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Editorial/preface

Engineering proteolytically-degradable artificial extracellular matrices

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ABSTRACT

Hydrogels are widely used as provisional matrices for tissue engineering and regenerative medicine, showing also great promise as platforms for 3D cell culture. Different bio-functionalization strategies have been proposed to enhance the biological performance of hydrogels, particularly when they lack intrinsic bioactivity. In this context, the design of artificial materials that mimic structural and functional features of the natural extracellular matrix (ECM) has been pursued. This review presents an overview on bioengineering approaches of integrating protease-sensitive motifs into hydrogels, for the creation of cell-responsive biomimetic scaffolding materials that degrade in response to their proteolytic microenvironment. The successful incorporation of protease-sensitive motifs in several synthetic and natural polymers, which has been achieved using various chemical routes, is described. In each case, the selected peptide sequences and their target proteases are highlighted, along with the main achievements of the study. A critical analysis of current limitations and recent advances is also provided, along with suggestions for further improvements.

Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional; A, Alanine; AAc, Acrylic acid; ADAM-TS, ADAMs with thrombospondin motifs; ADAMs, Metalloprotease-disintegrins; Arg, Alanine; AFM, atomic force microscopy; Asp, Aspartic acid; BIS, N,N'-methylenebis(acrylamide); BISAM, Bisacrylamide; BMP-2, Bone morphogenetic protein-2; BMSC, bone marrow stromal cells; C, Cysteine; CLSM, Confocal laser scanning microscopy; CryoSEM, Cryogenic scanning electron microscopy; D, Aspartic acid; Dex, Dextran; Dex-PMPI, p-maleimidophenyl isocyanate-dextran; E, Glutamic acid; ECM, Extracellular matrix; ELPs, Elastin-like polymers; F, Phenylalanine; G or Gly, Glycine; H, Histidine; HA, Hyaluronic acid; hESC, Human embryonic stem cells; hMSC, Human mesenchymal stem cells; HFF, Human foreskin fibroblasts; HPLC, High-performance liquid chromatography; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; MDPs, Multidomain peptides; MMPs, Matrix Metalloproteinases; MS, Mass spectroscopy; MSC, Mesenchymal stem cells; MT1-MMP, Membrane-type matrix metalloproteinase 1; N, Aspargine; OM, original magnification; P, Proline; NIPAAm, N-isopropylacrylamide; P(NIPAAm-co-AAc), Poly(N-isopropylacrylamide-co-acrylic acid); Pas, Peptide amphiphiles; PEG, Poly(ethylene glycol); PHEMA, Poly(2-hydroxyethyl methacrylate); PLA, poly(L-lactide); PLEOF, Poly(lactide-co-ethylene oxide-co-fumarate; PMPI, p-maleimidophenyl isocyanate; Q, Glutamine; R, Arginine; RADA16, (Arg-Ala-Asp-Ala)4; RGD, Arginine-glycine-aspartic acid paptide sequence; RGD-PEG, PEG hydrogels modified with RGD; RCO, Rat calvarian osteoblasts; S, Serine; SAPs, Self-assembled peptides; SELPs, Silk-ekastin like polymers; sIPN, Semi-interpenetrating polymer networks; SCID, Severe combined immunodeficiency; SMC, smooth muscle cells; SPARC, Secreted protein acidic and rich in cysteine; T, Threonine; TIMP, Tissue inhibitors of metalloproteinases; TNF-alfa, Tumor necrosis factor alfa; V, Valine; VEGF, Vascular endothelial growth factor; W, Tryptophan; Y, Tyrosine.

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Contents

1. Introduction

Artificial three-dimensional (3D) matrices that mimic the natural extracellular matrix (ECM) hold great promise for tissue engineering applications, where they are mainly explored as temporary scaffolds for tissue regeneration and as carriers for the delivery of transplanted cells [1]. These ECM analogs are also suitable to create advanced substrates for 3D cell culture, which have been gaining increasing popularity as they bridge the gap between 2D *in vitro* studies and *in vivo* animal model systems [2].

By intrinsically exhibiting some structural features of native ECMs, hydrogels emerge as appealing materials for the development of such matrices. These highly hydrated and permeable polymeric 3D networks provide adequate cellular microenvironments, promoting an efficient exchange of nutrients, oxygen and cellular metabolites with the extracellular milieu [1,3,4]. Hydrogels are pliable, presenting viscoelastic properties that can be tuned to match those of natural tissues [1,3,4], and can often be formed under mild and cytocompatible conditions, which makes them ideal for cell encapsulation or entrapment [1,3,4]. A wide range of hydrogel-forming natural and synthetic polymers is currently available. Natural polymers are often distinguished as protein-based (e.g. elastin, collagen and fibrin) or polysaccharide-based (e.g. hyaluronic acid, dextran, chitosan and alginate). Examples of synthetic polymers frequently used to prepare hydrogels include poly(ethylene glycol) (PEG), poly(*N*-isopropylacrylamide-*co*-acrylic acid (P(NIPAAm-*co*-AAc)), poly(2-hydroxyethyl methacrylate) (PHEMA), poly(lactide-*co*-ethylene oxide-*co*-fumarate) (PLEOF), and self-assembled peptides (SAPs).

Protein-based hydrogels, such as collagen and fibrin that are commonly employed as substrates for 3D cell culture, provide cell-instructive matrices since they are intrinsically enriched with different bioactive moieties that specifically interact with cells, modulating their behavior [1]. However, these materials also face several shortcomings limiting their applicability. Being biologically-derived, they are often ill-defined in terms of composition, and do not afford enough control over their biochemical properties. Along with the anticipated biofunctional moieties, protein-based matrices often concomitantly present other binding sites for non-targeted biological ligands, which may activate unwanted and/or interfering cell responses [1,4–6]. Moreover, protein-based hydrogels usually posses a narrow range of mechanical properties that are generally difficult to tune in a precise way, which further restricts their use as biomaterials [1,4–6]. Alternatively, hydrogels based on synthetic polymers have also been explored. These hydrogels are generally well defined and present a broad and flexible range of properties, in terms of composition, microstructure and mechanics. Yet, they are generally bio-inert, as they lack biofunctional sites for cell recognition and fail to elicit specific cell–matrix interactions [1,4–6]. The properties of polysaccharide-based natural hydrogels fall somewhere in the middle of the previous categories. Human ECM components like the hyaluronic acid (HA), a glycosaminoglycan that is specifically recognized by cell-surface receptors like CD44 [7], take part in important physiological events. Specific mammalian enzymes are able to recognize and degrade HA, as well as other biopolymers, such as chitosan, a polymer of marine origin [8,9]. Other polysaccharides, including alginate, are essentially bio-inert, as far as it is currently known [10]. In general, the mechanical properties of polysaccharide-based hydrogels are relatively easy to tune, generally by changing the polymer mass and/or the crosslinking density.

The limitations presented by the previous types of hydrogels have motivated the use of biomimetic approaches toward the development of hybrid materials that combine their advantages. Building on their versatility, cell-instructive domains can be incorporated into bio-inert hydrogels creating multifunctional materials with unique properties that integrate both structural and biofunctional features of natural ECMs. In this sense, the bio-inertness of some types of hydrogels can actually be regarded as an advantage in terms of biomaterials design, allowing them to be used as blank slates in bio-functionalization schemes. This way, cell interactions with modified hydrogels are expected to be essentially dependent on the added bioactive moieties, providing a means to promote and modulate specific cell responses [1,4–6].

Hybrid hydrogels have been commonly prepared by conjugating polymers to many different types of proteins and peptides, molecules that play a major role in biological recognition phenomena [1,4–6]. Probably the most common and well-studied example is the short amino acid sequence arginine–glycine–aspartic acid (RGD), the prototypical cell–adhesion domain of fibronectin and other cell–adhesive proteins, which has been used to promote integrin-mediated cell adhesion to different types of natural and synthetic hydrogels [9,11,12]. The modification

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