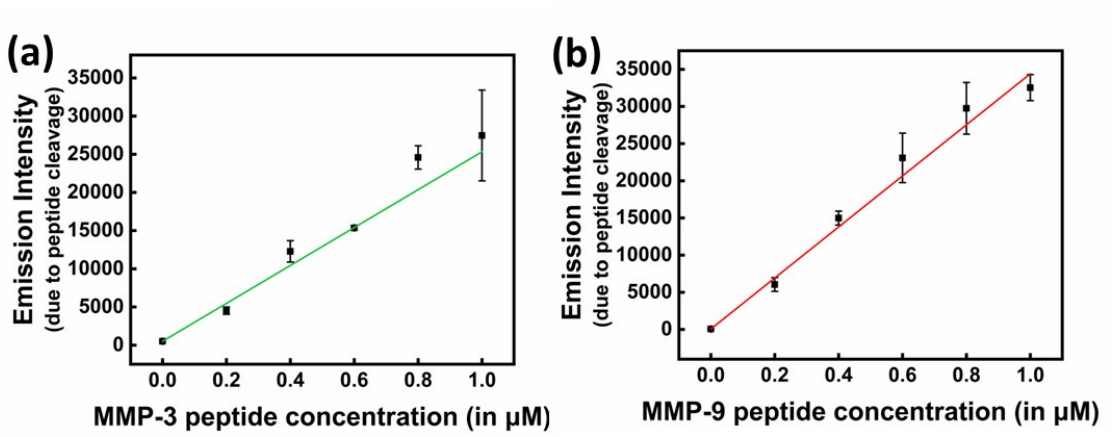

Figure S1: (a) Fluorescence intensity plot recorded at λ_{em} = 520 nm for known concentrations of MMP-3 peptide substrate in 1 ng/mL MMP-3 enzyme containing media. **(b)** Fluorescence intensity plot recorded at λ_{em} = 576 nm for known concentrations of MMP-9 peptide substrate in 1 ng/mL MMP-9 enzyme containing media.

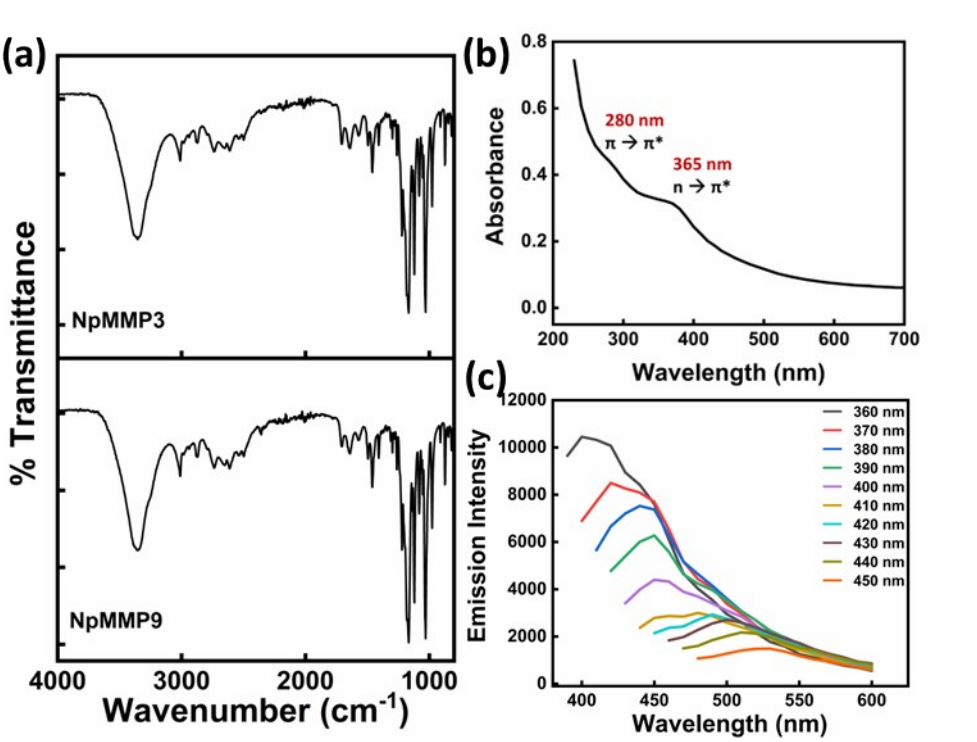

Figure S2: Characterisation of nanoprobe **(a)** FTIR spectra for NpMMP3 and NpMMP9; **(b)** Absorbance spectra for nanoprobe showing 2 peaks at 280 nm and 365 nm; **(c)** Plot showing excitation-dependent fluorescence emission behaviour of nanoprobes.

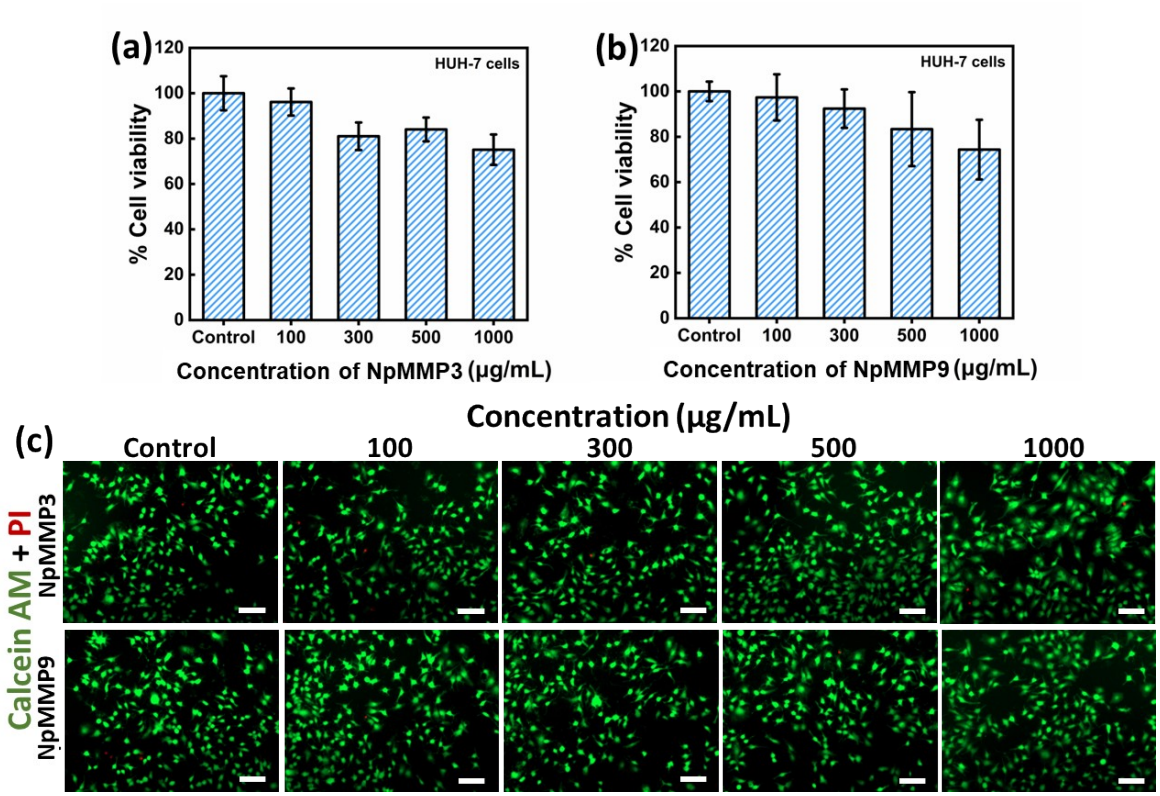

Figure S3: *In vitro* cytocompatibility for NpMMP3 and NpMMP9 nanoprobes in Huh-7 cell line using (a) MTT assay and (b) Live-dead assay by staining cells with Calcein-AM and PI dyes. (Scale bar: 100 µm)

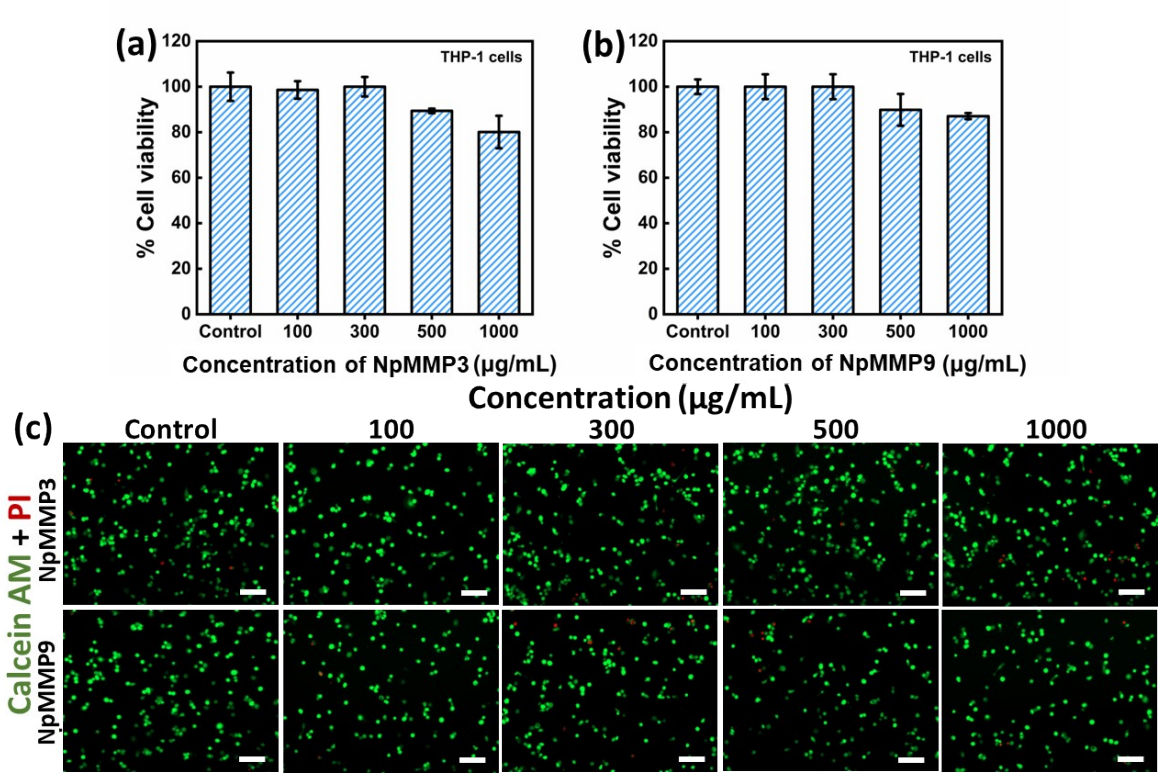

Figure S4: *In vitro* cytocompatibility for NpMMP3 and NpMMP9 nanoprobes in THP-1 cell line using (a) MTT assay and (b) Live-dead assay by staining cells with Calcein-AM and PI dyes. (Scale bar: 100 µm)

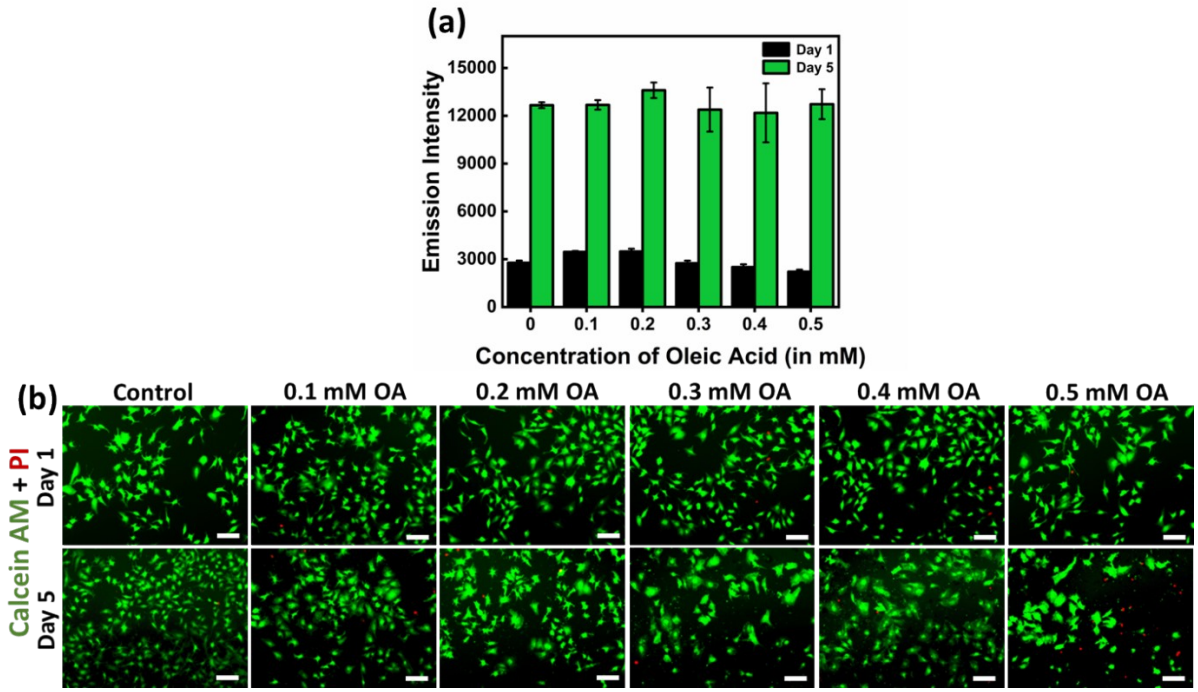

Figure S5: *In vitro* cytocompatibility of cells treated with varying concentrations of oleic acid using (a) Alamar blue assay and (b) Live-dead assay by staining cells with Calcein-AM and PI dyes. (Scale bar: 100 µm)

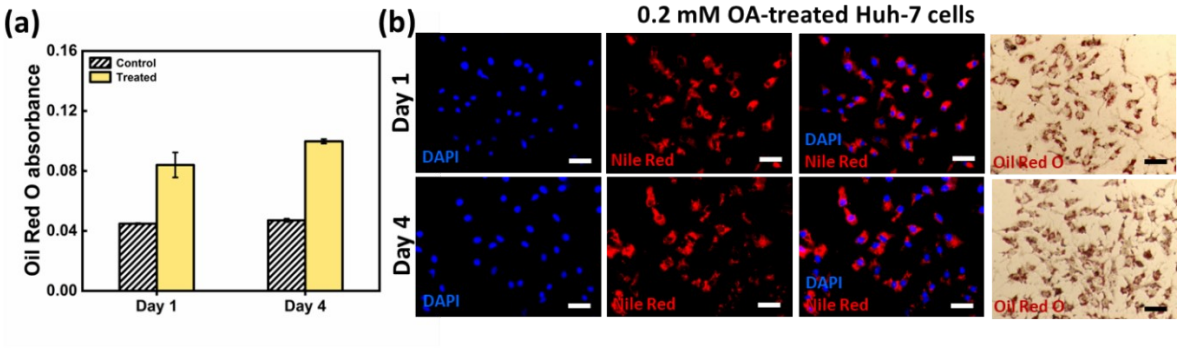

Figure S6: (a) Quantifying intracellular lipid accumulation in Oil Red O-stained 0.2 mM OA-treated Huh-7 cells; **(b)** visualising lipid accumulation in OA-treated cells by using Nile red/DAPI stains and Oil red O stains (Scale bar: 50 µm)

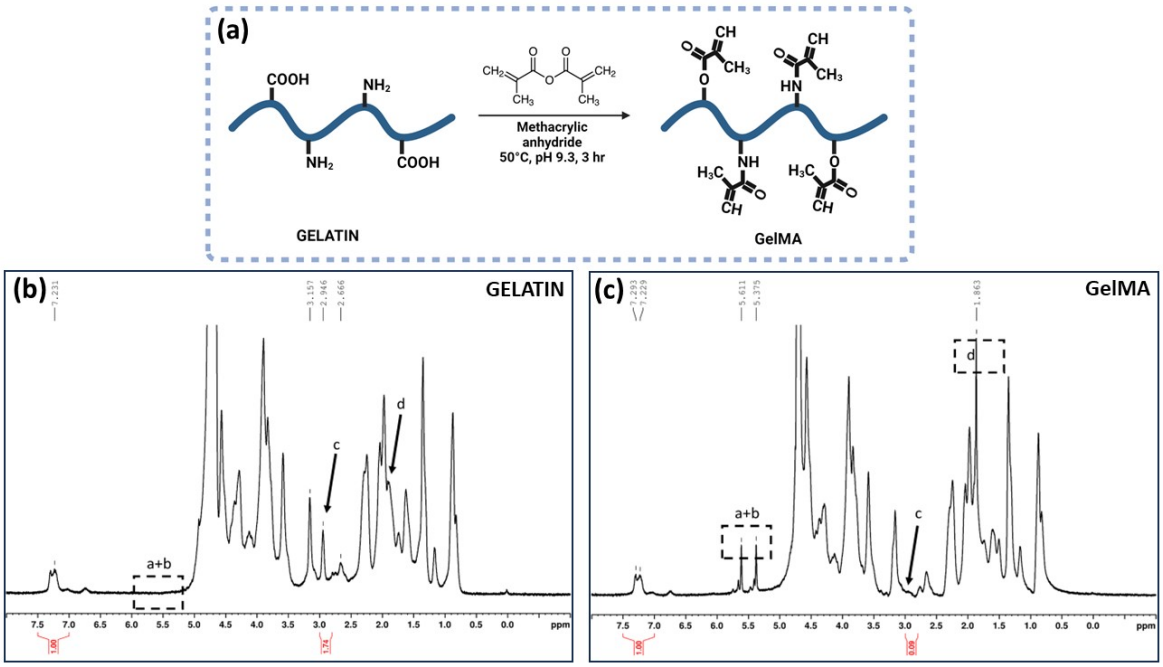

Figure S7: (a) Schematic showing synthesis protocol for GelMA using gelatin and methacrylic anhydride; ^{1}H NMR spectra for **(b)** gelatin and **(c)** GelMA.

Pore diameter (nm)	Pore volume (cc/g)	Surface area (m^2/g)
2.98	0.006	3.434

Figure S8: Table showing pore diameter, pore volume and surface area of microscaffolds determined *via* BJH analysis of nitrogen adsorption desorption isotherms.

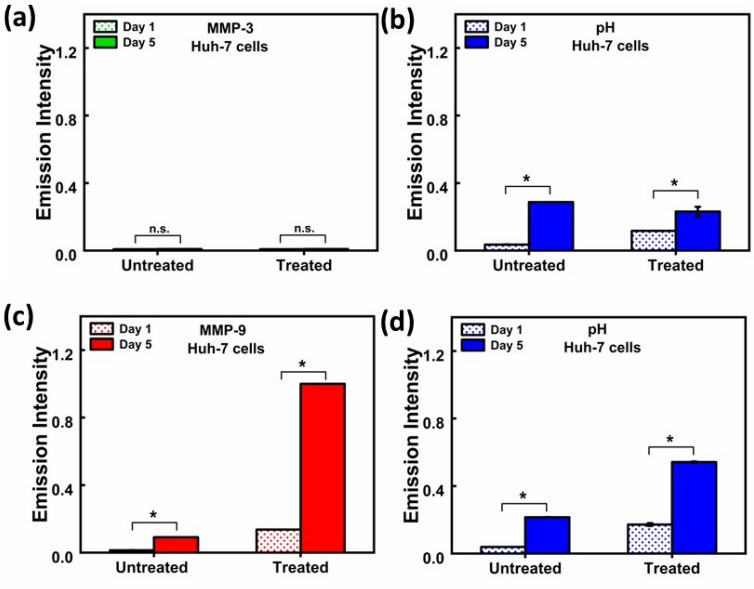

Figure S9: Normalised fluorescence emission intensity plots (corresponding to fluorescence microscopy images) showing change in expression of **(a)** MMP-3 & **(b)** pH; and **(c)** MMP-9 & **(d)** pH in 3D microscaffolds encapsulating untreated or treated Huh-7 cells at days 1 and 5. (*$p < 0.05$)

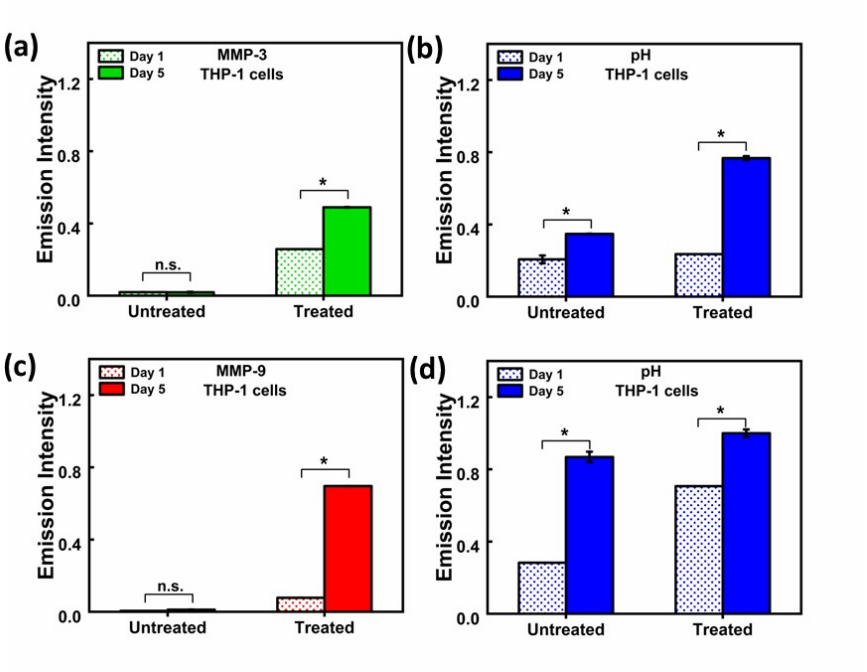

Figure S10: Normalised fluorescence emission intensity plots (corresponding to fluorescence microscopy images) showing change in expression of **(a)** MMP-3 & **(b)** pH; and **(c)** MMP-9 & **(d)** pH in 3D microscaffolds encapsulating untreated or treated THP-1 cells at days 1 and 5. (*$p < 0.05$)

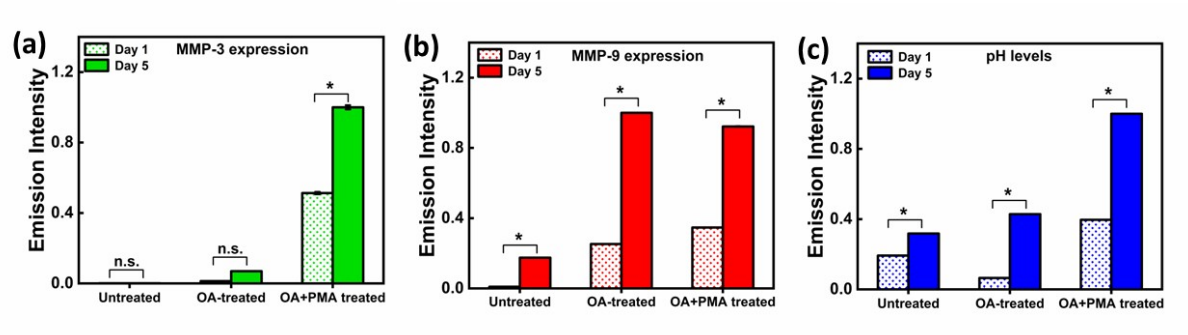

Figure S11: Normalised fluorescence emission intensity plots (corresponding to fluorescence microscopy images) showing change in expression of **(a)** MMP-3, **(b)** MMP-9 and **(c)** pH in 3D microscaffolds encapsulating Huh-7 and THP-1 cells at days 1 and 5. (*$p < 0.05$)