SUPPLEMENTARY INFORMATION

Nanoengineered biomimetic hydrogels for guiding human stem cell osteogenesis in three dimensional microenvironments

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Running title: Nanoengineered osteoinductive hydrogel

SUPPLEMENTARY FIGURES AND TABLE

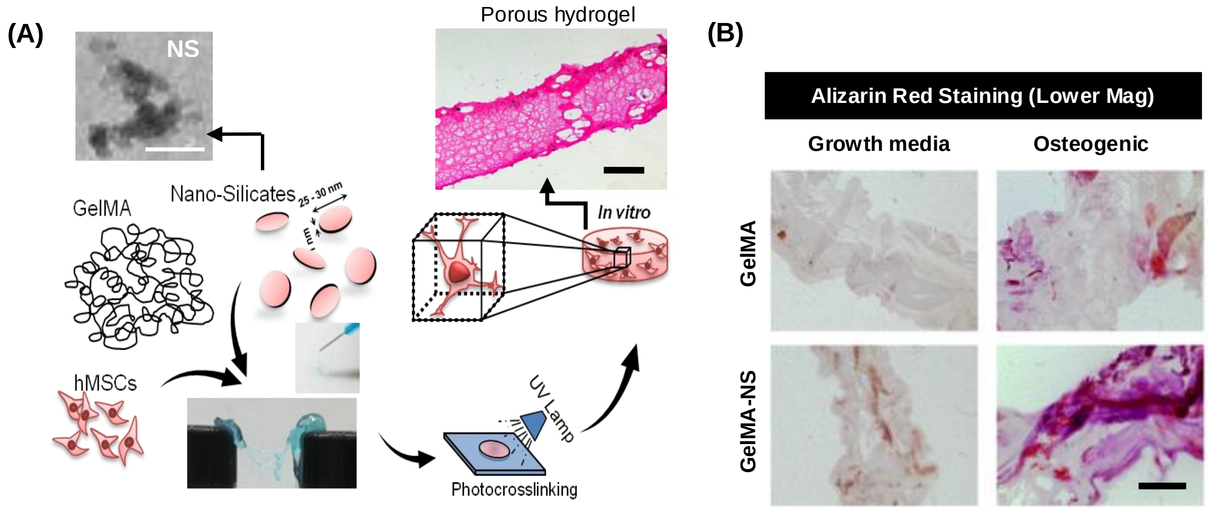

Figure S1: Fabrication of hMSC encapsulated GelMA-nSi hydrogels for 3D osteogenic differentiation. **(A)** Schematic representation of *in vitro* hydrogel fabrication method using GelMA, nanosilicates and hMSCs by covalent crosslinking under UV radiation. Transmission Electron Microscope (TEM) image of nSi particles dispersed in water (Scale: 100nm). Bright field picture of porous hydrogel (Scale bar: 200µm). **(B)** Confirmation of 3D osteogenic differentiation of hMSCs after 21 days of culture in growth media and osteogenic media (with no drugs) by alizarin red staining of cross-section of the hydrogels to trace calcium deposits. Scale bar: 200µm.

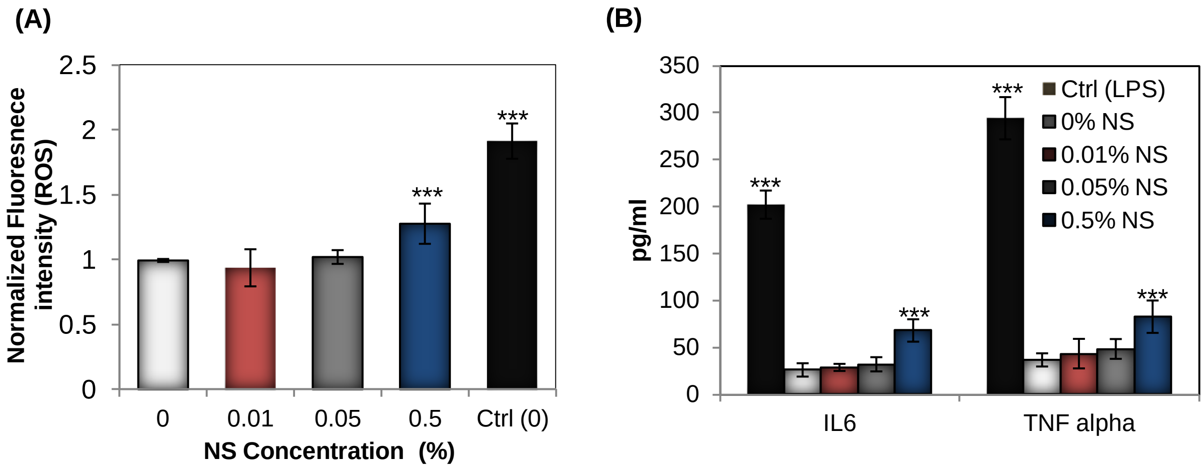

Figure S2: (A) Effect of the GelMA-nSi hydrogel on production of reactive oxide species (ROS) in encapsulated hMSCs. The formation of radicals as a measure of intracellular stress that generates a cytotoxic response was determined. The intracellular production of ROS was evaluated after hMSCs incubation in the presence of different NSi concentrations in GelMA-nSi hydrogel. As the silicate concentration increased, no intracellular oxidative stress (ROS) was noticed until 0.05% NS. However, at higher silicate concentrations (0.5% NS), a significant increase in ROS was observed as quantified by ROS fluorescence assay in a plate reader and represented as fold change compared to 7% GelMA with 0% NS. Serum starved group with 7% GelMA hydrogel was used as the experimental control, Ctrl (0). **(B)** Secretion of pro-inflammatory cytokines, IL6 and TNFα, from RAW 264.7 macrophages encapsulated in hydrogels with different formulations (7% GelMA hydrogels with different percentages of NS) after 24h of exposure represented in the bar graph with different colors [Black: Ctrl (LPS), White: 0% NS, Red: 0.01% NSi, Grey: 0.05% NS, Blue: 0.5% NS] as obtained by ELISA analysis. As positive control group, RAW cells were treated with LPS. Data represent Mean ± SD (n=3). *****=p< 0.05 & *******=p< 0.001 compared to control GelMA group (0% NS).

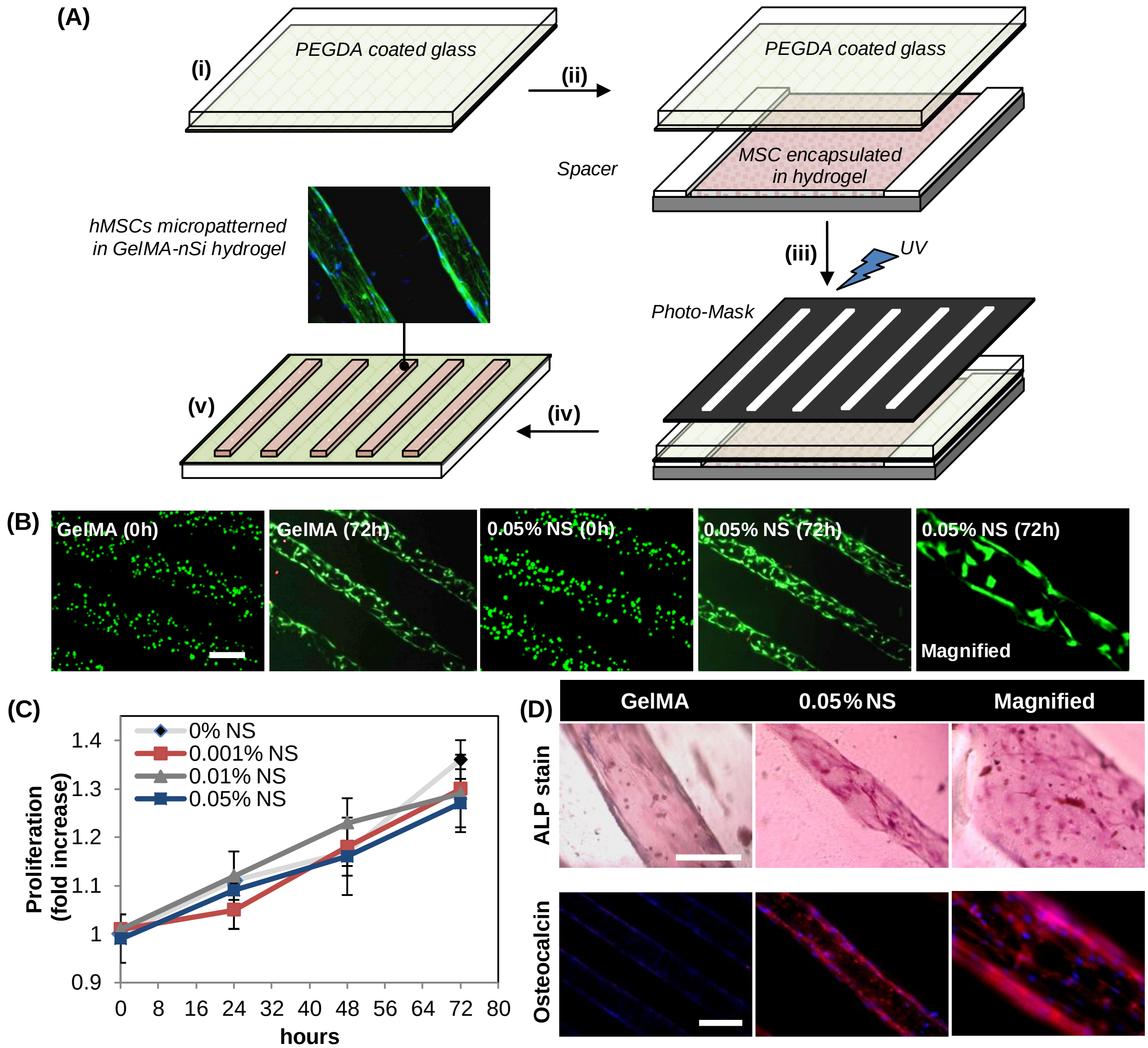

Figure S3: (**A**) Schematic to step-wise microfabricate hMSCs encapsulated in 3D GelMA-nSi hydrogels on PEGDA coated glass slides by UV photo-crosslinking. Inset shows fluorescence image of the micropatterned cells stained with F-actin (green) and nuclei (blue). (**B**) Biocompatibility of micropatterned GelMA-nSi hydrogel was confirmed by fluorescence microscope images of calcein stained hMSCs at 0h and 72h encapsulated in GelMA-nSi (0.05% NS) and control GelMA group (0%NS) in normal media. Additionally, **(C)** cell proliferation study by MTS assay demonstrated that all the micropatterned hydrogel groups had similar growth kinetics with no significant differences ($p>0.05$). **(D)** Increase in osteogenic differentiation potential of hMSCs in micropatterned GelMA-nSi hydrogel compared to GelMA group was confirmed by ALP staining (upper panel, in purple) and osteocalcin immunostaining (lower panel, in red) after 21 days of culture in osteoconductive media. Scale bar: 100μm.

Table S1: Synthesis and Biological activities of Encapsulated Stem Cells within GelMA-nSi and other Nanocomposite Hydrogels reported for 2D and 3D bone regeneration applications.

Nanocomposite Materials	Cell Type	Mode of Cell Culture	Growth Factors (Y/N)	Remarks/Characteristics
Photo-crosslinked gelatin and synthetic silicate nanoclay (GelMA-nSi)	Human mesenchymal stem cells	3D encapsulation	N	• Simple one-step fabrication method • Biomimetic behavior • GelMA-nSi hydrogel has strong osteoinductive properties • Hydrogels can be micropatterned to design diverse osteogenic structures with hMSCs in 3D • Biocompatible (tested in vitro and in vivo)
Chitosan + nanocrystalline hydroxyapatite + single-walled carbon nanotubes [1]	Human fetal osteoblasts	2D Seeding	N	• Complex three step fabrication method • Bioactivity of nanocrystalline hydroxyapatite • Nanofibrous matrix using carbon nanotube reinforced substrate
Gelatin + chitosan nanoparticles + recombinant human bone morphogenic proteins (rhBMPs) [2]	Human mesenchymal stem cells	2D Seeding	Y	• Two step fabrication • Multiple components needed • rhBMP-2 promoted differentiation • Chitosan was used to control delivery of rhBMP
GelMA + Gold nanoparticles [3]	Human mesenchymal stem cells	2D Seeding	N	• One step fabrication • Bioactive gold nanoparticles • Cells were not encapsulated
Hyaluronic acid methacrylate + growth & differentiation factor 5 [4]	MC3T3-E1 preosteoblasts	2D Seeding	Y	• One step fabrication • Application with human cells is not demonstrated
β-tricalcium phosphate scaffolds [5]	Human mesenchymal stem cells & endothelial cells	2D Seeding	N	• Cells seeded on prefabricated scaffold • Co-culture promoted osteogenesis
Alginate [6]	Human mesenchymal stem cells	3D encapsulation	N	• One step fabrication • Result depends on cell density
Alginate +anti-BMP2 monoclonal antibody [7]	Human mesenchymal stem cells	3D encapsulation	Y	• One step fabrication • Expensive Large scale production
GelMA + fibronectin + laminin + osteocalcin [8]	Human mesenchymal stem cells	3D encapsulation	N	• Rapid prototyping • Need for multiple and costly biomolecules

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