Biophotolithographic Method to Create Biomolecular Patterns in Porous Collagen-Glycosaminoglycan Scaffolds

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Statement of Purpose: Modern tissue engineering requires animated biomaterials that incorporate heterogeneous patterns and instructive cellular cues to better mimic native tissues. We have recently developed a suite of technologies that allows us fabricate homologous series of uniform collagen-glycosaminoglycan (CG) scaffold variants with independent control over scaffold microstructural and mechanical properties [1], aligned (anisotropic) CG scaffolds, and multicompartment CG scaffolds that mimic the cartilagenous, osseous, and interfacial regions within osteochondral tissue [2]. Here we describe an approach to generate surface-immobilized patterns and gradients of solution-phase biomolecules into our CG scaffold systems [3]. This technology enables full, independent control of microstructure, mechanics, and surface chemistry within our CG scaffold system.

Methods: CG scaffolds were fabricated by freeze-drying a suspension of type I collagen and chondroitin sulfate in acetic acid. We modified a benzophenone (BP) method recently developed to photochemically pattern 2D substrates to enable BP functionalization of the CG scaffold [4]. An Ar+ Ion laser with photomask was used to expose regions of the scaffold to enable BP-mediated attachment of concanavilin A-biotin (ConA-biotin), Fibronectin (Fn), as well as E- and N-cadherins; patterns were visualized via fluorescent secondary antibodies using an LSM 710 confocal microscope. XRD was used to assess material crystallinity. CG scaffolds (BP functionalized and blank controls) were pulled to failure using an MTS Instron 2 to determine tensile elastic modulus. Cell number and metabolic activity were measured using a fluorescent DNA-binding assay and alamarBlue reduction respectively.

Results: UV excitation of BP (conjugated to scaffold free amines) creates a transient diradical that allows covalent attachment of biomolecules via a C-H bond insertion reaction. Excited BP molecules that do not undergo this insertion reaction return to the ground state and can be excited again, enabling multiple biomolecules to be patterned on a single construct. Various biomolecular patterns were created in CG scaffolds including gradients, stripes, and multicomponent patterns (Figure 1).

XRD analysis of the CG scaffold, the BP modified (CG-BP) scaffold, and the Fn functionalized (CG-BP-Fn) scaffold showed small changes in the CG peak post modification, but the scaffold maintains its microstructural properties and the collagen was not denatured. CG-BP scaffolds displayed significantly higher tensile moduli than CG controls; this is likely due to crosslinking effects of solvents used during BP conjugation. There were no detrimental effects from BP conjugation observed for MC3T3-E1 pre-osteoblast attachment and metabolic activity after 2 and 7 days, indicating BP is not toxic to cells. CG-BP scaffolds actually had significantly higher cell number and metabolic activity at 7 days compared to CG controls; however this is likely due to differences in modulus.

The Fn ligand has recently been reported to mediate initial cell attachment (< 1 hr) to CG scaffolds [5]. Here we compared MC3T3 attachment to CG, CG-Fn (Fn passively adsorbed), and CG-BP-Fn scaffolds after 30 minutes; the experiment was performed in PBS to prevent non-specific adsorption of exogenous factors from serum. CG-BP-Fn scaffolds had more than twice as many cells attached as CG or CG-Fn scaffolds ($p < 0.0001$), suggesting that the BP photopatterning method can be used to confer specific bioactivity to CG scaffolds.

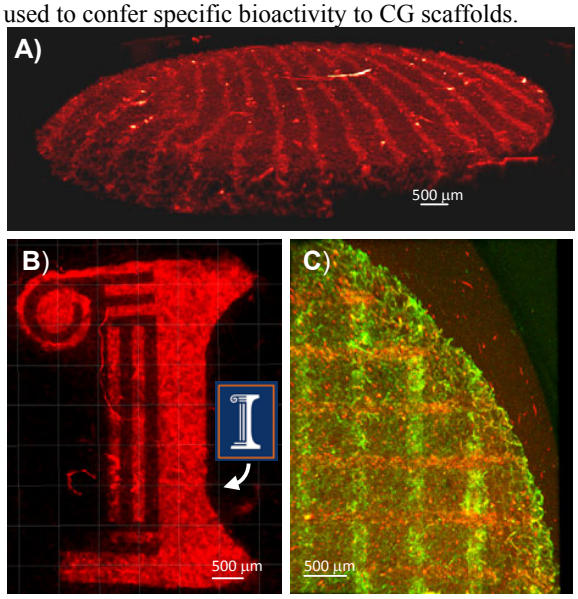

Figure 1. A) 1-component patterns of ConA-biotin visualized with Qdot 525 conjugated to streptavidin. B) 1-component pattern of ConA-biotin in Column I logo. C) 2-component patterns of N-cadherin (horizontal red stripes) and Fibronectin (vertical green stripes).

Conclusions: We have demonstrated the ability to conjugate BP to CG scaffolds and spatially pattern biomolecules in a controlled and defined manner. Our approach represents a general strategy to pattern any biomolecule of interest as it only requires a C-H bond for immobilization. This method is a direct, photolithographic approach to generate complex, multicomponent patterns or gradients of biomolecules to more accurately mimic the heterogeneity of native tissues. Ongoing work aims to generate multicomponent patterns and gradients of soluble factors (PDGF-BB, IGF-1) and proteoglycans (decorin, aggrecan) to investigate tendon cell behaviors in both single compartment (isotropic and anisotropic) and multicompartment (tendon-bone interface) CG scaffolds.

References:

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