# Synthetic Bioactuators for High Throughput Cell Massaging

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**Statement of Purpose:** Dynamic synthetic matrices, such as bioactuators, that can simultaneously provide 3D-structural support and dynamic mechanical cues in a spatiotemporal manner to the encapsulated cells will be advantageous to promote differentiation of stem cells to targeted cells/tissues.[1] In this study, we have developed hydrogel-based synthetic bioactuators using p[($MEO_2$MA-OEGMA)-*co*-PEGDA] hydrogels capable of exhibiting continuous volume changes in response to subtle changes in environmental temperature. Moreover, we have validated the effect of such dynamic multifunctional matrices on stem cell differentiation by examining osteogenic differentiation of human mesenchymal stem cells (hMSCs).

**Methods:** The synthetic bioactuating system involves a fast responsive heating-cooling device and a cytocompatible temperature sensitive hydrogel. The temperature sensitive hydrogel, p[($MEO_2$MA-OEGMA)-*co*-PEGDA], is designed to undergo reversible volume changes responding to perturbations in environmental temperature. $MEO_2$MA-OEGMA oligomers (Mn=10,000) were synthesized from $MEO_2$MA [di(ethylene glycol) methyl ether methacrylate] and OEGMA [oligo(ethylene glycol) methyl ether methacrylate] by using a RAFT polymerization as described by Lutz *et al.*[2] Passage 5 bone marrow-derived hMSCs were dispersed in the precursor solution and photopolymerized (UV light, 365nm, 50μW/cm$^2$) for 5 minutes (**Fig 1**). hMSC-laden oscillating hydrogels were subjected to an oscillatory volume strain of ~ 3% at a frequency of ~ 0.008Hz, by changing the environmental temperature 31.5 & 35.5°C for 1hour/day for 2weeks (**Fig 2**).

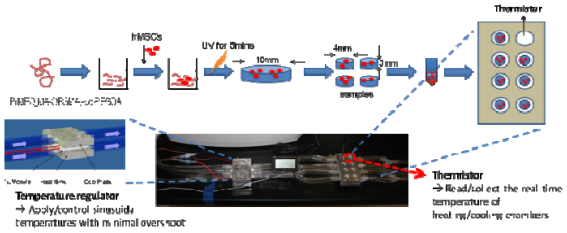


**Fig 1.** Schematics of photoencapsulation of hMSCs and fast-response heating-cooling device.

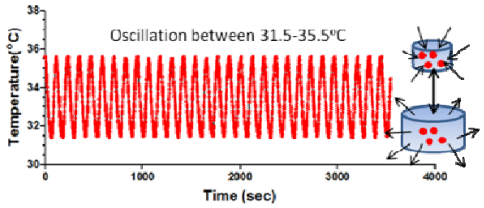


**Fig 2.** Oscillatory heating-cooling temperature profiles and schematics of swelling-deswelling behavior of cell-laden hydrogels.

**Results:** hMSCs in oscillating hydrogels exhibited similar cell viability to that of non-oscillating hydrogels (**Fig 3a-b**). Osteogenic differentiation of cell-laden hydrogels in the absence/presence of oscillation was assessed by Alizarin Red S staining and quantitative RT-PCR (**Fig 3c-d & Fig 4**). As seen from Fig. 4, hMSCs cultured using oscillating hydrogels showed higher osteogenic differentiation. In addition to osteogenic markers, hMSCs in oscillating gels showed upregulation of heat shock protein hsp72. Upregulation of hsp72 has been shown in cells exposed to various conditions such as heat, protein degradation, hypoxia, low amount of glucose, shear or mechanical stress.[3]

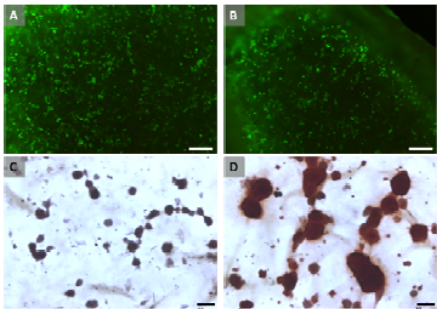


**Fig 3.** Live/Dead assay (A, B) and Alizarin Red S staining (C, D) of hMSC-laden hydrogels after 14 days of *in vitro* culture in the absence (A, C) and presence (B, D) of oscillation. (Scale bars are 200μm and 20μm for Live/Dead and Alizarin Red S staining, respectively.)

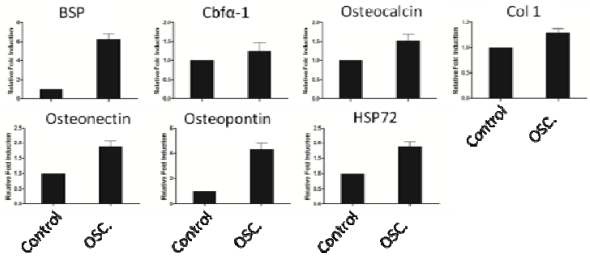


**Fig 4.** Quantitative RT-PCR analysis of osteogenic gene expressions of hMSCs cultured for 14 days in the absence (Control) and presence (OSC) of oscillation.

**Conclusions:** We synthesized p[($MEO_2$MA-OEGMA)-*co*-PEGDA] hydrogels-based synthetic multifunctional bioactuators, which can provide 3D structural support and oscillatory strains simultaneously to the encapsulated hMSCs. Our data further show the beneficial effect of synthetic bioactuators on osteogenic differentiation of hMSCs.

**References:**

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2. Lutz J. J Am Chem Soc, 2006, 128 (40): 13046-13047.
3. Febbraio MA. J Appl Physiol, 2000, 89: 1055-1060.