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In situ forming stereocomplexed and post-photocrosslinked acrylated star poly(ethylene glycol)-poly(lactide) hydrogels

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ABSTRACT

Biodegradable acrylate end-group functionalized poly(ethylene glycol)-poly(lactide) (PEG-PLA) star block copolymer hydrogels were formed by the consecutive physical gelation through stereocomplexation of star shaped PEG-$(PLLA)_8$ and PEG-$(PDLA)_8$ enantiomers and UV photopolymerization. The 8-armed PEG-PLA star block copolymers were prepared by ring opening polymerization of lactide onto an amine end-group functionalized PEG with a molecular weight of 20 kg/mol using stannous octoate as a catalyst. The degree of polymerization of the PLA blocks was 12 lactyl units and the end hydroxyl groups were reacted with acryloyl chloride to give the required acrylate end groups. Aqueous solutions of enantiomeric mixtures of the PEG-$(PLA)_8$ macromonomers formed physically crosslinked hydrogels above a critical gel concentration of 4 w/v%. Subsequent photopolymerization at 365 nm in the presence of Irgacure 2959 resulted in gels with improved mechanical properties and hydrolytic stability. With 40% polymer mass loss after 45 d *in vitro*, these hydrogels show excellent resistance against hydrolytic degradation and dissolution, which is believed to result from the combination of stable amide linkages between the PEG and PLA blocks and the high physical and chemical crosslink density owing to the star architecture.

1. Introduction

Hydrogels are highly water swollen polymer networks whose properties resemble those of natural soft tissues [1–3]. Biodegradable poly(ethylene glycol)-poly(lactide) (PEG-PLA) type hydrogels generally exhibit excellent biocompatibility and are accordingly widely investigated for their use in biomedical applications such as tissue engineering and systems for controlled delivery of biologically active agents. Thermo-sensitive amphiphilic block copolymers form hydrogels through physical crosslinking. Depending on their molecular architecture and molecular weight they provide a sol to gel transition upon a decrease or increase in temperature. Such systems offer the advantages of a simple injection method when the sol to gel transition is close to body temperature. In this way surgical procedures may be omitted, the shape can be properly adapted and cells or proteins can be easily incorporated [4–6]. Physical crosslinks, including stereocomplexation between enantiomeric PDLA and PLLA blocks in amphiphilic block copolymers [7,8], can be formed under mild conditions, but the resulting hydrogels are generally degraded and/or dissolved relatively fast. Alternatively, chemically crosslinked hydrogels have been prepared from various combinations of macromonomers

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endcapped with reactive groups. Well-known examples are hydrogels formed by Michael addition between thiols and vinylic groups [9,10] or by reaction between activated esters and amines [11,12]. Most often, however, chemically crosslinked hydrogels are prepared by photocrosslinking of (meth)acrylate endcapped block copolymers with the aid of UV or visible light. Pioneering work was conducted by the group of Hubbell, who prepared photocrosslinked hydrogels from end acrylated PLA-PEG-PLA and PLGA-PEG-PLGA triblock copolymers [13]. The degradation times of the hydrogels could be tuned from 1 to 120 d by altering the molecular weight of the PEG and the length and composition of the hydrophobic polyester block. West et al. reported on a photocrosslinked hydrogel based on PEG diacrylate that was rendered biodegradable by incorporation of a collagenase sensitive peptide sequence in the network [14]. Fibroblasts were encapsulated successfully in the hydrogel by exposing a cell containing macromonomer solution briefly to UV light. An acrylated RGD (arginine-glycine-aspartic acid) cell adhesive peptide was incorporated by light-induced reaction with remaining acrylate groups in a partially crosslinked PEG diacrylate network. By using a photolithographic technique, the precise location of RGD could be dictated and cells exhibited guided three-dimensional migration only into the RGD-patterned regions of the hydrogels. The group of Anseth prepared various photocrosslinked hydrogels based on (meth)acrylate terminated PEG copolymers. It was demonstrated that the macroscopic properties and degradation of hydrogels prepared from PLA-PEG-PLA triblock copolymers endcapped with acrylate moieties could be tuned by altering the polymerization conditions [15]. Furthermore, they showed that a hydrogel composed of a mixture of degradable and non-degradable PEG diacrylate macromonomers can serve as a scaffold for the engineering of cartilage [16]. After implantation of the construct in mice, encapsulated chondrocytes were capable of survival and proliferation and newly formed tissue was integrated with surrounding native cartilage over time. Photocrosslinked hydrogels have also been used for the controlled release of various hydrophobic and hydrophilic drugs, including hydrogels based on methacrylate terminated PEG [17] and PEG-poly(ε-caprolactone) multiblock copolymers [18]. In both systems, the drug release could be regulated via the composition of the hydrogel.

It is important that during the gelation process no flow of macromonomers or collapse of the gel takes place [19]. This issue has been addressed by a few research groups by combining physical and chemical crosslinking. Most dual gelling systems reported to date are based on thermosensitive polymers, such as poly(N-isopropylacrylamide) (pNIPAAm), poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (commercially known as Pluronics) and poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA) derivatives, which are modified with various chemically functional groups for post-gelation reactions [20]. For example, thermally induced physical crosslinking of an acrylated Pluronic was used to provide fast gelation [21]. The gel was then photocrosslinked to give a highly stable gel. More recently, Vermonden et al. combined temperature-induced physical gelation with native chemical ligation as a chemoselective crosslink strategy [22]. Triblock copolymers consisting of cysteine functionalities, thermo-responsive NIPAAm units and degradable moieties were mixed with thioester or N-hydroxysuccinimide functionalized PEG crosslinkers. The combined physical and chemical crosslinking resulted in fast network formation and mechanically strong hydrogels. These studies show that tandem gelation is a feasible concept, but the field is still in its infancy. For example, a controlled degradation is an important item that has yet received little attention in the design of dual gelling hydrogels.

Recently we showed that the mechanical stability and most importantly the degradation of star block copolymers of PEG and PLLA can be controlled by replacing the highly hydrolytically labile linking ester bond with an amide bond [23,24]. Furthermore it was demonstrated that stereocomplexation and photocrosslinking of methacrylate terminated enantiomeric PEG-PDLA and PEG-PLLA star block copolymers allows for respectively *in situ* formation of physical hydrogels and their subsequent chemical stabilization [25]. Although these stereocomplexed & photocrosslinked hydrogels were relatively stable with a degradation time of 3 weeks *in vitro*, for some applications, such as long-term drug delivery, a higher resistance against hydrolytic degradation is required. Inspired by the promising results of the two aforementioned papers, we hypothesized that stereocomplexation and photocrosslinking of PEG-PLA star block copolymers with amide groups between PEG and PLA may provide a tandem gelling system with a high resistance against hydrolytic degradation. In the present paper, we describe the synthesis and characterization of novel acrylate-terminated amide-linked PEG-PDLA and PEG-PLLA star block copolymers. The physical, mechanical and degradation properties of stereocomplexed and photocrosslinked hydrogels prepared from these macromonomers are presented with a particular focus on the concentration dependence of the gelation properties. Furthermore we propose a gelation mechanism for the formation and *in vitro* degradation of the stereocomplexed & photocrosslinked hydrogels starting from enantiomeric PEG-PDLA and PEG-PLLA star block copolymer solutions.

In summary, we show that stereocomplexation of these new macromonomers in aqueous solution and subsequent UV-initiated radical polymerization of the terminal acrylate groups result in hydrogels with excellent mechanical properties and a degradation time of several months, confirming our hypothesis that this system may be well applicable as an injectable, robust hydrogel with a high resistance against hydrolytic degradation.

2. Experimental section

2.1. Materials

Hydroxyl terminated 8-armed poly(ethylene glycol) (PEG-$(OH)_8$, $M_{n,NMR}$ = 21,400 g/mol) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. PEG-$(OH)_8$ was converted to PEG-$(NH_2)_8$ using a two-step procedure analogous to that described by Elbert et al. for linear hydroxyl terminated PEGs [26]. Eight-armed PEG-PDLA and PEG-PLLA star block copolymers with a PEG core and 12 lactyl units in each PLA block (PEG-$(PDLA_{12})_8$ and PEG-$(PLLA_{12})_8$ respectively) as well as their acrylate end functionalized analogs (PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC) were synthesized as described previously [23,27]. L-lactide and D-lactide were obtained from Corbion Purac