European Polymer Journal 72 (2015) 566–576

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Gelation characteristics, physico-mechanical properties and degradation kinetics of micellar hydrogels

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 18 April 2015
Accepted 20 April 2015
Available online 25 April 2015

Keywords:
Micellar hydrogel
Aliphatic hydroxy acid chain extension
Cell encapsulation
Gelation
Degradation
Elasticity

ABSTRACT

Due to their high water content and diffusivity of nutrients and biomolecules, hydrogels are very attractive as a matrix for growth factor immobilization and *in situ* delivery of cells to the site of regeneration in tissue engineering. The formation of micellar structures at the nanoscale in hydrogels alters the spatial distribution of the reactive groups and affects the rate and extent of crosslinking and mechanical properties of the hydrogel. Further, the degradation rate of a hydrogel is strongly affected by the proximity of water molecules to the hydrolytically degradable segments at the nanoscale. The objective of this review is to summarize the unique properties of micellar hydrogels with a focus on our previous work on star polyethylene glycol (PEG) macromonomers chain extended with short aliphatic hydroxy acid (HA) segments (SPEXA hydrogels). Micellar SPEXA hydrogels have faster gelation rates and higher compressive moduli compared to their non-micellar counterpart. Owing to their micellar structure, SPEXA hydrogels have a wide range of degradation rates from a few days to many months as opposed to non-degradable PEG gels while both gels possess similar water contents. Furthermore, the viability and differentiation of mesenchymal stem cells (MSCs) is enhanced when the cells are encapsulated in degradable micellar SPEXA gels compared with those cells encapsulated in non-micellar PEG gels.

1. Introduction

Hydrogels are hydrophilic polymeric networks that retain a significant fraction of water in the equilibrium state without dissolving. Owing to their high water content and high permeability to small nutrient molecules and large proteins, hydrogels are used as a carrier for delivery of cells to the site of regeneration in cell based therapies and tissue regeneration [1–5]. In that approach after injection and *in situ* hardening, the gel is gradually degraded to provide new volume for neo-tissue formation and replacement by the patient's own tissue [2]. Natural hydrogels like collagen, chitosan, and alginate as well as synthetic polyethylene glycol (PEG) and polypeptide gels are used as a carrier in stem cell delivery in regenerative medicine [6–13]. Neural stem cells (NSCs) encapsulated in alginate gels differentiated into neuronal lineages only in gels with an elastic modulus similar to that of brain tissue (100–1000 Pa) [14]. Likewise natural and synthetic hydrogels like PEG [15], collagen [16], chitosan [17], mixture of PEG and agarose [18], and mixture of hyaluronic acid and chitosan [19] have been used as a matrix for cell delivery in cartilage regeneration. Tissue engineered constructs require composite, multi-phasic, micro-patterned gels with a wide range of elasticity and degradability to support neurogenesis, vascularization, and

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http://dx.doi.org/10.1016/j.eurpolymj.2015.04.028
0014-3057/

structural stability. As an example, osteogenesis requires a highly elastic and slowly degrading matrix whereas a compliant fast-degrading matrix is essential for vasculogenesis [20–22]. Aside from biocompatibility, hydrogels used in cell delivery should have fast gelation kinetics to reduce the exposure of cells to reactive macromers and low molecular weight initiators, provide a wide range of elasticity, and degrade concurrent with tissue formation [6]. In that regard, synthetic macromonomers and more specifically the inert non-immunogenic PEG macromonomers generate hydrogels with a wide range of elasticity and stiffness [23,24] and the extent of interaction and adhesion of the encapsulated cells with the matrix can be controlled by conjugation of integrin- and heparin-binding peptides to the gel [25,26]. However, most synthetic hydrogels like PEG, polyvinyl alcohol (PVA), polyacrylamide (PAM), and poly(hydroxyethyl methacrylate) (PHEMA) are non-degradable and their use as a cell delivery matrix is limited by their persistence at the site of delivery, thus limiting the rate of tissue regeneration [27–29]. Co-polymerization of hydrophilic macromers with degradable hydrophobic monomers generates macromonomers which form micellar structures in aqueous solution [30–32]. These micellar structures affect the proximity of water molecules to the hydrolytically degradable segments of the copolymer chains at the nanoscale leading to a noticeable change in gelation kinetics, elasticity, and degradation of the hydrogel [32]. In this work we review the unique properties of micellar hydrogels specifically those based on star polyethylene glycol (PEG) macromonomers chain extended with short aliphatic hydroxy acid (HA) segments (SPEXA hydrogels) with respect to water–copolymer interaction, water content, gelation kinetics, elasticity, degradation, and cell–matrix interaction.

2. Physically versus covalently bonded micellar gels

It is well known that surfactant-like amphiphilic diblock copolymers such as PEG–polypropylene oxide (PEG–PEO), PEG–polylactide (PEG–PLA), and PEG–poly(lactide-co-glycolide) (PEG–PLGA) form micelles in aqueous solution at low concentrations and undergo physical gelation at high concentrations [33,34]. Hydrogel formation by diblock copolymers is attributed to the packing of micelles into a crystal-like macro-lattice with body-centered cubic symmetry and the interpenetration of polymer chains in the corona of the neighboring micelles [33]. A–B–A triblock copolymers are known to form stimuli-responsive micellar gels in aqueous solution [35,36]. Typically in micelle forming A–B–A copolymers, one of the "A" or "B" blocks is hydrophilic and the other block is hydrophobic or becomes hydrophobic in response to an external stimulus like temperature, pH, ionic strength or enzyme concentration [32,37–39]. When the "A" block is permanently hydrophilic, A–B–A copolymers form star-like micelles at low concentrations [36]. At high concentrations (>20 wt%), a hydrogel is formed through the packing of micelles into an ordered phase of the permanently hydrophilic "A" blocks [35,40]. Conversely, when the "B" block is permanently hydrophilic, gelation takes place by bridge formation between the micelles [38,41]. In that case, aggregation of hydrophobic "A" blocks forms the core of flower-like micelles when the concentration of block copolymers exceeds the critical micelle concentration (CSC). The hydrophilic "B" blocks with a loop conformation form the corona of the flower-like micelles at low concentrations [32]. With increasing polymer concentration, the density of micelles increases, the average density between micelles decreases and some of the loop forming "B" blocks transform to inter-micellar bridges [32]. The density of bridges and the bridge/loop ratio increases with increasing the macromer concentration [42]. A transient micellar network, crosslinked physically by inter-micellar bridges, is formed when the polymer concentration exceeds the percolation threshold [32]. In addition to A–B–A block copolymers, the A–B–C block copolymers have been used for the synthesis of stimuli-responsive micellar gels. For example, pH and temperature-responsive gels are formed in aqueous solutions of polystyrene-b-poly(2-vinylpyridine)-b-poly(ethylene oxide) (PS–PVP–PEG) at a macromer concentration of 8 wt% [43].

In addition to the above block copolymers, several peptides and peptide conjugated macromers have been shown to form physically bonded micellar hydrogels. For example, peptides with alternating Arginine–Alanine–Aspartate (RAD) residues with a total of 16 amino acids, $(RADA)_4$ and $(RARADADA)_2$, self-assemble to form fibrous gels with a β-sheet structure at peptide concentrations of 1–10 mg/mL [44]. Similarly, fibrous gels are formed in the aqueous solutions of β-sheet forming $(AEAEAKAK)_2$ peptides with alternating alanine (A), glutamic acid (E) and lysine (K) residues in the concentration range of 0.1–1 wt% [45,46]. Peptide amphiphiles (PA) with a hydrophilic head group conjugated to a hydrophobic alkyl tail group are used to synthesize micellar gels [47–49]. For example, PAs with a head composed of 4 consecutive cysteines, 3 glycines, a serine and a segment of arginine–glycine–aspartic acid (RGD) and an alkyl tail of 16 carbon atoms formed nanofibrous micellar gels with decreasing pH to below 4 [47]. Long peptides (~230 amino acids) with α-helical end blocks and hydrophilic middle blocks are shown to form pH and temperature-sensitive micellar gels due to coiled-coil aggregation of the terminal blocks [50].

The crosslinks (bridges) in physically bonded micellar gels have a finite residence time within the micelles depending on the hydrophobicity of the micelles' core and bridging blocks. Therefore, the physically bonded gels are dynamic at the molecular scale and mechanically soft at the macro-scale [32,38]. Physically bonded micellar gels can be mechanically reinforced by incorporation of covalent bonds within the micelles. The confinement of the crosslinking reaction to the micellar phase imparts special properties to the hydrogel with respect to gelation kinetics, elasticity, water content, and cell–matrix interactions in cell encapsulation [32]. To test that, we synthesized a series of degradable covalently crosslinkable micelle-forming macromonomers by chain extension of star 4-arm PEG macromers with short aliphatic hydroxy acid (HA) segments including L-lactide (L), glycolide (G) and ε-caprolactone (C) followed by termination of the arms with a reactive acrylate (Ac) group (SPEXA macromonomer with X = L, G or C) (Fig. 1) [30,31,51–53]. The SPELA, SPEGA and SPECA macromonomers are hereafter denoted by L, G and C, respectively. The star PEG acrylate without chain extension with HA is denoted by "w/o