Supporting information

Microgel-based Modular 3D in vitro microfluidic cell culture platform

Manleen Kaur[a1], *Mayuri Dutta*[a1], *Soutik Betal*[c] *Neetu Singh*[a,b*]

[a]Centre for Biomedical Engineering, Indian Institute of Technology Delhi, New Delhi, 110016, India

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

[c] Department of Electrical Engineering, Indian Institute of Technology Delhi, New Delhi, 110016, India

Email: sneetu@iitd.ac.in

Type	Size
Small D: 3 cm F: 90 mL/min P: 10%	1. 515.625 µm 2. 531.250 µm 3. 580.423 µm 4. 585.950 µm 5. 606.794 µm ---------- Average size = 564 ± 38.71 µm
Medium D: 4.5 cm F: 60 mL/min P: 20%	1. 791.746 µm 2. 835.938 µm 3. 865.586 µm 4. 882.809 µm 5. 885.416 µm ---------- Average size = 852.29 ± 39.17 µm
Large D: 6 cm F: 45 mL/min P: 25%	1. 1546.875 µm 2. 1572.917 µm 3. 1625 µm 4. 1651.042 µm 5. 1656.250 µm ---------- Average size = 1610.41 ± 48.49 µm

Table S1: Average diameters for various sizes of microgels fabricated using

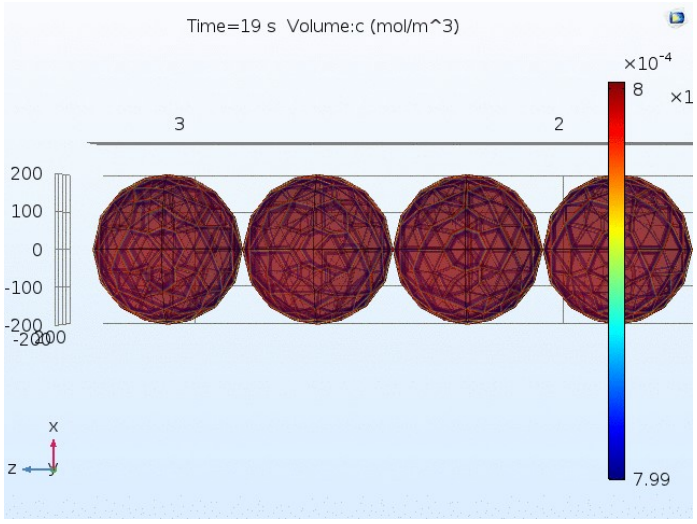
	Chip 1 (pixels)	Chip 2 (pixels)
Day 2	12.04 to 119.94 Av : 57.3	13.23 to 109.12 Av: 62.4
Day 6	5.83 to 84.77 Av: 34.1	7.89 to 77.42 Av: 36.2
Day 14	9.43 to 64.63 Av: 39.1	6.23 to 58.23 Av: 23.7

Table S2: Distance of Huh-7 (red) to nearest NIH-3T3 (green fluorescence) in two different sets of microgels as chip 1 and chip 2 using FIJI image analyzer distance tool.

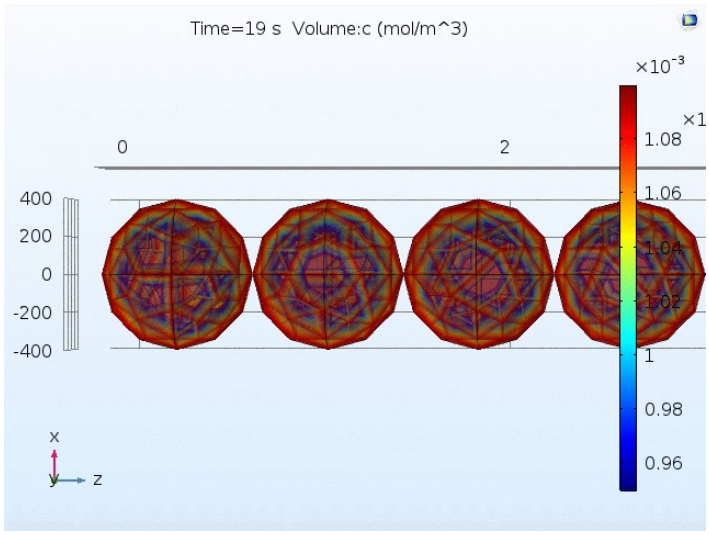
Day	1:1	1:2	1:3	2:3
2	2.18	4.23	3.43	4.51
6	3.92	4.39	4.64	27.86
8	3.94	4.63	4.29	25.74

Table S3: Albumin (pg/mL) production in HF conditions using different cell density ratios over 8 days time-period.

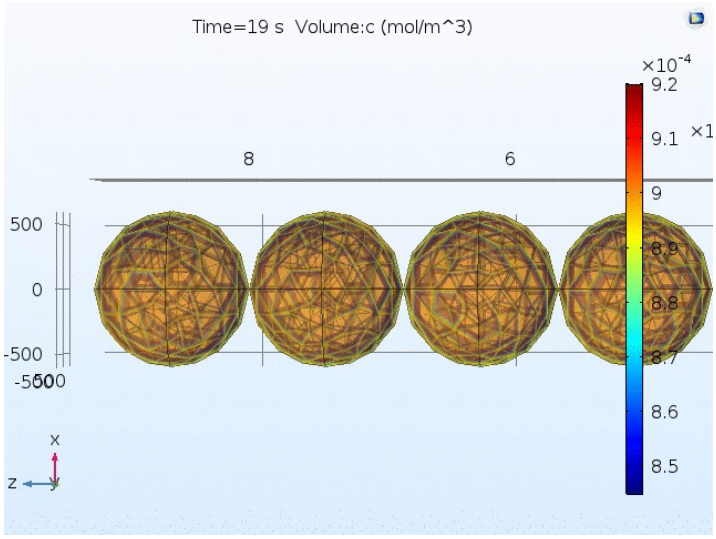
Figure S1: COMSOL simulation of diffusion across three sizes of microgels



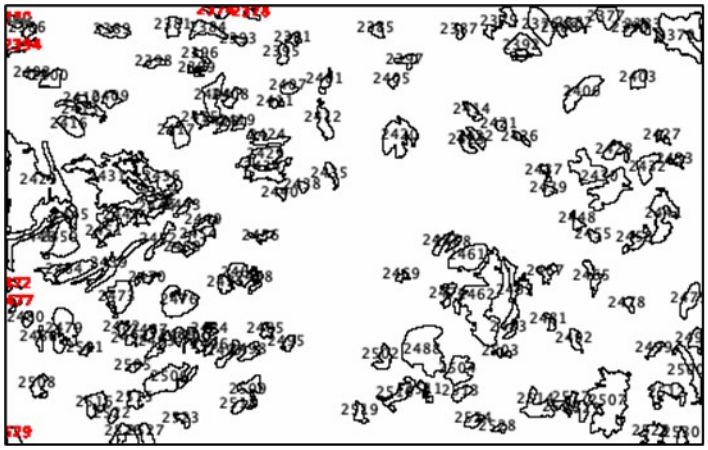
Small Microgels



Medium Microgels



Large Microgels



	Count	Area %	Min Feret Diameter (um)
Picture1-1.png	157	**16.179**	**18.428**

Figure S2: Binary for the SEM in Figure 1E to calculate the pore size**.**

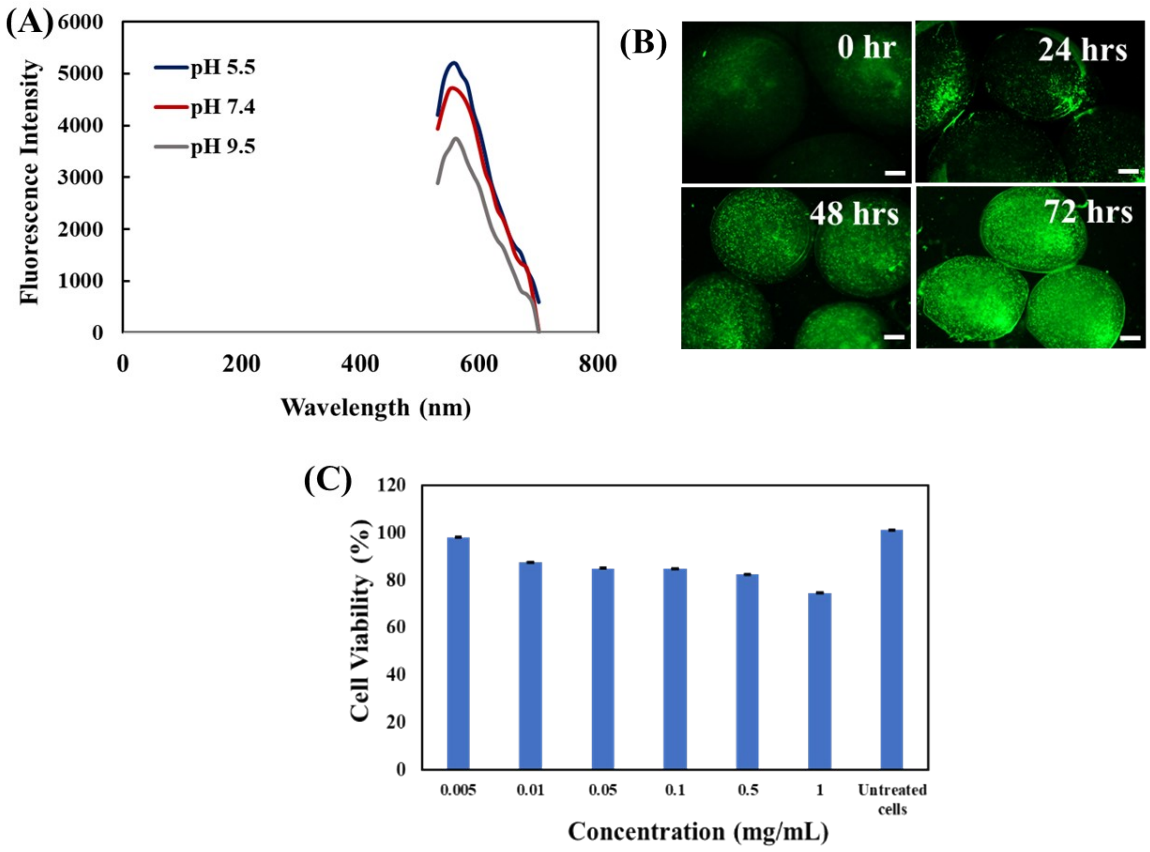


Figure S3: (A) Fluorescence emission spectra at excitation 500nm for carbon dots (0.5mg/mL) dispersed in PBS at pH 9.5, pH 7.4 and pH 5.5; (B) Fluorescence microscopic images of Carbon dots encapsulated with cells for Cell proliferation and (C) Cytocompatibility assay of Carbon dots using various concentrations from 0.005-1mg/mL using Huh-7 cells

Optimization of the chip design:

The microfluidic chamber design was based on the intrinsic architecture of a hepatic lobule, the functional building block of the liver. Each lobule consists of a network of sinusoids linking the hepatic portal triad to the central vein. Initially, a hexagonal chamber **(Figure S2A)** was designed to emulate this structure, comprising six inlet ports converging into a common outlet port through several microfluidic channels. These channels were dimensioned to accommodate single medium-sized microgels linearly without overlap. At the end of each channel, a cylindrical post was constructed to sustain dynamic flow while preventing the microgels from escaping into the outlet. However, during manufacturing, the radial convergence of the channels at the outlet led to unintended inter-channel mixing at the outlet openings. To address this flaw, the chamber design was improved into a rectangular arrangement with 10 inlet ports connecting perpendicularly via microfluidic channels into a central outlet **(Figure S2B)**. The new design retained all prior dimensions and features while providing the flexibility of including additional inlets, facilitating the inclusion of a greater amount of cell-encapsulated microgels, and thereby enhancing the efficiency of functionality evaluations within the chip.

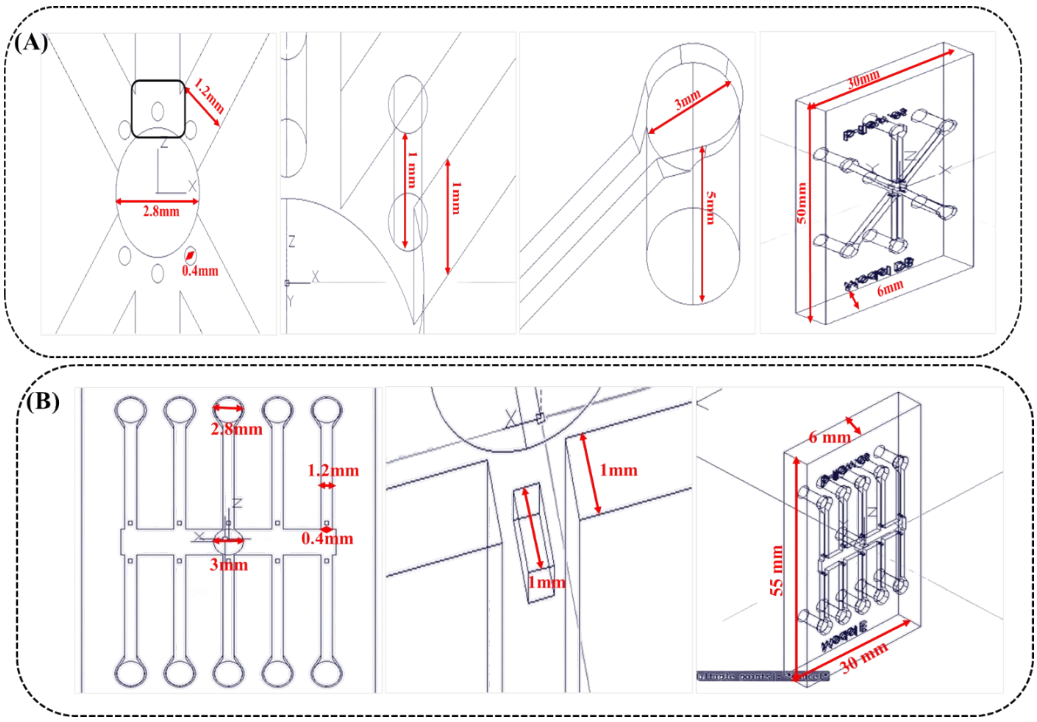


Figure S4: **Evaluating the design of microfluidic chip:** (A) represents design comprising hexagonal lobular architecture and (B) The rectangular design with 10 inlet pores connecting perpendicularly via microfluidic channels into a central outlet.

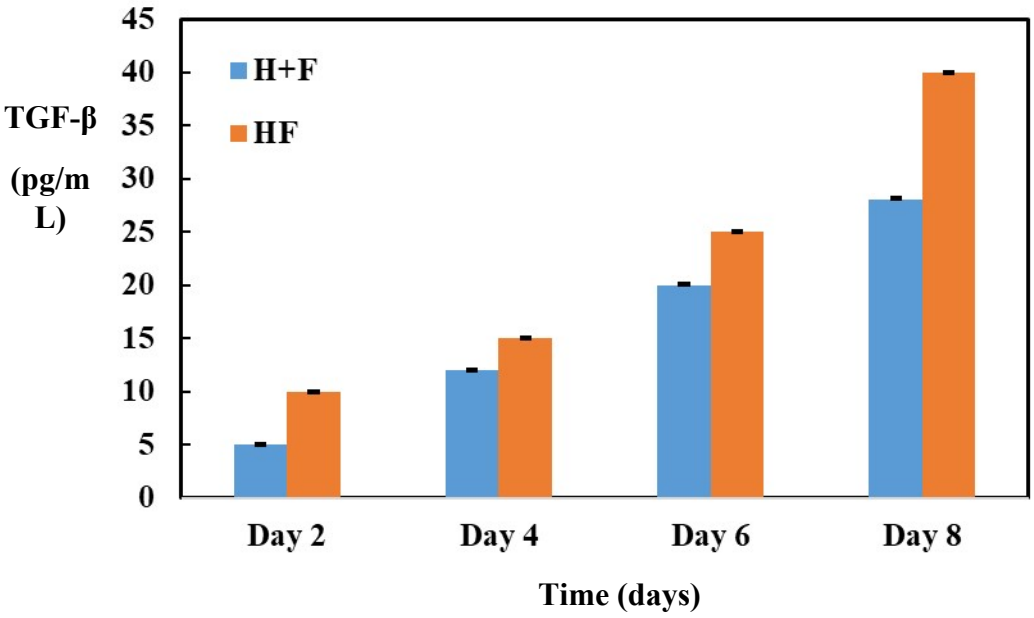


Figure S5: TGF-β (Transforming growth factor-β) Assay for the combinations (H+F) and (HF) microgels in dynamic condition.

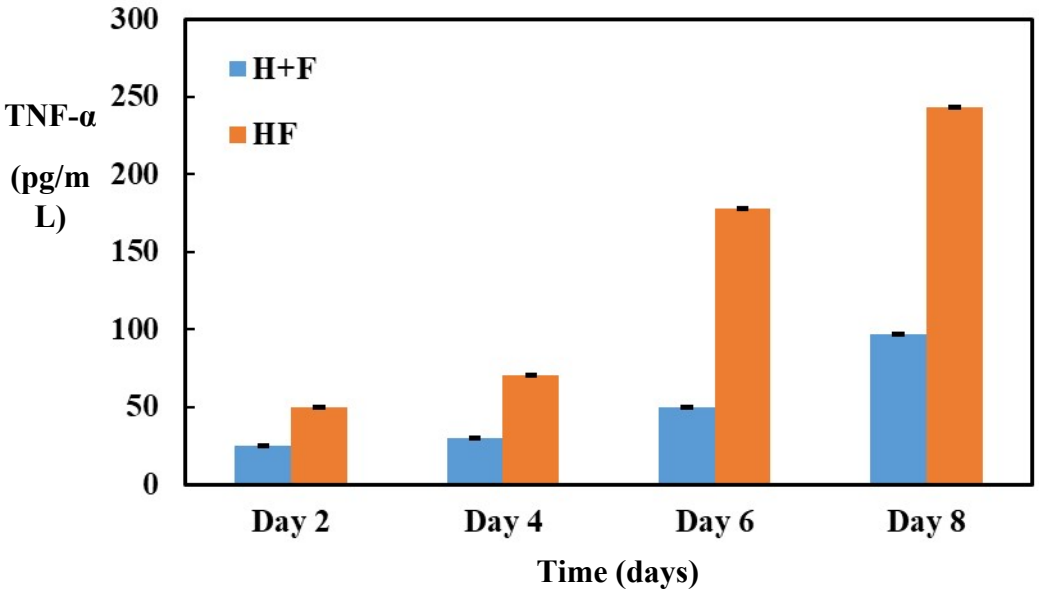


Figure S6: TNF-α (Tumor Necrosis factor-α) Assay for the combinations (H+F) and (HF) microgels in dynamic condition.

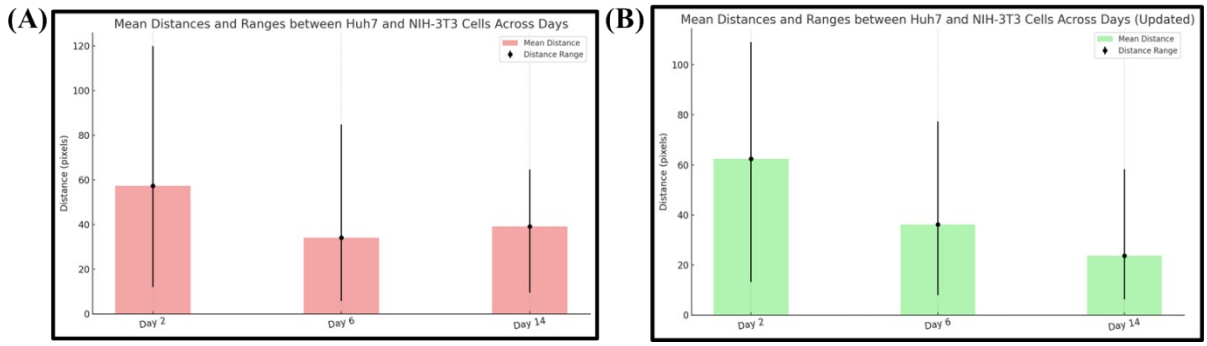


Figure S7: (A) Distance of Huh-7 to nearest NIH-3T3 over 14 days time-period in chip 1and (B) distance of Huh-7 to nearest NIH-3T3 over 14 days time period in chip 2.

The chip distance range refers to the minimum and maximum distance of Huh-7 to the NIH-3T3.