A Dynamic Ligand Presentation System to Examine Adhesive Ligand "Temporal Threshold"

Timothy A. Petrie[1,2], Edward A. Phelps[1,2], Melanie Wirkner[3], Aranzazu del Campo[3], Andrés J. García[1,2]
[1] Woodruff School of Mechanical Engineering; [2]Petit Institute for Bioengineering and Bioscience;
Georgia Institute of Technology, Atlanta GA 30332; [3]Max-Planck-Institut für Metallforschung, Stuttgart, Germany.

Introduction: Cell adhesive interactions with ECMs often rely on time-dependent binding and activation [1]. It is unknown whether adhesion to a ligand is required during the entire differentiation process, or only during a specified time period ("temporal threshold"). Most studies to date have been restricted to static bioadhesive ligand densities. Current stimuli-responsive surfaces, which can respond to thermal and photochemical triggers, lack precise control over exposed ligand densities and are limited to model substrates [2,3]. In order to examine the time-dependent effects of adhesive ligand density on cell function, we have developed a unique caged RGD peptide that is UV-phototriggerable for controlled dynamic presentation of bioadhesive RGD peptides [4]. In this ligand, a photoremovable group adjacent to the aspartic acid residue of RGD effectively "cages" this cyclo(RGDfk) peptide. UV irradiation (360 nm) releases the caging group thereby restoring the adhesive activity of the peptide. Optimization of light intensity and dose time has afforded us precise control over exposed cyclo(RGD) density and kinetics. By tethering this caged ligand onto mixed alkanethiol self-assembled monolayers (SAMs), we demonstrate a powerful *in vitro* system to examine the role of dynamic temporal ligand presentation on cell activities

Materials and Methods: The caged RGD peptide has an UV-labile DMNPB group linked to the carboxylic acid side of the aspartic acid residue of the cyclo(RGD) peptide [4]. Caged RGD was tethered onto mixed SAMs of alkanethiols on Au (1:20 EG_6-COOH:EG_3) via NHS/EDC chemistry [5]. The system is shown schematically in **Fig 1A**. Initial ligand density and adhesive activity were characterized using surface plasmon resonance (SPR) and NIH3T3 fibroblast cell adhesion (1 hr) wash assays. Adhesive strength to mixed static "basal" RGD (0.8 pmol/cm^2) and caged RGD (2.8 pmol/cm^2) densities was quantified with a spinning disk assay [6]. A basal level of linear RGD was tethered to each sample to ensure cell adhesion on all samples. NIH3T3 fibroblasts were cultured (2 hr) in serum, and adhesion strength measured.

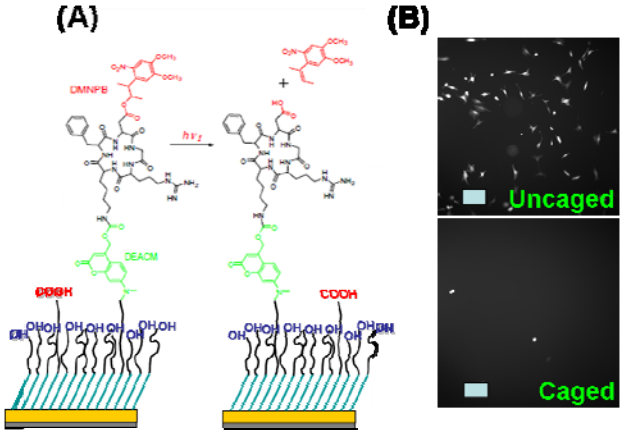

Fig.1: A) Schematic of phototriggerable RGD presentation system, B) Irradiated (10 min) caged RGD surfaces promote robust cell adhesion. Scale bar = 30 µm. Non-irradiated surfaces remain non adhesive for at least 5 days.

Results and Discussion: There are several advantages of using mixed SAMs of alkanethiols for our caged ligand presentation system: 1) the non-ligand tethered base is non-fouling, 2) tethered ligand density can be easily controlled, and 3) ease of uncaging characterization via UV absorbance and SPR. The efficacy of this dynamic caged RGD system in controlling cell adhesion was verified through fibroblast adhesion on irradiated and non-irradiated caged RGD surfaces. Cells did not adhere or spread on non-irradiated samples, but adhered in high numbers and were well spread on irradiated (10 min) samples (**Fig. 1B**). Caged RGD surfaces incubated in cell media for 5d without irradiation remained non adhesive. Moreover, UV irradiation did no detectable damage to adhered cells. To examine the adhesive activity of caged and uncaged substrates, a spinning disk assay was used to quantify the adhesion strength of NIH3T3-E1 fibroblasts on seeded surfaces presenting mixed "basal" densities of static linear RGD + saturating levels of caged cycloRGD containing separate regions of caged and uncaged RGD. Cells on uncaged regions displayed significantly higher adhesive strength (6-fold) than uncaged regions, which were only slightly higher than "basal" linear RGD strength levels (**Fig. 2**). Taken together, this data confirms the robust ability of this phototriggerable system to temporally and spatially present bioactive RGD as well as the physiological stability for longer-term functional studies.

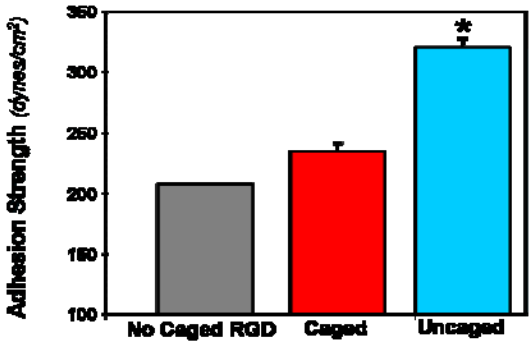

Fig.2: Fibroblasts on uncaged surfaces (with "basal" levels of linear RGD) display higher adhesive strength than on caged RGD surfaces. No caged RGD surfaces consisted of just "basal" static RGD density (0.8 pmol/cm^2)

Conclusions: We have developed a robust, *in vitro* dynamic ligand presentation system which can spatiotemporally control RGD density through a simple phototriggerization mechanism. We are currently evaluating the effect of time-dependent exposure of ligand density on myoblast proliferation, signaling, and differentiation.

References : [1] Rowley et al., *JBMR*, 60, 217-23 (2002); [2] Okano et al., *Biomaterials*,16 :297-303 (1995) ; [3] Yeo et al, *JACS*, 125 :14995-5 (2003) ; [4] Petersen et al., *Angew Chem.*, 47:3192-95 (2008); [5] Petrie et al. *Biomaterials*, 27(31):5459-70 (2006); [6] Garcia et al. *Biomaterials*, 18:1091-98 (1997).

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