Tissue Engineering

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Dynamic 3D Patterning of Biochemical Cues by using Photoinduced Bioorthogonal Reactions**

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Recreating an appropriate microenvironment for the purpose of regenerating tissues or organs poses clinically relevant issues that intrigue every mind, regardless of background. Living tissue, however, is an enigmatic amalgamation of cells that is surrounded by the extracellular matrix, a hierarchically organized hydrogel that is made of polysaccharides and proteins with specific biochemical and biomechanical functions.[1] Controlling the 3D microenvironment has been an important aspect, not only for tissue engineering, but also to study the cells in more biologically relevant systems. Researchers have approached this problem with clever designs by combining materials with concepts from cell biology to associate specific cellular functions with controllable cues.[2] These cues range from cell adhesion ligands, soluble cytokines/growth factors, mechanical properties of the substrate (that is, stiffness, porosity, etc.), 2D versus 3D architectures, and many more variations that are still being explored with diverse cell types.

Whereas the advent of some earlier 3D materials forged a path towards a greater understanding of the cells, the limitations of these materials became quite apparent. The typical platform consists of a static architecture with fixed cues, like a mashed network of electrospun fibers or a porous hydrogel that contains randomly embedded bioactive molecules.[2–4] In most cases, these previous systems possess poorly defined spatial patterns of biological moieties. Alternatively, an ideal scaffold should address the dynamic nature of the cells and their microenvironment. Therefore, a platform that possesses both high spatial resolutions and precise temporal control of the signaling motifs would indeed be useful for studying how cells work in dynamic networks.

As demonstrated by DeForest and Anseth,[5] 3D spatial programming of a hydrogel platform can be achieved through bioorthogonal photochemistries to attach or remove functional groups by using multiphoton light, which allows dynamic control of the pericellular region to test how cells would respond to biochemical changes in their local environment. Although using multiphoton chemistry to achieve a 3D immobilization of proteins or other signaling motifs in hydrogels has been reported by a number of groups,[6,7] DeForest and Anseth further advance this technology by employing a combination of three bioorthogonal chemical processes (Figure 1). In the first step, the strain-promoted azide–alkyne cycloaddition (SPAAC) reaction forms the hydrogel.[8] The bis-azido crosslinker contains an alkenyl tag which can undergo a photocontrolled thiol-ene "click" reaction[9] to pin down the cell-binding peptide within the hydrogel network. Each peptide contains a photocleavable *o*-nitrobenzyl ether linker that can be removed to provide spatiotemporal control over the ligands. The application of two click processes, that is, the SPAAC and the thiol-ene reactions, with multifunctional molecular designs provide a clever approach to explore a broad array of bioactive motifs with good spatiotemporal control.

Furthermore, this process is used to present gradients of cell-binding moieties, either linear or exponential, by applying a user-defined gradient of light exposure. Such functionally graded materials with spatiotemporal control provide powerful methods to study many biological processes, which include directionality for neuron outgrowth from the mammalian retina, and bone–muscle transitions with tendon/ligament regeneration.[10,11] The concept of positional information, that a cell knows its position in the gradient and determines its developmental fate accordingly, could be further elaborated with the 3D model reported by DeForest and Anseth. For example, as the cell transitions from one position to another (that is, as the ligament transitions from bone to muscle), the surrounding signaling moieties follow specific gradients and patterns in the natural tissue growth or even embryo development.[12,13] The use of static 3D matrices would limit the cells to one artificial stage of development unless bioactive signals can be altered in time and space. With the spatiotemporal control, molecules would be presented at the "right time and right place", and possibly with the proper concentrations to recognize the threshold levels that are required to initiate signal recognition.

A platform that has an added level of sophistication takes in vitro experiments one step closer towards physiologically relevant systems. The beauty of the system reported by DeForest and Anseth comes from the simple chemistries that can be performed even in a living cell system. These

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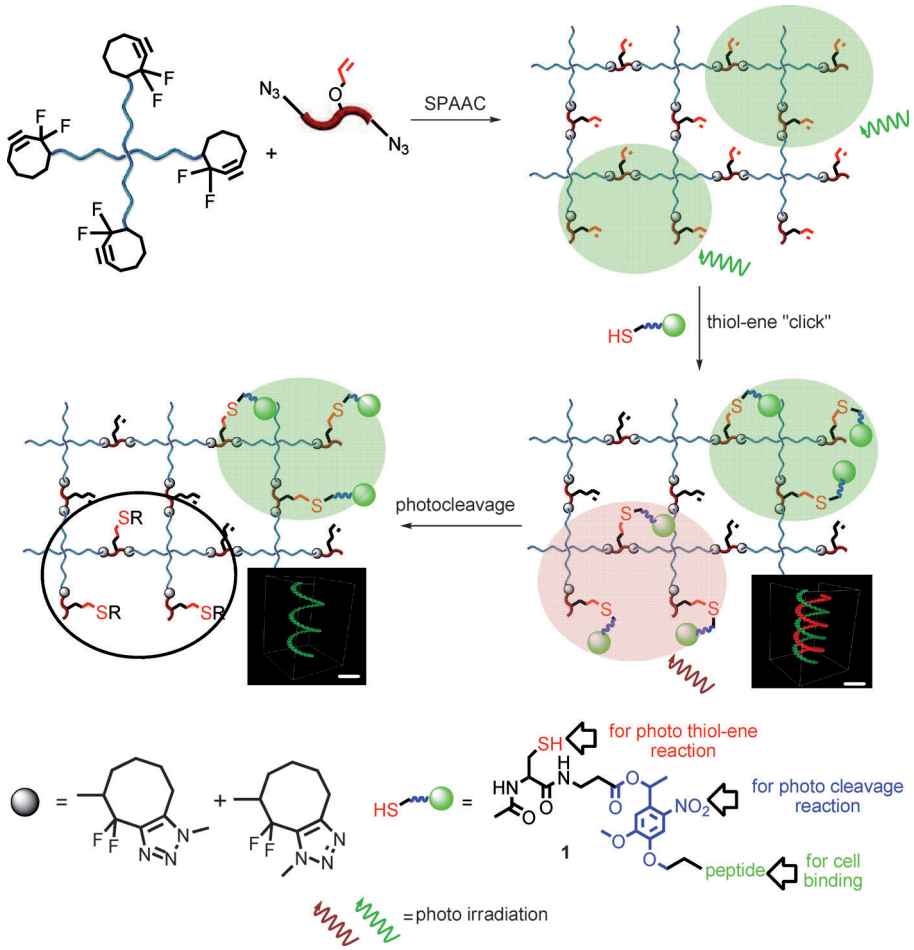

Figure 1. Illustration of the synthetic approach, which involves three bioorthogonal chemistries. The hydrogel network is formed by a SPAAC reaction between difluorocyclooctyne-terminated polyethylene glycol and bis-azido crosslinkers. These anchor points (as indicated by gray spheres and red highlighted line) also contain an overhanging alkenyl group for photoinduced thiol-ene chemistry to regioselectively confine the bioactive ligands. The ligand **1** contains a reactive thiol group (red) for thiol-ene chemistry, and a biorelevant, fluorescent peptide group (green) bridged by a photocleavable *o*-nitrophenyl ether (blue). To demonstrate the chemical selectivity and spatial resolution, two different, fluorescently labeled ligands (red and green) are positioned in a helical arrangement by thiol-ene "click" (bottom right inset). The ligands with the red fluorophore peptide conjugates are then selectively removed by cleavage of the *o*-nitrobenzyl group, which leaves only the ligands with the green fluorophore (bottom left inset).

approaches should ultimately help us design smarter biomaterials with a range of chemical tools for a better understanding of the interactions between cells and their microenvironment.

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