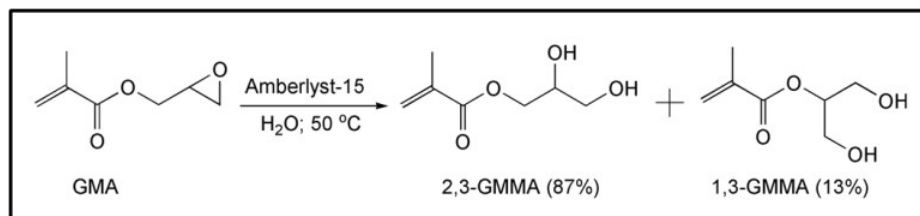


Inverse high internal phase emulsion polymerization (i-HIPE) of GMMA, HEMA and GDMA for preparation of superporous hydrogels as tissue engineering scaffold. TB-ART-09-2015-001873

(Electronic Supplementary Information)

1. Synthesis of Glycerol monomethacrylate

Glycerol Monomethacrylate was synthesised according to scheme S1. In a typical experiment, GMA (25 g, 0.176 mol) and water (6.33 g, 0.352 mol) were stirred at 10 °C for 10 min. before adding 4.32 g pre-activated Amberlyst-15 resin (H⁺ form) and the stirring was continued for five hours at 50 °C. The clear solution was extracted in 50 mL dichloromethane, filtered through sintered crucible to remove solid acid catalyst, dried with anhydrous sodium sulphate and concentrated under reduced pressure which yielded a crude product (clear viscous liquid, mainly a mixture of 2,3- and 1,3-GMMA isomers). The crude product was purified by the column chromatography (silica gel; 50:50 petroleum ether and ethyl acetate). Finally 22.0 g of purified clear viscous liquid was obtained which comprised of 2, 3- and 1, 3-GMMA.



Scheme S1. Facile synthesis of GMMA from GMA.

The purified GMMA was characterized by FTIR, ¹H NMR (200 MHz, D₂O: δ values (ppm) - 6.12 (s,1H), 5.68 (s,1H), 4.16 (m,2H), 3.97 (m,2H), 3.62, (m,2H), 1.88 (m,3H) and ¹³C NMR (200 MHz, D₂O: δ values (ppm) - 169.16, 135.58, 127.08, 75.63*, 69.46, 65.55,

62.26, 60.15*, 17.43; * 1, 3- GMMA isomer). It was found that the hydrolysis of GMA catalyzed by Amberlyst-15 gave a mixture of approximately 87% 2,3-GMMA and 13% 1, 3-GMMA as determined by ^{13}C NMR (Fig. S1).

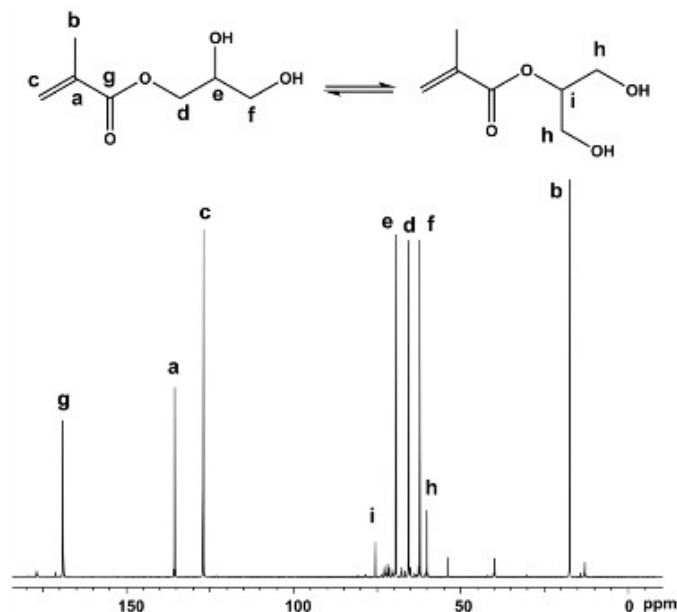


Fig. S1 ^{13}C NMR spectrum of GMMA isomers.

2. Characterization

NMR spectroscopy

^1H and ^{13}C spectra were recorded on Bruker NMR spectrometer (200 MHz, Billerica, USA) at room temperature with CDCl_3 as a solvent with tetramethylsilane (TMS) as an internal standard.

FTIR analysis

Fourier Transform Infra Red (FTIR) spectra were obtained with a Perkin Elmer infrared spectrometer (Spectrum One, USA) between 400 and 4000 cm^{-1} in KBr pellets prepared by grinding the dried polyHIPE hydrogel samples with potassium bromide (~1:99).

Scanning Electron Microscopy (SEM)

The internal porous architecture of the polyHIPE hydrogels samples was investigated with the help of scanning electron microscope (Quanta 200: 3D, dual beam ESEM microscope with thermionic emission source, tungsten filament electrode) operated at 15kV accelerating voltage. The dried hydrogel samples were mounted on the stub and then gold coated using sputter coater for 30s prior to SEM imaging.

Surface area

The surface area of the polyHIPE hydrogels was determined with BET (Brunauer, Emmett and Teller) method (Quantachrome Nova 2000e, USA). All the samples were evacuated at 65 °C prior to surface area measurement.

Mercury intrusion porosimetry

Mercury intrusion porosimetry was performed (Quantachrome Pore Master 60-17,USA) at 2758 kPa with intrusion/extrusion mercury contact angles of 130°, penetrometers stem volume of 1.533 mL and bulb volume of 5.0 mL.

Thermal analyses

Thermogravimetric (TGA and DTA) analysis was carried out in the nitrogen atmosphere (TGA-STA 6000 analyzer, PerkinElmer, USA) in the temperature range 30-900 °C at a heating rate of 20 °C min⁻¹. The thermal properties viz., glass transition temperature (T_g) and melting temperature (T_m) were investigated with the help of differential scanning calorimetry (DSC, Q100 calorimeter, TA Instruments, USA). The samples were heated from -50 to 200 °C at a rate of 10 °C min⁻¹ under the nitrogen atmosphere.

X- ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis of polyHIPE hydrogel samples was carried out on X-ray diffractometer (Rigaku MicroMax-007 HF, Japan) in the reflection mode using CuK α X-ray beam excited at 40 kV and 40 mA.

Swelling ratio measurement

The swelling kinetics of polyHIPE hydrogels was studied by gravimetric measurement of swelling ratio (Q). The specimens of dried hydrogels of known weight were immersed in deionized water at ambient temperature. The swollen hydrogel specimens were then taken out carefully at regular intervals, placed on a concave tea strainer (kept on a beaker filled with water to maintain humidity) and tightly covered with aluminium foil to allow removal of excess water by dripping. The swollen hydrogels were lightly blotted with a filter paper to remove surface water before weighing on an analytical balance (BSA 2245-CW, Sartorius, Switzerland) and then returned to the respective beakers. The swelling ratio Q was calculated as follows:

$$Q = w_s / w_d \quad (1)$$

Where, w_d is initial dry sample weight and w_s swollen sample weight of polyHIPE hydrogel. These experiments were run in duplicate.

Degradation study

In vitro degradation study was conducted by measuring the percent weight loss in 0.007 M NaOH and 1.0 mg mL⁻¹ lipase solutions. The weight loss was determined as follows:

$$\text{Weight loss} = (w_o - w_D) / w_o \quad (2)$$

Where w_o is original dry weight and w_D residual dry weight (after the degradation period) of polyHIPE hydrogel. The specimens of dried polyHIPE hydrogels of known weight (w_o) were placed in 0.007 M NaOH and shaken on a rotary shaker at 60 rpm. The samples were removed after 2, 5 and 10 days, washed thoroughly with deionized water and dried overnight under vacuum at 65 °C to constant weight (w_D). The degradation study in lipase was conducted as described above but only for polyHIPE hydrogel GHG-5.

Biological studies

Cytotoxicity

Mouse embryonic fibroblast cells line (NIH3T3) purchased from National Centre for Cell Science (NCCS, Pune, India) were used to assess cytotoxicity (loss of viable cells) in accordance with ISO 10993-5 (2009). The cells were cultured at 37°C with 5% CO₂ in complete medium (changed every 3rd day) composed of Minimum Essential Medium α (MEM α , High Media, India) and harvested with 0.25% trypsin solution when grown to confluence. The cells were then plated in sterilized 96-well tissue culture polystyrene (TCPS) plates at 20000 cells cm⁻² and incubated for 2 days to form the subconfluent monolayer. The subconfluent NIH3T3 monolayer was cultured in the complete medium in the presence of purified polyHIPE hydrogels and in each case the cell viability assessed for 5 days with the help of MTT assay. The optical microscopic images were taken after 5th day. The polyHIPE hydrogels were purified by thorough washing with deionized water, dried overnight under vacuum (65°C), sterilized with 70 % ethanol (30 min.), washed three times with sterile phosphate buffer saline (PBS) and finally pretreated with complete medium for 1 day.

Cell proliferation and growth

The purified polyHIPE hydrogel samples were placed in 24-well plates and pre-incubated for 24 h in the culture medium. The cells were seeded on the polyHIPE hydrogels/TCPS control with 0.5 mL culture medium containing 1×10^4 cells and the culture medium (without cells) was replenished every alternate day. The cell adhesion and proliferation were evaluated after 1st and 7th day with the help of MTT assay. The cells seeded and cultured on TCPS were taken as a positive control. The cell adhesion on the hydrogels/TCPS and their proliferation were visualized with the help of an inverted microscope (Axioscope-A1, Carl Zeiss, Germany) in phase contrast mode. Whereas, the cell viability was assessed by examining the live/dead cells under the microscope in fluorescence mode after staining with Calcein AM-propidium iodide solution ($2 \mu\text{g mL}^{-1}$ PBS), incubation (10 min. at room temperature) and washing with PBS solution.

MTT assay

The colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, USA) assay, in which formazan is produced, was performed in triplicate to quantify the cell viability in both the studies. The MTT assay determines mitochondrial succinate dehydrogenase activity produced by live cells and thus indirectly measures the number of viable (living) cells. MTT solution (5.0 mg L^{-1}) was added to each well plate and the culture incubated for 4 h at 37°C after that the culture medium was removed and washed with PBS. The intra cellular formazan (a purple colour dye) in the well plates was dissolved in dimethyl sulfoxide ($150 \mu\text{L}$), incubated for 5 min. and the absorbance was measured at 570 nm.

3. Results and discussion: Figures

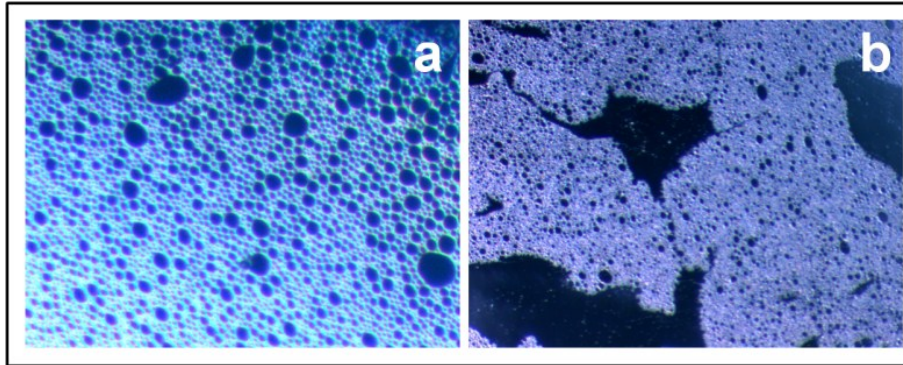


Fig. S2 Representative optical microscopic images: A) stable HIPE (HEMA \leq 60 %), and B) unstable HIPE (HEMA $>$ 60%).

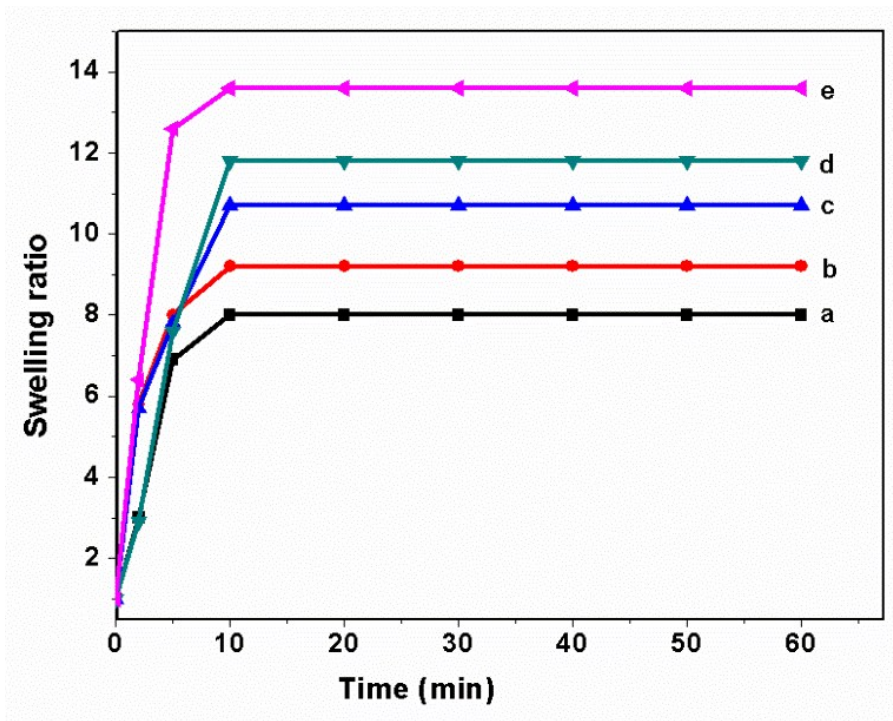


Fig. S3 Swelling kinetics of polyHIPE hydrogels in water: a) GHG-1, b) GHG-2, c) GHG-3, d) GHG-4, and e) GHG-5.

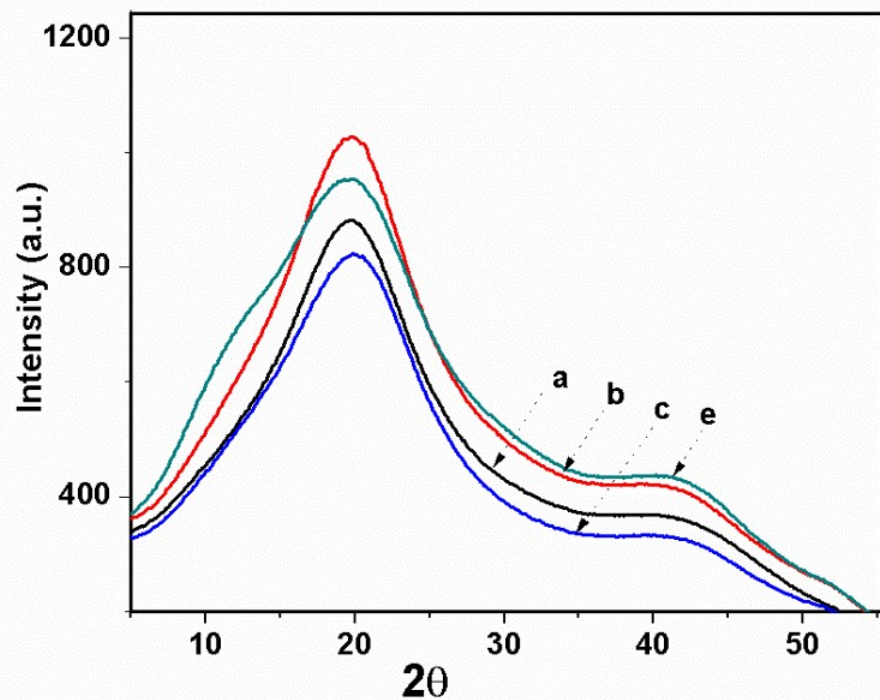


Fig.S4 XRD patterns of polyHIPE hydrogel samples: a) GHG-1, b) GHG-2, c) GHG-3, and e) GHG-5 (GHG-4, not recorded).

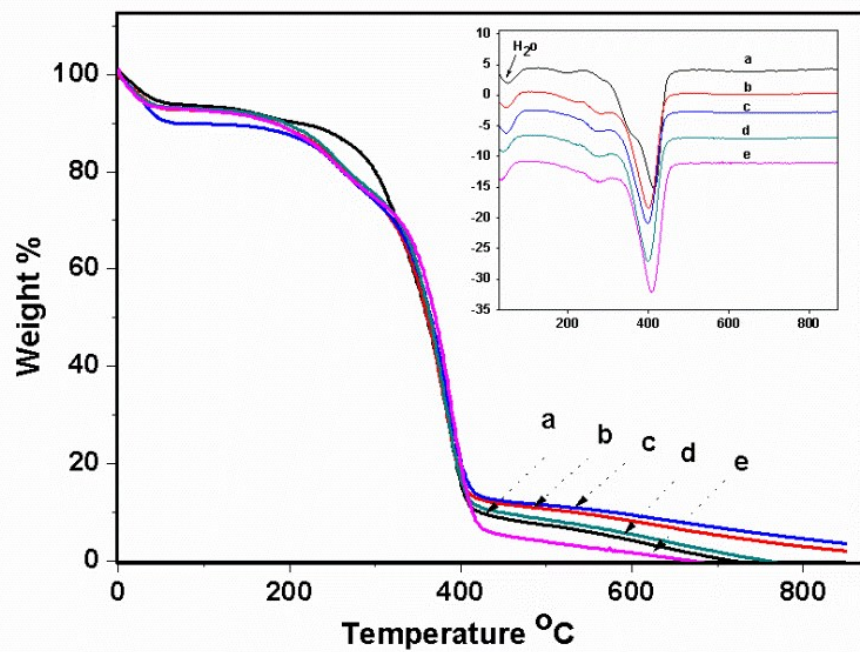


Fig. S5 TGA and DTA (inset) curves of polyHIPE hydrogels: a) GHG-1, b) GHG-2, c) GHG-3, d) GHG-4 and e) GHG-5.

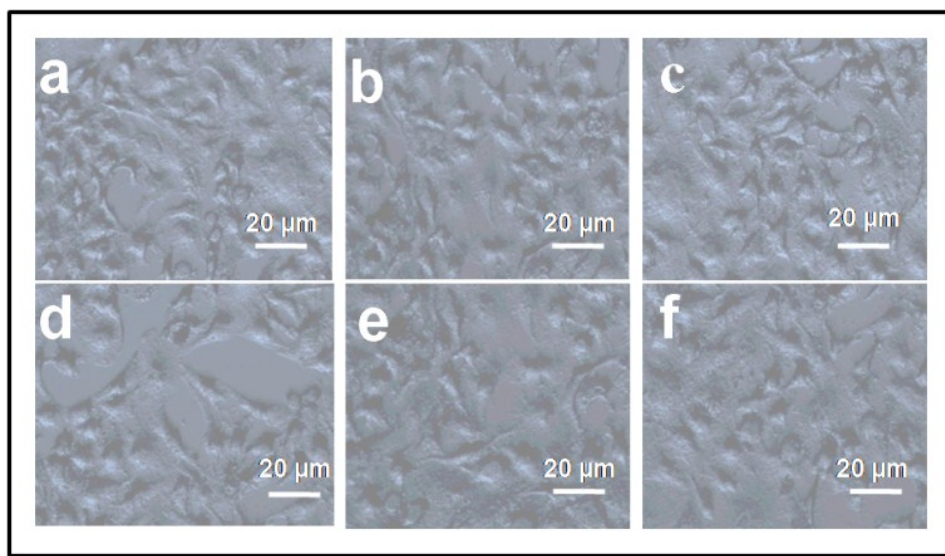


Fig. S6 Optical microscopic images of NIH3T3 cells on TCPS in presence of polyHIPE hydrogels: a) control, b) GHG-1, c) GHG-2, d) GHG-3, e) GHG-4 and f) GHG-5) after 5 days.

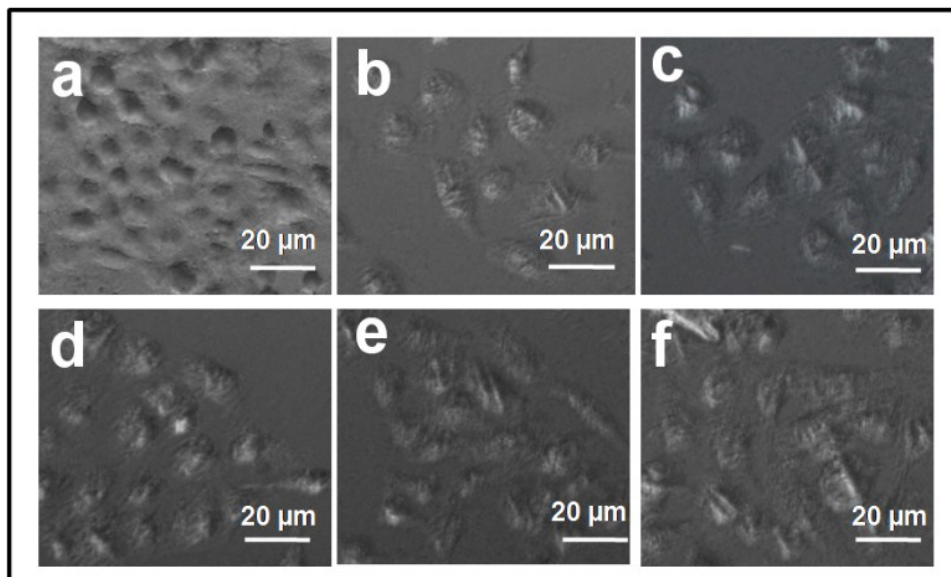


Fig.S7 Phase contrast microscopic images of NIH3T3 cells seeded on polyHIPE hydrogels: a) control, b) GHG-1, c) GHG-2, d) GHG-3, e) GHG-4 and f) GHG-5 after 7 days.