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Reversing Adhesion with light: A General Method for Functionalized Bead Release from Cells

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Coated beads retain great importance in the study of cell adhesion and intracellular communication; we present a generally applicable method permitting spatiotemporal control of bead adhesion from cells. Herein we demonstrate *in vitro* release of a Poly-D-Lysine layer from anionic polystyrene beads, allowing complete bead release from rat cortical neurons post-adhesion.

Synthetic beads have emerged as a useful tool to present adhesive molecules to cells in a geometrically controlled and localized fashion.[1] Coating sepharose beads with poly-basic compounds – not neutral or anionic compounds – and presenting them to neurons forms pre-synaptic elements, as demonstrated by Burry and co-workers ~30 years ago.[2,3] In 2009, Lucido et. al., showed that PDL-coated beads in simultaneous contact with axons and dendrites stimulate the formation of native synapse and remain irreversibly attached to the neurons,[4] and that these connections are functional.[5] Beads are also used to sort and analyze cells,[6] and have additionally most recently been used to mechanically manipulate the formation of neural networks.[7] However, tuning and controlling the PDL bead adhesion to the neurites remains a challenge, especially the question of how to release the beads from the sample without affecting the cellular response to PDL, which could allow future access to a differentiated cell end.[8,9] Remarkable progress in synthesizing specific biomaterials that can control cell adhesion under various stimuli has been made.[10–15] A general protocol for surface functionalization yielding a releasable charged layer however, via an external trigger, would provide a valuable tool for biological and materials science in that any poly-cationic coating could be subsequently coated, tested, and released with ease on demand.

Photo-response in biologically relevant materials has been demonstrated with many different chromophores,[16] such as azobenzenes,[17,18] coumarins,[19,20] spiropyrans,[21] and nitrobenzoyl ethers (NBEs).[22] Upon near-UV irradiation, NBE derivatives undergo a Norrish type II rearrangement resulting in an irreversible cleavage of the bond linking the heteroatom *ortho* to the nitro group from the ring. The use of these compounds is favourable due to their high two-photon cross-section and absorption tunability.[23] Engineering and design of nitrobenzoyl moieties has been applied to afford irreversible and drastic changes to macromolecular architectures,[22] surface properties,[24] cell capture and release in hydrogels[25] as well as control over polyelectrolyte multilayer decomposition.[26,27] The photodecomposition pathway of NBEs has also been extensively studied,[28] and presents an attractive choice for reliable, irreversible and relatively benign material control. The new protocol presented here offers a rapid and simple method to photo-release polycation coated microparticles from their adhesion by cells.

Candidate molecules (Figure 1) were designed based on previously reported photo-cleavable architectures for biological applications.[13,29] Chromophore (**1**) was coupled to the carboxylic acid functionalization of polystyrene microparticles, replacing the outermost negative charge with a similar carboxylic acid, allowing it to be employed without modification of usual protocols. The negatively charged bead was then coated by immersion in a PDL solution resulting in charge reversal through electrostatic interactions between the carboxylate end of **1** and the amine side chains of the poly-peptide. This formed polyelectrolyte complex remains stable in solution while the bead is adhered by a neuron. Irradiation with violet light to cleave the linker results in the release of the outer PDL layer from the bead and release of the bead from the cell. A scheme of this process can be seen in Figure 1.

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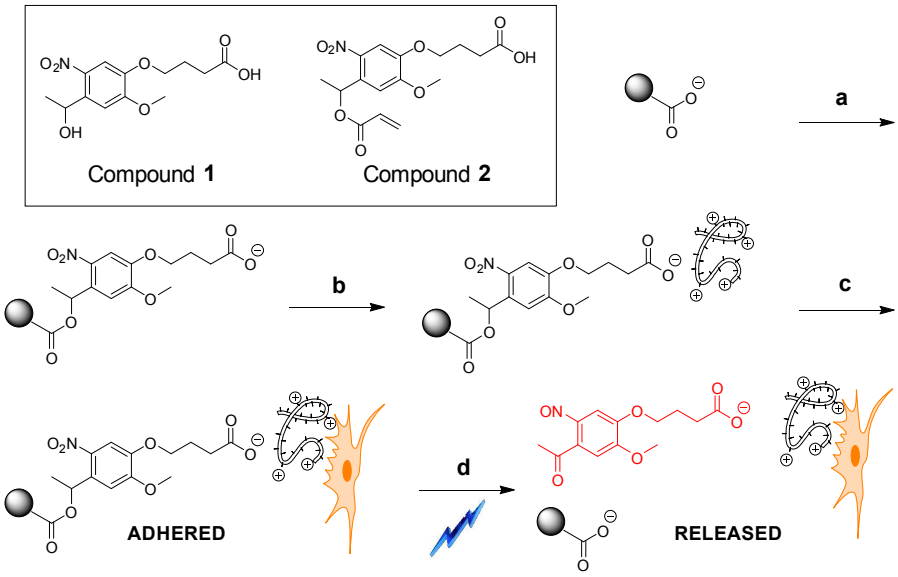

Fig. 1 Structures used in this study and scheme for use in controlled bead release. **(a)** A carboxylated polystyrene microsphere is treated with photo-release Compound **1** (using EDC, NHS, and DMAP in acetonitrile) (**b**) the functionalized bead is coated in poly-D-lysine (**c**) the coated bead is brought in contact with a cell (**d**) and irradiated with 405 nm light, releasing the bead from the cell.

In designing light-responsive materials for use in contact with biological systems, avoiding irradiation that is harmful to cellular systems is critical. The synthesis of molecule **1** yields a chromophore with significant absorption in the visible region (>380 nm). As can be seen in Figure 2, this is due to the spectral tunability of NBEs and would not be evident from the starting material acetovanillone. The resulting bathochromic shift of the n→π* transition renders it accessible to readily-available microscope 405 nm light sources.

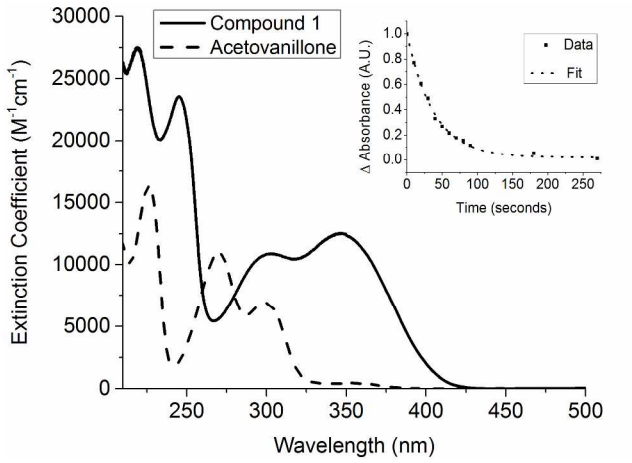

Fig. 2 UV-vis absorption traces in acetonitrile of photo-release Compound **1** showing an extinction coefficient of 1,300 $M^{-1}cm^{-1}$ at 405 nm, compared to effectively no absorbance at 405 nm in the parent acetovanillone. Inset: first-order decay kinetics of **2** in Neurobasal cell media upon irradiation of 405 nm light, with full spectra in the ESI.

Along with a non-damaging absorption spectrum, a second criterium of a successful system for biological applications is rapid actuation. Although the photo-decomposition of NBEs has been studied previously, these assays were performed in organic solvent or buffer and not in a biologically relevant environment such as cell media. Acrylic, itaconic, and methacrylic acid were identified as the three potential co-monomers present during the emulsion copolymerization of the beads,[30] resulting in the generation of the surface-functionalized beads. As such a similar ester leaving group synthesized from an acrylate monomer of Compound **1** was prepared to determine photo-physical parameters in solution (Compound **2**). These measurements (Figure S1) show ready decomposition of **2** when irradiated with 405 nm light with a half-life of 26 seconds at a power of 103 mW/cm^2.

Several experimental conditions were evaluated to ensure successful coupling of the photo-release molecule, which is only sparingly water soluble. Due to the majority polystyrene composition of the beads and their density (1.05 g/mL), solvent and reagent selection was limited, leaving acetonitrile as our choice solvent. The selection of a base in coupling reactions was also limited as we observed that liquid bases common in these coupling reactions such as Hunig's base, triethylamine, collidine, and pyridine dissolve or swell polystyrene to some extent, leaving DMAP (4-Dimethylaminopyridine) as the best choice for a base. The presence of an alcohol and carboxylic acid on **1** called for a two-step coupling process. Coupling agents DCC (N,N'-Dicyclohexylcarbodiimide), EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and HATU were tested, all in the presence of NHS (N-Hydroxysuccinimide) to provide an activated ester which was displaced by **1**, forming a photo-labile

coating on which PDL was coated (see ESI for full details).

To test the release of beads from neurons, hippocampal neurons from Sprague Dawley rat embryos of either sex were prepared as described previously in the literature,[4,31] and added to custom-made microfluidic chambers featuring patterned channels for the axons to grow. These dishes were used as opposed to normal culture dishes to to isolate soma and neurites, which can be localized in the channels, for a schematic please see reference 7. The neurons were tested after 14–18 days in culture, whereby functionalized beads were incubated with neurons in Neurobasal cell media at a10 bead/neuron ratio for 1 h in a humidified 5% CO_2 environment at 37 °C. The dishes were washed twice with Hank's Balanced Salt Solution and imaged in bright field using a LSM 710 Zeiss laser scanning confocal microscope with a 10X objective to detect the beads attached to the cells. After that, samples were exposed to 405 nm laser light for 3 minutes, washed twice with Hank's Balanced Salt Solution and imaged again to evaluate the photo-release efficiency. These washing steps were crucial to observing efficient bead release from the cell bodies, as we suppose that insufficient rinsing allows the cell to remain attached to the bead through a reorganization of the PEM complex.

All of the beads from the EDC-coupled photo-release functionalized microspheres were released from the cells, while none of the beads from the control dish were released. In all cases, 2.72×10^6 beads were treated and coated, of which ~18,000 beads were added to each dish and tested over 8 trials with an average adhesion efficiency (beads adhering to cells initially) of 2.3%. While this is admittedly somewhat low, the statistical probability of a bead landing on a cell leading to it being adhered by the cell is equally low. An average of 46 ± 27 beads were observed in each field of view on the microscope stage adhered to cells after a rinsing cycle with PBS at the beginning of each photo-release experiment. These data are summarized in Table 1, and as expected, demonstrate that all three coupling agents show improvement over the control in releasing cell adhesion. However, EDC is the only coupling agent providing a satisfactory reaction ensuring a thorough functionalization of each bead with photo-release Compound **1**.

Table 1 Bead release from cells after light irradiation as a function of coupling agent.

Coupling agent	DCC	EDC	HATU	Control
Bead release	7%	100%	8%	0%

From this data, we conclude that the beads are successfully coated with a photo-responsive functionality that releases adhesion from a living cell. It is important to note that even the mild irritation of a 3 minute dose of relatively powerful light is not lethal, which could trigger a loss of cell function that would damage adhesion pathways, resulting in a non-specific loss of adhesion across all samples. Also of note is that the native anionic charge on the beads does not provide enough adhesion to retain the polycationic polymer adhered to the bead after photo-release.

The experiment additionally showed that the cells largely preferred to adhere beads on their cell bodies, and less so on the neurites, which grow in the microgrooves oriented vertically towards the bottom of Figure 3. Working with the beads in solution presented many challenges with bead aggregation as well as adhesion to the side walls of centrifuge tubes during manipulation. These issues only became apparent once the beads were transferred from organic to aqueous media, and result in a different number of beads being available to adhere to the cells in the first step of these experiments. Improving these methods to achieve a higher recovery of coated cells as well as a higher number of adhered cells is the subject of future work.

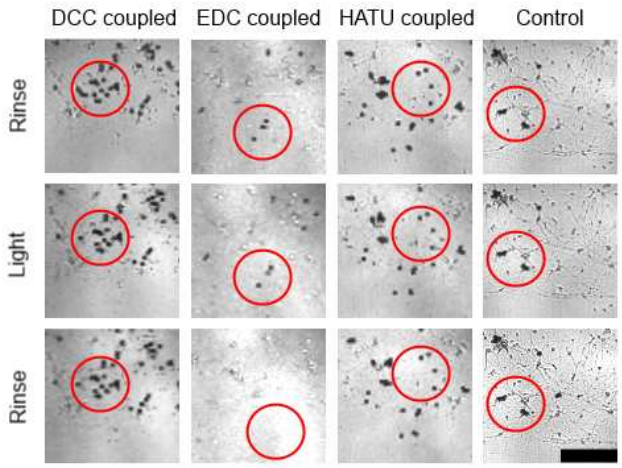

Fig. 3 Representative images showing bead photo-release results from rat cortical neurons. The top row shows bead adhesion after a PBS rinse, the second row shows the beads after irradiation with 405 nm light and the bottom row shows the effect of the final rinse, demonstrating that the EDC treatment is the only one leading to full photo-release of beads. The red circle is added to guide the eye following microscope readjustement after each step, the scale bar is 100 μm.

In summary, we have developed a method which replaces a native negative charge of a bead with a photo-releasable negative charge. While remarkable progress has been made in generating biomaterials that can control cell fate under various controlled stimuli,[21,32,33] we sought to develop a more general approach. Since a cationic surface is known to be critical for optimal cell adhesion, a protocol for surface functionalization yielding a photo-releasable negative charge would provide a valuable tool for biological and materials science in that any poly-cationic coating could be subsequently tested with ease. When a PDL-covered coating of Compound **1** coupled to the PS-COOH beads with EDC/NHS was irradiated with light, it allowed for complete release of structures adhered to cells. Furthering discovery at the materials-biology interface requires simple methods and versatile tools can be easily adapted to a required experiment. Our findings offer a ready and simple method to photo-release polycationically-coated microparticles from their adhesion to cells or other biological tissues.

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All animal experimentation was approved by the institutional animal care committee of McGill University and conformed to the guidelines of the Canadian Council of Animal Care.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details. Material synthesis, coating protocols and *in vitro* testing. See DOI: 10.1039/b000000x/

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