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Direct laser writing of synthetic poly(amino acid) hydrogels and poly(ethylene glycol) diacrylates by two-photon polymerization

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ABSTRACT

The additive manufacturing technique of direct laser writing by two-photon polymerization (2PP-DLW) enables the fabrication of three-dimensional microstructures with superior accuracy and flexibility. When combined with biomimetic hydrogel materials, 2PP-DLW can be used to recreate the microarchitectures of the extracellular matrix. However, there are currently only a limited number of hydrogels applicable for 2PP-DLW. In order to widen the selection of synthetic biodegradable hydrogels, in this work we studied the 2PP-DLW of methacryloylated and acryloylated poly(α-amino acid)s (poly(AA)s). The performance of these materials was compared to widely used poly(ethylene glycol) diacrylates (PEGdas) in terms of polymerization and damage thresholds, voxel size, line width, post-polymerization swelling and deformation. We found that both methacryloylated and acryloylated poly(AA) hydrogels are suitable to 2PP-DLW with a wider processing window than PEGdas. The poly(AA) with the highest degree of acryloylation showed the greatest potential for 3D microfabrication.

1. Introduction

Hydrogels are promising matrix candidates for tissue engineering due to their biocompatibility, high water content and tunable biomimetic properties [1]. Combining hydrogels with advanced microfabrication approaches holds great potential for recreating complex extracellular microarchitectures [2]. Among these approaches, direct laser writing by two-photon polymerization (2PP-DLW) offers 3D microfabrication with superior accuracy compared to the widely used methods of UV laser stereolithography and 3D printing [3]. 2PP-DLW is based on the nonlinear optical phenomenon of two-photon absorption (2PA) and the fabricated microstructures are formed by overlapping ellipsoidal voxels (volumetric pixels). Voxel size can be varied by adjusting the processing parameters, such as laser power, exposure time and numerical aperture of the objective lens [4]. With optimal processing conditions, 2PP-DLW enables feature sizes of less than 100 nm and beyond the diffraction limit of light [5]. Furthermore, structures with multiple length scales ranging from sub-micron to millimeter can be fabricated [6,7].

2PP-DLW can be used for the processing of a variety of materials ranging from traditional photoresists to biopolymers [8]. The 2PP-DLW of hydrogels was first demonstrated with proteins when Pitts et al. reported the crosslinking of bovine serum albumin (BSA) and type I collagen [9,10]. Since then, the processing of various other protein hydrogels, such as fibronectin [11], fibrinogen [12] and BSA combined with laminin [13] has been demonstrated. However, despite their beneficial properties, such as biodegradability, natural hydrogels often suffer from batch-to-batch variation and relatively poor mechanical properties, which limit their use as tissue engineering scaffolds [14]. In order to tune the mechanical properties and photoreactivity, chemically modified natural hydrogels, such as methacrylamide-modified gelatin [15–18], vinyl ester-modified gelatin [17], methacrylate-modified dextran and hyaluronan (HA) [19] have been recently studied. However, these materials are still biological in origin, which makes them prone to batch-to-batch variability.

Synthetic hydrogels are attractive alternatives to naturally derived materials due to their adjustable properties and customizable chemistry [14]. Polyethylene glycol diacrylate (PEGda), which is the acrylated form of poly(ethylene glycol) (PEG), is one of the most widely used synthetic hydrogels and has been approved by the FDA for several medical applications due to its low toxicity and biocompatibility [2, 20]. PEGda has also been shown well suitable for scaffold fabrication

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by 2PP-DLW [21,22]. However, a drawback of PEGda is that it is not inherently biodegradable [1,2].

In order to realize the full potential of creating biomimetic microstructures for cell culturing, it is essential to expand the selection of synthetic, degradable hydrogels applicable for 2PP-DLW. The aim of this work was to study the 2PP-DLW of synthetic poly(α-amino acid) hydrogels (poly(AA)s) for the first time. Due to their polypeptide backbone, poly(AA) hydrogels are cleavable in a biological environment by enzyme-catalyzed hydrolysis and the rate and enzyme specificity of the degradation can be controlled through copolymerization [23–25]. Hydrogels based on synthetic poly(AA)s have been previously shown suitable for the culturing of porcine mesenchymal stem cells [26]. In this study, we prepared poly(AA)s based on methacryloylated and acryloylated poly[N^5-(2-hydroxyethyl) L-glutamine]s (PHEGs) and compared their 2PP-DLW performance to commercial PEGda hydrogels. The 2PP-DLW of four different PHEG poly(AA) hydrogels and three PEGdas was studied in terms of the fundamental parameters of hydrogel 2PP-DLW, namely the polymerization and damage threshold values, voxel size, line width and post-development swelling and deformation.

2. Materials and methods

2.1. Preparation of macromers

The synthesis of macromers for 2PP-DLW, methacryloylated and acryloylated poly[N^5-(2-hydroxyethyl) L-glutamine]s (PHEGs), is illustrated in Fig. 1(a) and consists of monomer synthesis, polymerization and two-step modification of the resulting polymers (aminolysis and methacryloylation).

2.1.1. Materials

Tetrahydrofuran, 1,4-dioxane, chloroform, hexane (all from Lach-Ner, Czech Republic), 2-aminoethanol and dimethylacetamide (both from Sigma-Aldrich) were dried with appropriate drying agents, distilled and stored over molecular sieves. Sodium hydrogen carbonate (Fluka), pyridine (Lachema, Czech Republic) and acetic acid (Lach-Ner) were of an analytical grade and were used as obtained. Triphosgene (Chemos, Czech Republic or TCI Europe), methacryloyl chloride and acryloyl chloride (Fluka) were used as obtained [25]. γ-Benzyl L-glutamate (Emmenar Group, India) was recrystallized from distilled water. Poly(ethylene oxide) standards (PEO) were purchased from Polymer Standard Service GmbH (Germany).

2.1.2. Monomer synthesis and polymerization

The synthesis of the monomer, N-carboxyanhydride of γ-benzyl L-glutamate (Fig. 1(a), NCA-BLG) was carried out by the reaction of γ-benzyl L-glutamate (BLG) with triphosgene, basically according to [27], and has been described in detail in [23]. Crude NCA-BLG was crystallized from tetrahydrofuran with the addition of chloroform, recrystallized from chloroform if necessary and stored under nitrogen in a freezer. NCA-BLG was polymerized in dry 1,4-dioxane (0.2 mol/l) with sodium methanolate as an initiator with the initiator-to-monomer ratio of 1/200 [28]. The polymer, hydrophobic poly(γ-benzyl L-glutamate) (PBLG, Fig. 1(a), I.), was precipitated in ethanol and dried under vacuum at 40 °C.

2.1.3. Aminolysis of PBLG

PBLG was aminolyzed in heterogeneous phase with 50-mole excess of 2-aminoethanol at 60 °C for two days [24]. The resulting water-soluble polymer, poly[N^5-(2-hydroxyethyl) L-glutamine] (PHEG, Fig. 1(a), II.) was precipitated in absolute ethanol, isolated by filtration and dissolved in water. The aqueous polymer solution was neutralized with acetic acid and dialyzed against water (Spectra/Por® 1 with cutoff of 6000–8000). The dialyzed polymer solution was filtered (Whatman 0.2 μm nylon membrane), frozen and freeze-dried.

2.1.4. Methacryloylation and acryloylation of PHEG

Polymerizable methacryloyl or acryloyl groups were introduced in PHEGs by the reaction of the hydroxyethyl side chains of PHEG with methacryloyl chloride (MA-Cl) or acryloyl chloride, respectively. The reaction with MA-Cl was carried out in dry dimethylacetamide (DMA, 2.5% w/w) by modifying the procedure described in [29]. First, the amount of MA-Cl required to obtain a desired degree of methacryloylation was estimated from the dependence shown in Fig. 1(b), which was based on preliminary model experiments. Second, lithium chloride (5% w/w to PHEG) was added to prevent physical association of polymer chains. Third, the reaction was cooled by ice to prevent spontaneous crosslinking through radical polymerization. An equimolar amount of pyridine with respect to the chloride reagent was added to capture hydrochloride released by the reaction and to shift the equilibrium in favor of the products. The reaction was stopped after 2 h by adding sodium carbonate solution in excess. The reaction mixture was filtered from salts and the filtrate was dialyzed against water, then filtered (Whatman 0.2 μm nylon membrane), frozen and lyophilized. The product (Fig. 1(a), III.) was stored under nitrogen in a freezer. Practically the same procedure was successfully used for acryloylation.

The molecular weight averages of the methacryloylated and acryloylated PHEGs were determined by size exclusion chromatography (SEC) analysis on a PolySep-GFC-P Linear column (Phenomenex®) with a gradient Knauer system with diode array detection (DAD) and Alltech 3300 evaporative light scattering detection (ELSD). PEO standards were used for calibration and an isocratic system of 0.05 M

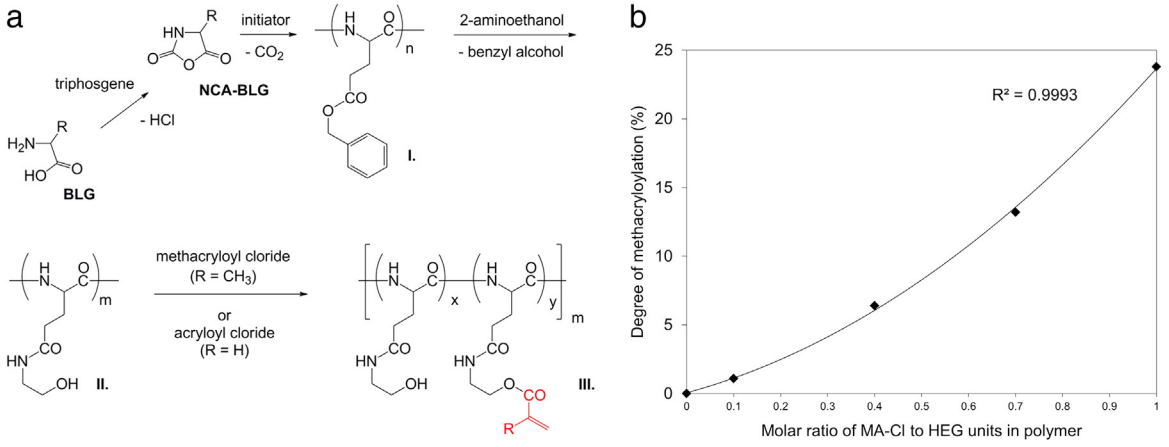

Fig. 1. (a) Scheme of preparation of PHEG macromers. (b) Dependence of the degree of methacryloylation on the amount of methacryloyl chloride (MA-Cl) added to the reaction mixture, expressed as a molar ratio of MA-Cl to HEG units in the polymer chain.