Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Supporting Information

## Molecular Beacon-Loaded Polymeric Nanoparticles for Non-Invasive Imaging of mRNA Expression

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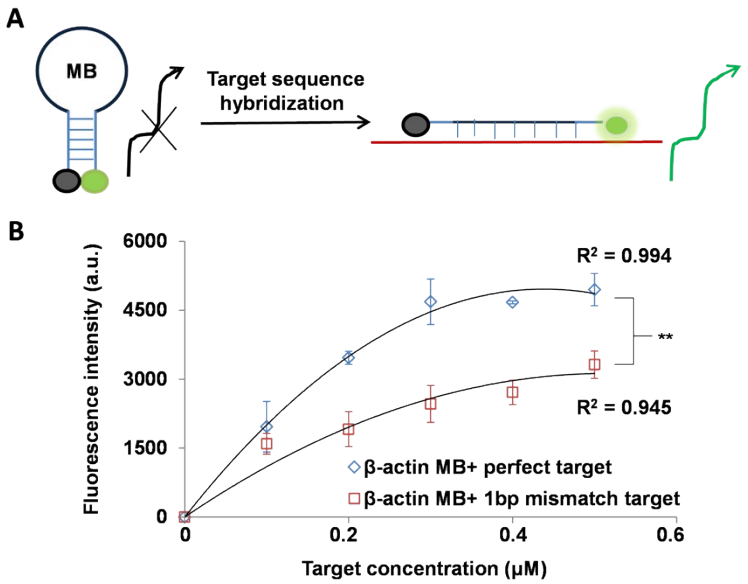


**Figure S1**. MB hybridization with β-actin mRNA. **A**) Schematic illustration of the working principle of MBs. **B**) Hybridization of β-actin mRNA MBs (0.5 μM) with various concentrations of complementary target sequence or one-base mismatch sequence. n≥4, ** indicates $p<0.01$ comparing the normalized fluorescence intensity following perfect or 1 base pair mismatch target hybridization, at various concentrations.

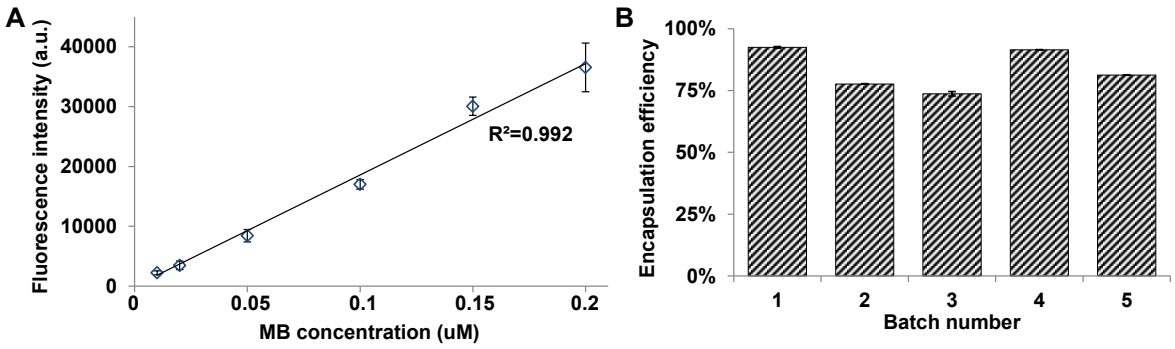


**Figure S2**. Characterization of β-actin nanosensors: **A**) Calibration curve of fluorescence intensity versus MB concentration. **B**) Encapsulation efficiency of MBs in MB-NPs for five batches.

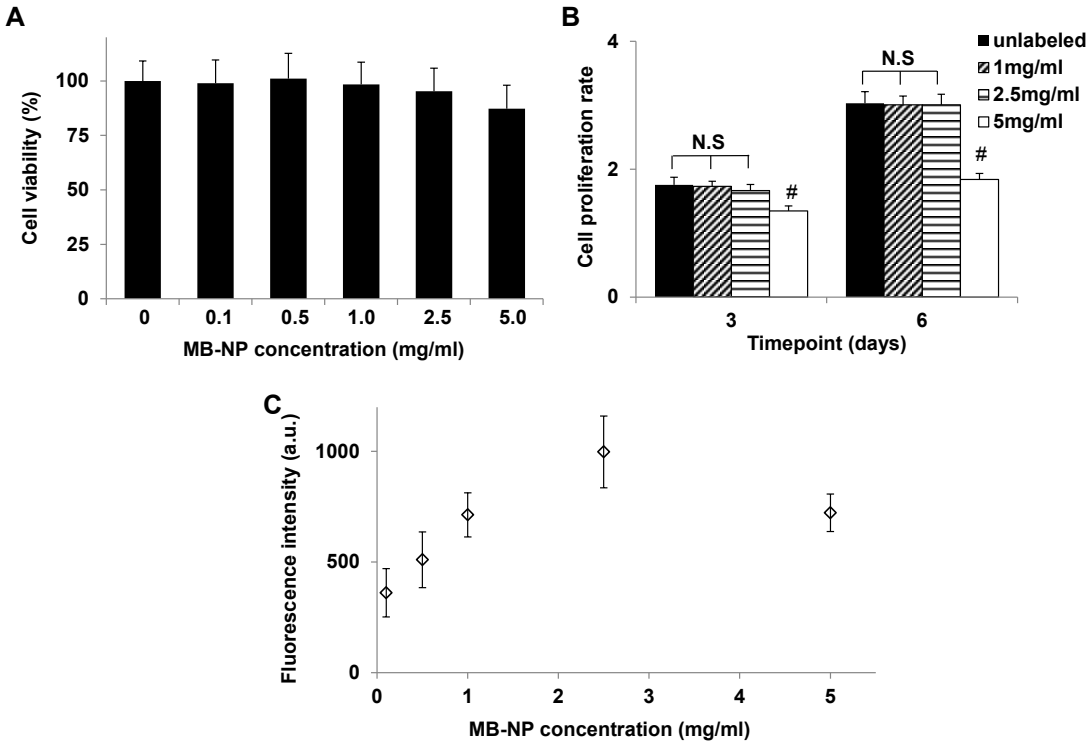


**Figure S3**. Cytotoxicity study of MB-NPs. **A**) MSCs viability assay under the incubation with MB-NPs at the concentrations ranging from 0 to 5 mg/ml for 24 hours. **B**) MSC proliferation assay under the incubation with MB-NPs at the concentrations of 0, 1, 2.5, and 5 mg/ml for 3 and 6 days. **C**) Fluorescence intensity plot of MSCs following MB-NP labeling at various concentrations. Mean ± std, n = 6. N.S: non-significant, # indicates $p<0.001$ to unlabeled group.

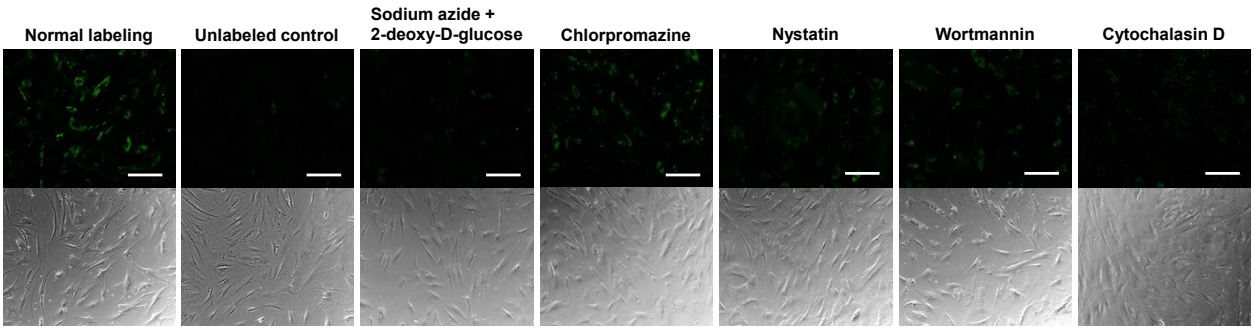


**Figure S4.** Representative fluorescence and bright field images of MSCs following co-incubation with MB-NPs and specific endocytosis inhibitors. Scale bar: 100μm.

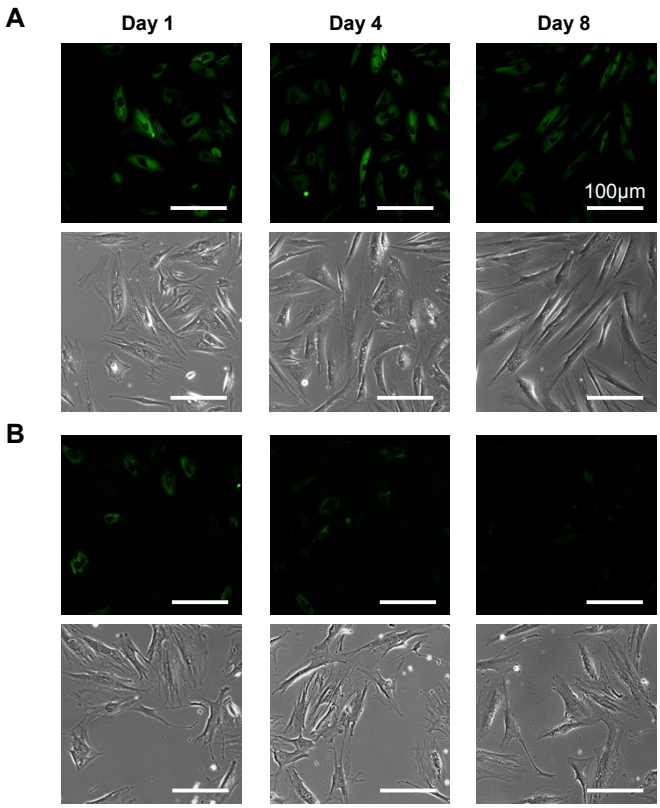


**Figure S5.** Representative fluorescence and bright field images of MSCs labeled with MB-NPs (**A**) and free MBs (**B**) on day 1, 4, and 8 post labeling. Scale bar= 100μm.

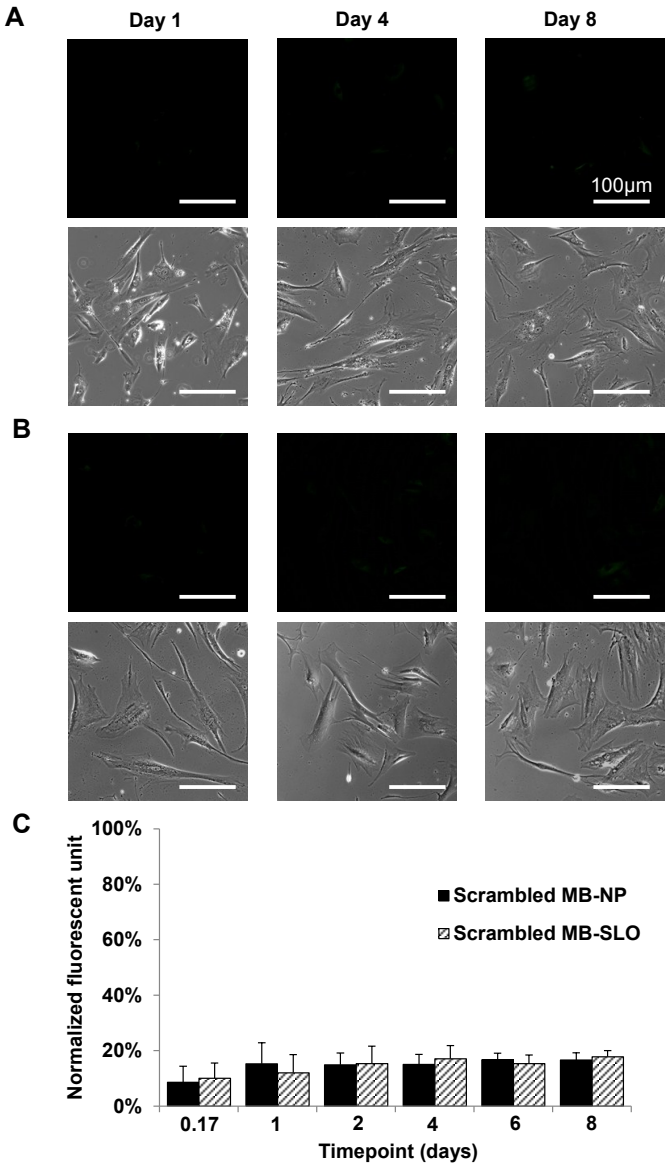


**Figure S6**. Longitudinal imaging of MSCs labeled with scrambled-MB-NPs or free scrambled-MBs. Representative fluorescence and bright field images of MSCs labeled with scrambled-MB-NPs (**A**) or free scrambled-MBs (**B**) over a period of 8 days post labeling. Scale bar= 100µm. **C**) Quantification of fluorescence intensity from MSCs labeled with free scrambled-MBs or scrambled-MB-NPs over the period of 8 days post labeling. Each point is shown as mean ± std of signal intensity of over 150 cells, following the normalization to control β-actin MB-SLO 4 hour group.

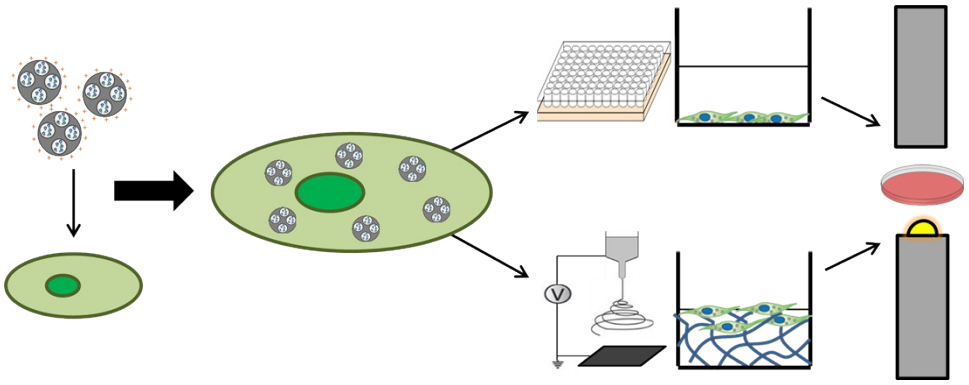

**Figure S7**. Schematic illustration of the β-actin mRNA expression in MSCs with MB-NPs. Following the labeling with MB-NPs, MSCs were seeded on the 2D culture plate or 3D PCL scaffold. Cells were imaged with the fluorescence microscope at day 1, 2, 4, 6, and 8.

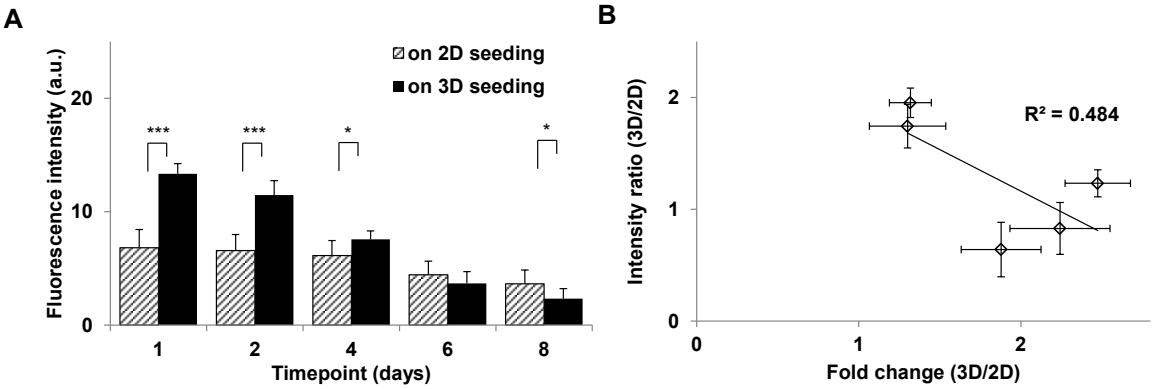


**Figure S8**. Free MBs for monitoring the cellular expression of β-actin mRNA in MSCs cultured on 2D tissue culture plate and 3D PCL scaffold. **A**) Quantification of the average fluorescence intensity per cell at various time point post labeling with free MBs. Each data point is generated by averaging signal intensity of ≥150 cells. **B**) A correlation plot between the ratio of MB fluorescence intensity of MSCs on 3D scaffold and 2D plate, versus the ratio of β-actin fold change in MSCs on 3D scaffold and on 2D plate from RT-qPCR. *: $p<0.05$; ***: $p<0.001$