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Thiol-ene and photo-cleavage chemistry for controlled presentation of biomolecules in hydrogels

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ABSTRACT

Hydrogels have emerged as promising scaffolds in regenerative medicine for the delivery of biomolecules to promote healing. However, increasing evidence suggests that the context that biomolecules are presented to cells (e.g., as soluble verses tethered signals) can influence their bioactivity. A common approach to deliver biomolecules in hydrogels involves physically entrapping them within the network, such that they diffuse out over time to the surrounding tissues. While simple and versatile, the release profiles in such system are highly dependent on the molecular weight of the entrapped molecule relative to the network structure, and it can be difficult to control the release of two different signals at independent rates. In some cases, supraphysiologically high loadings are used to achieve therapeutic local concentrations, but uncontrolled release can then cause deleterious off-target side effects. *In vivo*, many growth factors and cytokines are stored in the extracellular matrix (ECM) and released on demand as needed during development, growth, and wound healing. Thus, emerging strategies in biomaterial chemistry have focused on ways to tether or sequester biological signals and engineer these bioactive scaffolds to signal to delivered cells or endogenous cells. While many strategies exist to achieve tethering of peptides, protein, and small molecules, this review focuses on photochemical methods, and their usefulness as a mild reaction that proceeds with fast kinetics in aqueous solutions and at physiological conditions. Photo-click and photo-caging methods are particularly useful because one can direct light to specific regions of the hydrogel to achieve spatial patterning. Recent methods have even demonstrated reversible introduction of biomolecules to mimic the dynamic changes of native ECM, enabling researchers to explore how the spatial and dynamic context of biomolecular signals influences important cell functions. This review will highlight how two photochemical methods have led to important advances in the tissue regeneration community, namely the thiol-ene photo-click reaction for bioconjugation and photocleavage reactions that allow for the removal of protecting groups. Specific examples will be highlighted where these methodologies have been used to engineer hydrogels that control and direct cell function with the aim of inspiring their use in regenerative medicine.

1. Introduction

Scaffolding matrices have emerged as promising platforms for the delivery of therapeutics and cells *in vivo* [1]. Matrices loaded with releasable biomolecules can be implanted *in vivo* to promote tissue regeneration by signaling to endogenous cells or to cells delivered along with the matrix [2]. A common strategy for controlling the release of biomolecules involves their physical entrapment within scaffolding matrices [3–5], causing many high molecular weight molecules, such as cytokines and growth factors, to slowly diffuse from the matrix where they can direct cellular migration, differentiation and proliferation to promote tissue regeneration [6]. For example, Hubbell et al. demonstrated the promotion of bone regeneration *in vivo* through implantation of a poly(ethylene glycol) (PEG) hydrogel that was designed to elute bone morphogenetic protein 2 (BMP-2) to the surrounding tissues as the gel degraded [7]. Diffusion controlled release systems are versatile and can be used to deliver large doses without any chemical modification of the biological signal. However, there is limited control over the release profile, especially when loading multiple biomolecules, and maintaining a physiologically relevant concentration over extended time periods typically necessitates supraphysiological loading that can lead to deleterious off-target side-effects [8]. Indeed, the high levels of BMP-2 loaded in scaffolds used for regenerating bone tissue via diffusion controlled delivery systems led to severe ectopic bone growth [9]. With the rising use of regenerative medicine

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inspired therapeutics, there is a significant need for new biomaterials that allow versatile and precise control over the delivery of multiple biomolecules, from small molecules to proteins, in a manner that maintains their activity and ability to signal to targeted cells.

In designing biomaterial platforms to direct cellular function, it is necessary to consider the role that the extracellular matrix (ECM) plays in influencing cells *in vivo* (Fig. 1). Tissues are composed of cells and their associated ECM—the complex, tissue-specific, three-dimensional structure that provides cells with the requisite mechanical and biological signals for healthy function [6]. In native tissues, the ECM sequesters soluble biomolecules [11], a process that functions to shield them from proteolytic degradation [12]. Moreover, the bioactivity of many proteins, such as transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF), is potentiated by affinity binding to the ECM [11–13]. This suggests that cells can interact with biomolecules through the ECM directly and that the context in which a cell interacts with biomolecules can affect its function. Thus, an emerging approach to mimic biomolecule-ECM interactions is to use affinity binding [14,15] or covalent immobilization [16] of biomolecules to matrices.

In addition to how biomolecules are presented to cells, the choice in the biomaterial scaffold itself can influence cellular function. In fact, there is a growing appreciation for the role of biophysical cues, and their potential synergies with biochemical cues, on numerous cell processes [17–19]. One striking example comes from the Blau group, where they demonstrated that muscle satellite stem cells cultured on hydrogels with mechanical properties similar to skeletal muscle engrafted more efficiently and led to significantly more muscle regeneration than those cultured on gels in which the cells could sense the stiffness of the underlying tissue culture polystyrene (TCPS) [20]. Further, the authors discovered that satellite stem cells from aged mice require both the appropriate biophysical (substrate stiffness) and biochemical (mitogen activated protein kinase inhibitor) signals to efficiently reprogram them to a proliferative phenotype capable of muscle repair [21]. Taken together, these results demonstrate how both the context in which biomolecules are presented to cells and the scaffold on which they are presented can affect a biological response.

The emerging role of scaffold interactions with cells and biomolecules have led the field of regenerative medicine to focus on the design of ECM mimics capable of delivering viable cells and biomolecules to promote healing *in vivo* [22]. Hydrogels hold promise to fulfill these needs because they allow for complete user control over their material properties [23]. Hydrogels are synthetic scaffolds formed by the crosslinking of hydrophilic synthetic polymers or biomacromolecules such as poly(ethylene glycol) [24], poly(vinyl alcohol) [25], collagen [26], hyaluronan [27], and alginate [28] to afford matrices. Many mild crosslinking strategies have been developed, so hydrogels can be synthesized directly in the presence of tissues [29], cells [30], and biologics [7]. Physical or chemical crosslinks render the hydrogels insoluble, but they typically imbibe large amounts of water [31]. The high water content imparts mechanical properties that are similar to many soft tissues and transport properties that are desirable for delivering signals to cells [32]. Numerous material properties can be adjusted by changing the crosslinking density of the polymer network to engineer gels with properties of interest for selected applications [33].

Of the many materials from which a hydrogel can be assembled, there has been significant interest in PEG hydrogels. From an application standpoint, PEG is widely used in clinical medicine, and from a fundamental standpoint, PEG minimizes non-specific adsorption of proteins found in culture media and *in vivo* [34]. When designing systems to signal to delivered exogenous cells or endogenous cells, these properties are particularly advantageous and afford the user better control over the biological signals that are presented to cells. PEG hydrogels also allow one to directly study the effects of biological signals on cells without confounding results from biologically active hydrogel matrices, such as collagen or hyaluronan.

Since PEG itself is bioinert, strategies to functionalize PEG hydrogels are increasingly important and are the focus of this review article. PEG macromolecules are easily amenable to a wide variety of chemistries that can be used to attach biomolecules and impart biological signals within the hydrogel. Specifically, 'click' chemistries have been widely adopted within the field of regenerative medicine because they are mild, selective, high yielding, and possess rapid reaction kinetics [35]. Importantly, a subset of click reactions is bioorthogonal (i.e., inert to functional groups found in cells and free of toxic reagents and byproducts), so they can be performed in the presence of cells [36,37]. The most commonly employed bioorthogonal click reactions include thiol-Michael conjugate addition [38], strain-promoted azide-alkyne cycloaddition (SPAAC) [39], oxime formation [40], and inverse electron

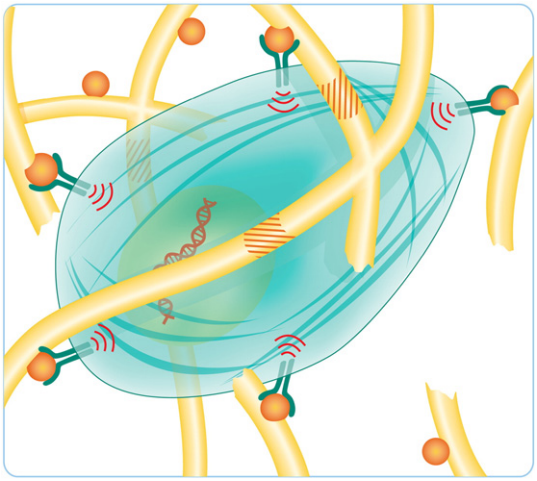

Fig. 1. A cell in its microenvironment. ECM proteins (shown as yellow rods) sequester biomolecules, such as growth factors and cytokines (shown as orange spheres). Cells engage these biomolecules through cell surface receptors to elicit signaling. Signaling can lead to cell spreading and proliferation, and the production of enzymes that enable the cell to remodel and degrade the ECM (ECM degradation sequences shown as orange stripes). Figure adapted from Kyburz et al. [10].

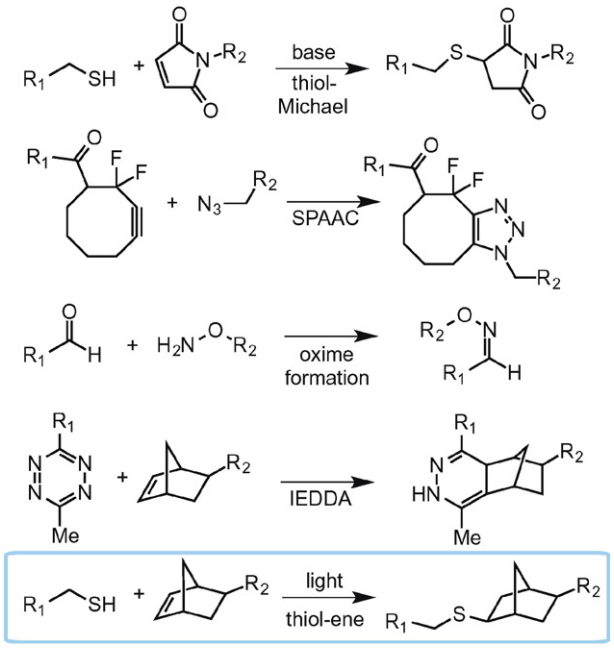

Fig. 2. Bioorthogonal click reactions include the thiol-Michael reaction, strain-promoted azide-alkyne cycloaddition (SPAAC), oxime formation, inverse electron demand Diels Alder (IEDDA) reaction, and the radical-mediated thiol-ene reaction. R_1 and R_2 can represent either a biomolecule or a hydrogel backbone.