ELSEVIER

The matrix reloaded: the evolution of regenerative hydrogels

Esmaiel Jabbari[1,8,*], Jeroen Leijten[2,3,8], Qiaobing Xu[4,8,*] and Ali Khademhosseini[2,3,5,6,7,*]

[1] Biomimetic Materials and Tissue Engineering Laboratory, Department of Chemical Engineering, University of South Carolina, Columbia, SC, USA
[2] Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3] Department of Medicine, Biomaterials Innovation Research Center, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA
[4] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA
[5] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
[6] Department of Bioindustrial Technologies, College of Animal Bioscience and Technology, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea
[7] Department of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia

Cell-laden hydrogels can regenerate lost, damaged or malfunctioning tissues. Clinical success of such hydrogels is strongly dependent on the ability to tune their chemical, physico-mechanical, and biological properties to a specific application. In particular, mimicking the intricate arrangement of cell-interactive ligands of natural tissues is crucial to proper tissue function. Natural extracellular matrix elements represent a unique source for generating such interactions. A plethora of extracellular matrix-based approaches have been explored to augment the regenerative potential of hydrogels. These efforts include the development of matrix-like hydrogels, hydrogels containing matrix-like molecules, hydrogels containing decellularized matrix, hydrogels derived from decellularized matrix, and decellularized tissues as reimplantable matrix hydrogels. Here we review the evolution, strengths and weaknesses of these developments from the perspective of creating tissue regenerating hydrogels.

Introduction

Biological tissues often contain highly complex hydrogels [1,2]. They contain dynamic, heterogeneous and spatially defined mixtures of cell types, growth factors, nutrients, and intricate extracellular matrices (ECMs) [3]. Importantly, the matrices of natural tissues have complex structures that start with the defined arrangement of amino acids that compose ECM proteins at the nanoscale, to the formation of fibrils and fiber bundles at the microscale, and to the alignment of fibers in a specific direction and crosslinking of the fibers at the macroscale [4]. The hierarchical structure of the ECM not only controls the tissue's biochemical and physico-mechanical properties, but also the concentration, location and distribution of cells and growth factors, cytokines, and hormones within the tissue. The ECM thus acts as a key element in inducing, orchestrating and maintaining the multifaceted processes that govern tissue phenotype, function, and fate [5–8]. Naturally derived hydrogels are used in engineered constructs to support the growth and maturation of implanted cells, but lack the minimum stiffness required to resist soft tissue compression [9,10]. Conversely, synthetic hydrogels provide the required mechanical support but lack the intricate arrangement of ligands that regulate cell fate. Not surprisingly, much effort has been dedicated to recreate or incorporate ECM – or their derivatives or biomimetic counterparts – in hydrogels. Here, we review the evermore sophisticated approaches to integrate ECMs in hydrogels by orthogonal conjugation of cell-interactive ligands, copolymerization with functionalized ECM molecules, doping with decellularized ECM, or hybridization with digested

*Corresponding authors: Jabbari, E. (jabbari@mailbox.sc.edu), Xu, Q. (Qiaobing.Xu@tufts.edu), Khademhosseini, A. (alik@rics.bwh.harvard.edu)
[8] Shared first authors.

 http://dx.doi.org/10.1016/j.mattod.2015.10.005

tissue ECM, designed for the regeneration of complex multicellular tissues.

Hydrogels as extracellular matrices

Like natural ECMs, hydrogels consist of hydrophilic networks of nano- and microfibers that provide a mechanically robust shelter for cells, while retaining a large fraction of water in their structure [11]. Consequently, they allow for nearly free diffusion of oxygen, carbon dioxide, and nutrients and proteins to maintain the viability and function of encapsulated cells [12]. Hydrogels can be segregated in two broad categories: natural and synthetic hydrogels.

Natural hydrogels are as suggested by its name, isolated from biological sources that include amongst others collagen [13], gelatin [14], silk [15], alginate [16], hyaluronic acid [17] and dextran [18]. Hydrogels of natural sources typically support cell adhesion and proliferation, but are mechanically weak and provide little control over remodeling. Advanced modifications or processing strategies are therefore often required to match the biomaterial with the injured tissue. In addition, minor changes in sequence distribution of natural gels can dramatically affect the fate and function of the encapsulated cells in the matrix giving rise to batch-to-batch variation [19,20].

Synthetic hydrogels such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP) have advantages that include well-defined composition and easily tunable physiochemical properties [21–23]. Synthetic hydrogels generate matrices with enormous range of physical, mechanical, and chemical properties for regeneration of complex multiphase tissues [3,24–27]. Reinforcement with fillers, nanofibers, nanotubes and optimization of network structure can improve the mechanical properties of hydrogels by several orders of magnitude [28–31]. Synthetic hydrogels are often characterized by slow degradation rates unless proteolytically degradable peptides are incorporated in the gel network [32]. However, additional modifications can remedy this challenge. For example, short hydroxy acid (HAc) segments can be polymerized to PEG chains to generate asymmetric HAc-chain extended PEG gels with tunable resorption times [33]. This enables a resorption time ranging from a few days for glycolic acid-chain extended gels, to a few weeks for lactic acid, to a few months for dioxanone, and to many months for caprolactone-chain extended gels (Fig. 1) [27]. Such modifications allow for the matching of hydrogel degradation with cellular invasion, vascularization, innervation and mineralization during tissue regeneration [33].

Extracellular matrix modified hydrogels

The regenerative potential of hydrogels heavily depends on our ability to develop man-made matrices that mimic the composition and microstructure of native tissues [34]. Although hydrogels resemble natural ECM on the abstract level, they do not incorporate the biological complexity derived from the vast variety of distinct ECM molecules [35]. To recapitulate this, hydrogels are commonly decorated with one or a few matrix molecule types [36–38]. These include amongst others hyaluronic acids [38], collagens [36,37], laminins [39], elastins [40], vitronectins and fibronectins [41]. It is well established that such modifications affect the function, proliferation and migration of cells. In addition, most of these ECM molecules can affect the biomaterials' porosity, swelling or degradation characteristic. In consequence, this often increases the difficulty of controlling the hydrogel's behavior [42]. As an alternative, numerous bioactive peptide sequences have been identified and conjugated to the polymer chains in the hydrogel network [28,43–45]. For example, cell-adhesive, vasculogenic and osteogenic hydrogels were generated by copolymerization of PEG macromonomers with acrylamide-terminated GRGD peptide (IP), propargyl acrylate and 4-pentenal (aldehyde moiety) monomers [46]. Aminooxy-functionalized vasculogenic SVVYGLRK peptide (VP) derived from osteopontin protein was conjugated to the PEG network by an aminooxy-aldehyde reaction whereas the azide-functionalized osteogenic KIPKA SSVPT ELSAI STLYL peptide (OP) derived from *recombinant human* bone morphogenetic protein-2 (rhBMP-2) was conjugated by a propargyl-azide reaction (Fig. 2) [46]. Functionalization of the hydrogels with IP, IP + OP, and IP + OP + VP significantly increased osteogenic

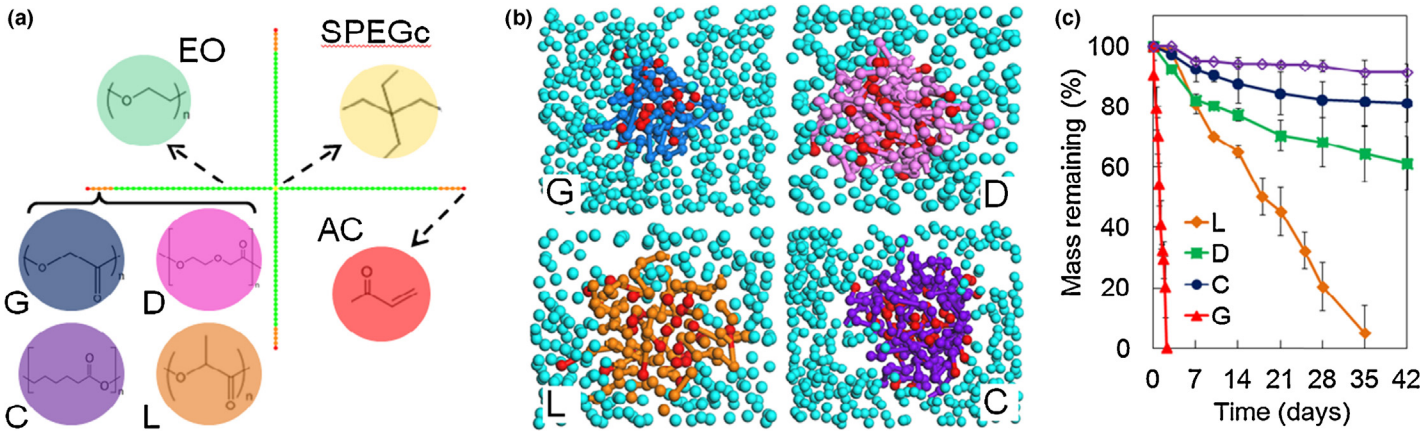

FIGURE 1

Representation of the SPEXA (X= L, G, C or D) macromonomer. Beads SPEGc (yellow), EO (green), G (blue), D (pink), L (orange), C (purple) and Ac (red) represent star PEG core, ethylene oxide repeat unit, glycolide, p-dioxanone, lactide, ε-caprolactone repeat unit, and acrylate functional group, respectively. (b) Simulation of the effect of degradable G (blue), L (orange), D (pink), and C (purple) monomers on the distribution of water beads around the micelles' core. Red and light blue beads in b are water and reactive acrylate beads, respectively. (c) Effect of monomer type G (red), L (orange), D (green), and C (blue) on the measured mass loss of SPEXA hydrogels with incubation time. The purple curve in C is the mass loss of PEGDA hydrogel without chain extension with a hydroxy acid. Reproduced with permission [27].