Azobenzene-based Scaffolds for Photomechanical Stimulation of Stem Cells

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Introduction

Biocompatible stimuli-responsive materials are attractive for creating dynamic platforms to study and manipulate cellular processes such as cell adhesion, migration and differentiation. With light as an external stimulus, photoswitchable molecules like azobenzenes have been utilized to develop light-responsive scaffolds that can reversibly stimulate cells.[1] These systems can have potential applications in the fields of regenerative medicine and biohybrid robotics.

Azobenzenes for Cell Stimulation

- We have developed a photoswitchable biointerface based on a push-pull azobenzene that oscillates between cis and trans states upon green light irradiation. (λ = 530 nm)[2]
- The azobenzene was functionalized with an cRGDfK moiety to bind cell surface integrins, rapid isomerization of azobenzene exerts oscillatory nanoscale forces (~10^2-10^5 Hz) on integrins.[2]

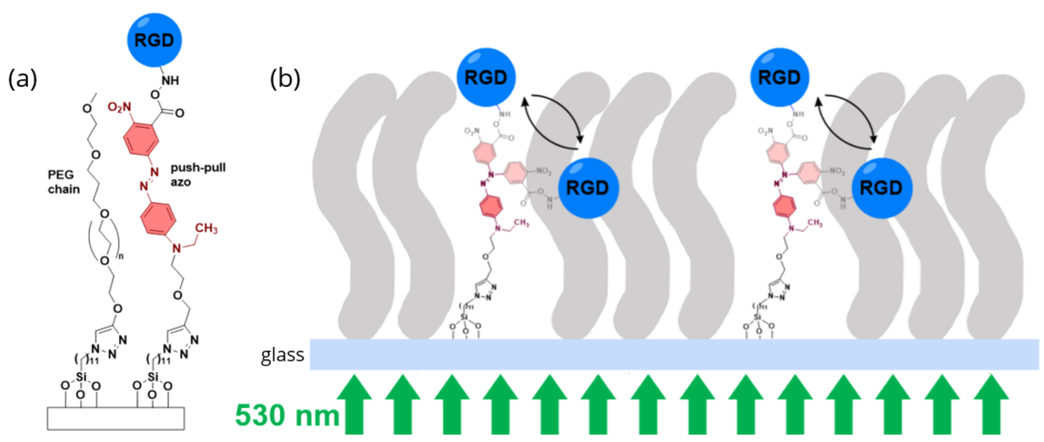


Figure 1: Schematic of the RGD push-pull azobenzene system. (a) Glass functionalized with PEG2000 and RGD push-pull azobenzene. (b) Irradiation of functionalized monolayer causes azobenzenes to rapidly oscillate and exert a mechanical pulling force on integrins responsible for cell adhesion.[2]

- **Fibroblasts respond to mechanostimulation by strengthening adhesion to substrate and upregulating focal adhesion proteins. [2]**

? Investigate the effects of azobenzene mechanostimulation on mesenchymal stem cell (hMSC) differentiation and other cellular processes such as cell migration and cell adhesion.

Surface and Photoswitching Characterization

- Functionalized surface was characterized using **Stimulated Raman Scattering (SRS) confocal microscopy**.
- **PEG forms aggregates, while azobenzene is uniformly present.**

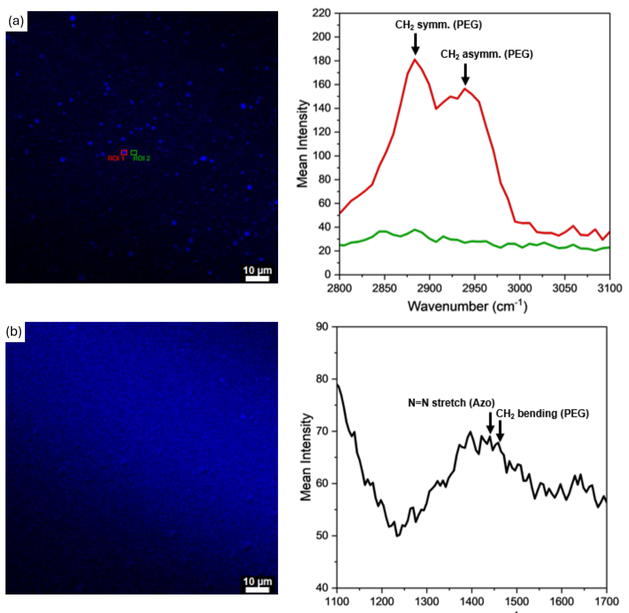


Figure 2: Characterization of azobenzene surface *

(a) SRS confocal image at 2883 cm^{-1} and Raman spectrum with peaks corresponding to PEG (CH_2 symmetric stretch ~2888 cm^{-1}, CH_2 asymmetric stretch ~2941 cm^{-1}). **Red: aggregate**, **green: non-aggregate**

(b) SRS confocal image at 1457 cm^{-1} and Raman spectrum with peaks corresponding to Azobenzene (N=N stretch ~1440 cm^{-1}) and PEG (CH_2 bending vibration ~1460 cm^{-1}).

*Measurements performed at Leica Imaging Center, Mannheim, Germany.

- Photoswitching (in solution) further studied by **Brillouin Spectroscopy**.[3]

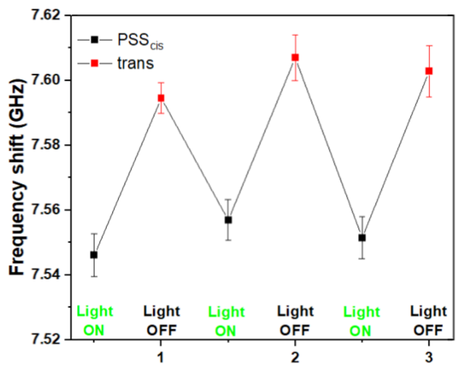
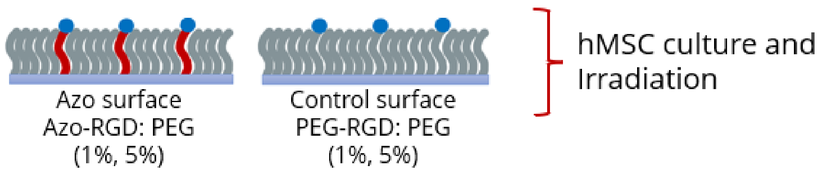


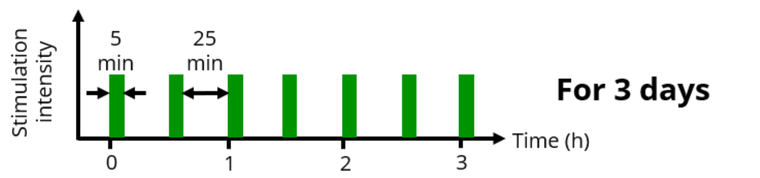
Figure 3: Photoswitching observed by Brillouin spectroscopy. Changes in Brillouin frequency shift over 3 cycles of photoswitching by alternating light (565 nm, trans → PSS_{cis}) and dark (PSS_{cis} → trans) periods.

Stimulation of Stem Cells on Azo Surfaces

Experimental samples



Minute-scale Irradiation using LEDs (530 nm)



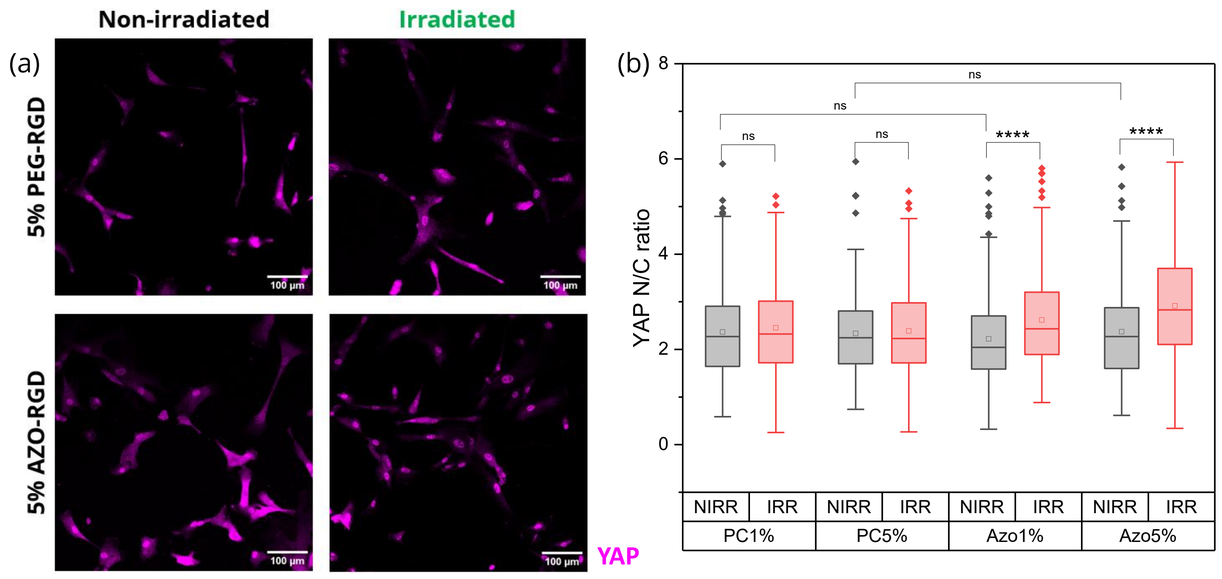
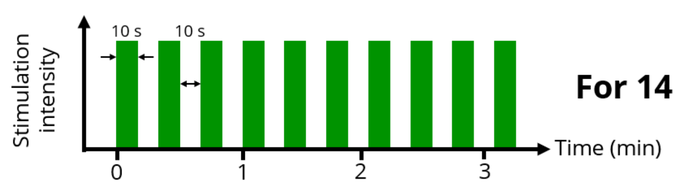


Figure 4: Quantification of YAP nuclear translocation upon photomechanical stimulation. (a) Representative immunofluorescence images of hMSCs stained for YAP. (b) Changes in YAP nuc/cyto ratios upon irradiation. (n>200 per group, ****p<0.0001, obtained using two-way ANOVA followed by Tukey's post hoc test.

Long-term photomechanical stimulation increases YAP nuclear localization in hMSCs, without significant light effects.

Second-scale Irradiation using laser (561 nm)



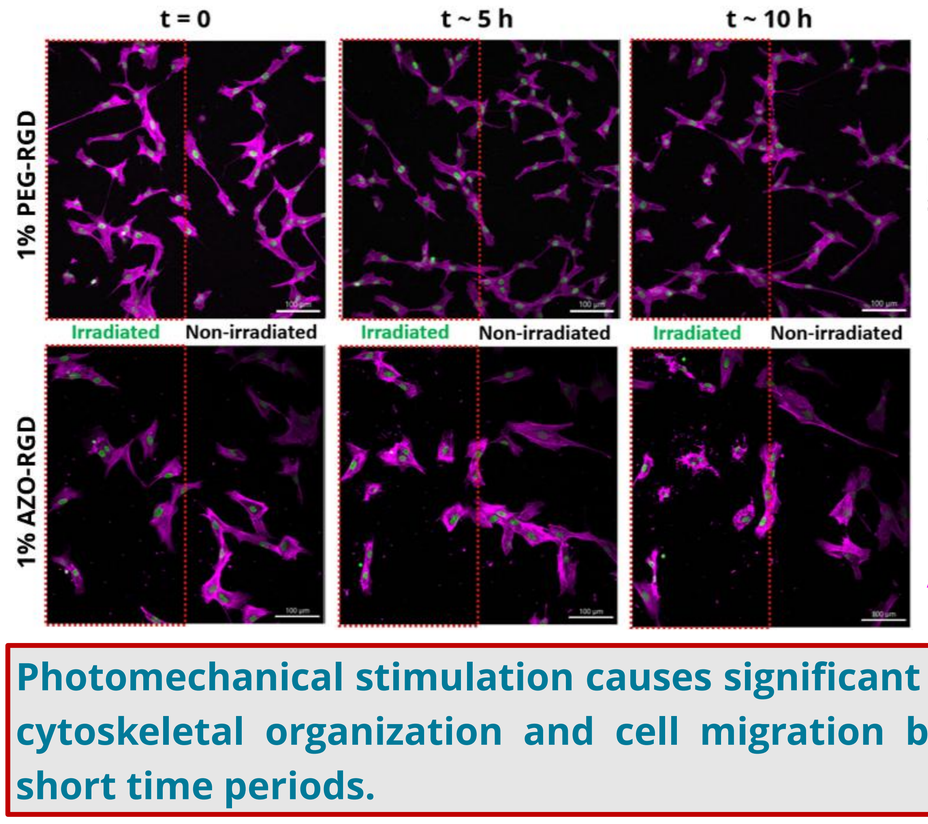


Figure 5: Live imaging of hMSCs upon photomechanical stimulation.
In each field of view (FOV), the left half is irradiated (561 nm, 5% laser power, 8 µs/pixel), the right half is non-irradiated. Entire FOV is imaged once every 10 min. Cells are stained with FastAct-640 (actin) and SPY DNA-505 (nucleus).

Actin
Nucleus

Photomechanical stimulation causes significant changes in actin cytoskeletal organization and cell migration behaviours over short time periods.

Outlook

- Study differentiation markers for second-scale and subcellular stimulation.
- Study spatial and temporal stimulation patterns.
- 3D photomechanical stimulation – using light-responsive hydrogels.

References

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2. Kadem, L. F., Suana, K. G., Holz, M., Wang, W., Westerhaus, H., Herges, R., Selhuber-Unkel, C. *Angew. Chem. Int. Ed.* **2017**, *56*, 225-229.
3. Wang, Z., Jiang, Q., Barwig, C., Mishra, A., Ramesh, K., Selhuber-Unkel, C. *J. Phys. Photonics.* **2024**, *6*, 035024.

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