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A tough, precision-porous hydrogel scaffold: Ophthalmologic applications

Wenqi Teng [a, b, c], Thomas J. Long [c], Qianru Zhang [a], Ke Yao [a], Tueng T. Shen [b, c], Buddy D. Ratner [c, *]

[a] Eye Center, Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang Provincial Key Lab of Ophthalmology, Hangzhou 310009, China
[b] Dept. of Ophthalmology, School of Medicine, University of Washington, Seattle, WA 98195, USA
[c] Dept. of Bioengineering, University of Washington, Seattle, WA 98195, USA

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ABSTRACT

Appropriate mechanical properties and highly interconnected porosity are important properties for tissue engineering scaffolds. However, most existing hydrogel scaffolds suffer from poor mechanical properties limiting their application. Furthermore, it is relatively infrequent that precision control is achieved over pore size and structure of the scaffold because there are relatively few current technologies that allow such control and there is not a general appreciation that such control is important. To address these shortcomings, by combining double network polymerization and sphere-templating fabrication techniques, we developed a tough, intelligent scaffold based on poly(acrylic acid) and poly(*N*-isopropyl acrylamide) with a controllable, uniform, and interconnected porous structure. A mechanical assessment showed the toughness of the hydrogel and scaffold to be up to ~1.4 $\times$ 10^7 Jm^{-3} and ~1.5 $\times$ 10^6 Jm^{-3} respectively, as compared with 10^4–10^5 Jm^{-3} for most synthetic hydrogels. The thermosensitivity and pH-sensitivity were explored in a swelling study. *In vitro* testing demonstrated the scaffold matrices supported NIH-3T3 cell adhesion, proliferation and infiltration. An *in vivo* rabbit study showed the scaffolds promote strong cellular integration by allowing cells to migrate into the porous structure from the surrounding tissues. These data suggest that the poly(acrylic acid)/poly(*N*-isopropyl acrylamide)-based scaffold could be an attractive candidate for tissue engineering.

1. Introduction

Many tissue engineering strategies involve the design of artificial scaffolds into which cells can migrate, proliferate, and differentiate to create new tissue that integrates with host tissue or replaces deficient tissue [1–3]. Numerous tissue engineering scaffolds have been designed and fabricated in recent decades [4–6]. The three-dimensional (3D) polymeric scaffolds with high porosities and homogeneous interconnected pore networks are increasingly applicable and useful for the repair and regeneration of various tissues and organs [7–9]. Many techniques have been developed to fabricate 3D porous scaffolds, such as particle leaching [10], self-assembly [11], fiber mesh [12] and electrospinning [13]. However, in general, these 3D scaffolds do not provide precision control of pore size, pore shape or interconnectivity [14]. Our group has developed a sphere-templating technique by which fabricated scaffolds possess a network of interconnected spherical pores of uniform size and display an inverted colloidal crystal geometry [15,16]. By modulating pore sizes and pore interconnects, the optimization of cell infiltration can be achieved for promoting biointegration, healing or vascularization. We have determined, that spherical, interconnected pores in the range 30–40 microns lead to vascularized, non-fibrotic, integrative healing while larger or smaller pores are associated with fibrotic healing and reduced angiogenesis [17]. The pore size range we have proposed as optimal differs significantly from that found in most tissue engineering scaffolds where large pores are thought to be essential for cell seeding and for tissue development. To date, the sphere-templated 3D porous scaffolds have been used for cardiac tissue engineering [18], bone tissue engineering [19] and percutaneous devices [20] and have received a CE Mark in Europe for use as a scleral implant for glaucoma surgery.

Sphere-templated 3D porous scaffolds may be made from a wide range of natural and synthetic polymers, such as proteins [21],

* Corresponding author. University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA. Tel.: +1 206 685 1005; fax: +1 206 616 9763.
E-mail address: ratner@uweb.engr.washington.edu (B.D. Ratner).

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silicone rubber [22] and hydrogels [18,22,23]. In recent decades, hydrogels have been seen as valuable as scaffold materials in tissue engineering because of their high water content (typically >20%), low interfacial tension, good biocompatibility and permeability. Hydrogels are water-swollen polymeric materials composed of three-dimensional cross-linked networks that have structural similarities to the ECM of many tissues [24,25]. In addition, some hydrogels such as poly(acrylic acid) (PAAc) and poly(*N*-isopropyl acrylamide) (PNIPAM), whose properties and volume changes in response to external stimuli such as pH or temperature, are called environmentally sensitive hydrogels or smart hydrogels [26]. Crosslinked PAAc is an anionic polymer that swells extensively in alkaline media. The carboxylic acid side groups of PAAc induce this pH-sensitivity with a pKa around 4.25. On the other hand, PNIPAM exhibits a volume phase transition temperature (VPTT) at around 32–34 °C in aqueous media. PNIPAM undergoes an abrupt, reversible swelling-deswelling process below and above the VPTT [27]. These responsive hydrogels are attractive candidates for various biomedical applications and artificial tissues. Also, synthetic polymers including PAAc and PNIPAM offer tunability with respect to biodegradation and biofunctionality. Our lab has synthesized and studied fully degradable PNIPAM scaffolds [14,19], and PAAc is well-suited to well-established protocols for heterobifunctional biomodification via its carboxylic acid group.

Despite many favorable properties, most synthetic hydrogels suffer from poor mechanical properties (low modulus, low strength), that limits their applications to strong tissues such as cornea, tendon, muscle and blood vessel. Efforts have been devoted to improve the mechanical properties of hydrogels including interpenetrating polymer network hydrogels [28], nanocomposite hydrogels [29], a macromolecular microsphere composite gel [30], and double network hydrogels [31]. In this work, a double network (DN) technique was developed based on a conventional interpenetrating network technique (IPN). Hydrogels fabricated by the DN technique (DN-hydrogels) consist of two polymer components that are referred to as the first network and the second network respectively. Unlike IPN-hydrogels, the DN-hydrogels are comprised of two types of polymers with contrasting physical natures: densely cross-linked, rigid and brittle polyelectrolytes at low concentration as the first network and sparsely cross-linked, soft and ductile neutral polymers at high concentration as second network [31,32]. DN-hydrogels are promising candidate materials for applications in tissue engineering and reconstructive medicine because of their high water content, high mechanical strength and toughness, good biocompatibility and low frictional resistance [33]. Such hydrogels have been explored for corneal prostheses [34].

Here we describe a preparation of a sphere-templated 3D porous hydrogel scaffold based on PAAc and PNIPAM by combining sphere-templating and double network polymerization techniques to enhance hydrogel mechanical strength and generate a controllable, uniform, interconnected porous structure that has demonstrated excellent biointegration and reconstruction. Scanning electron microscopy (SEM) was performed to characterize the morphology of the scaffolds. The mechanical properties were

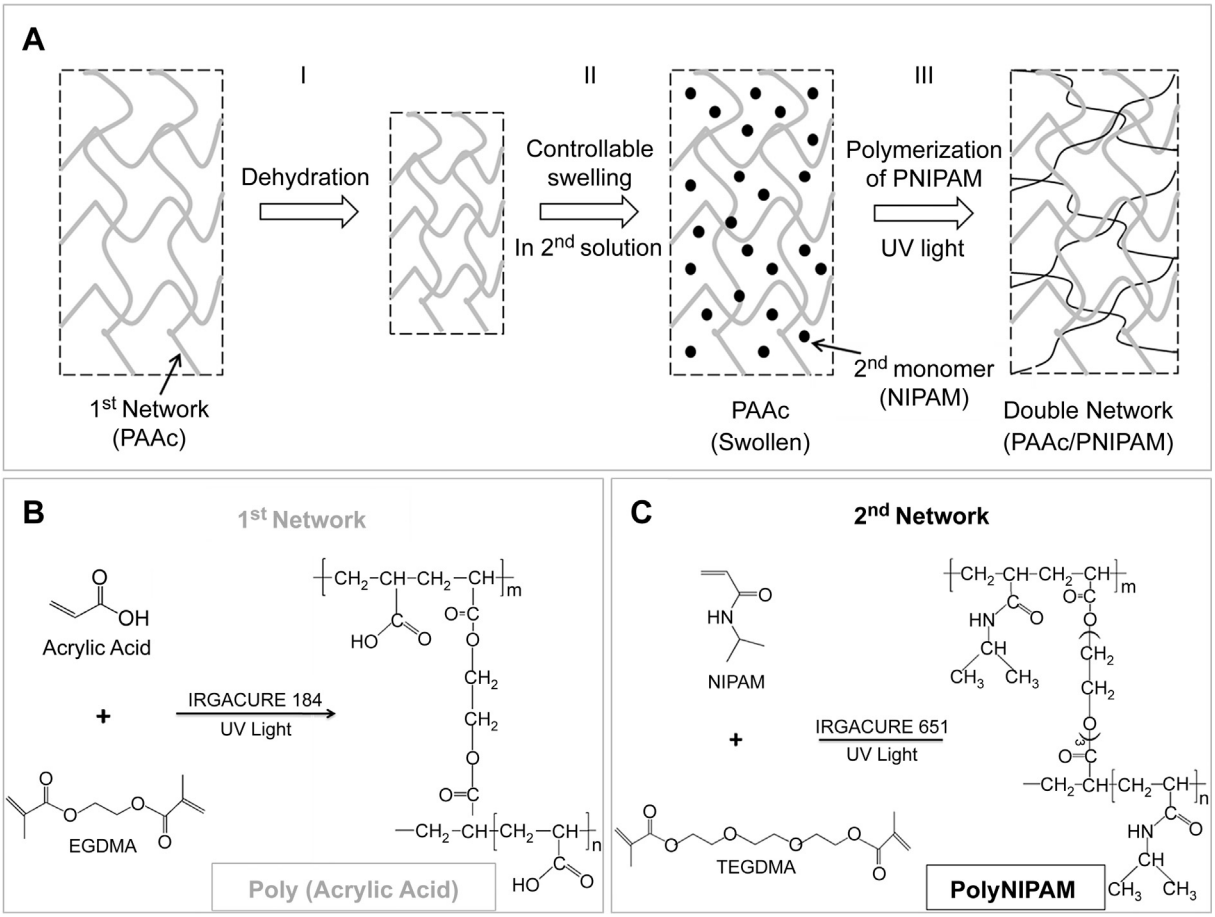

Fig. 1. Schematic of the formation of the PAAc/PNIPAM-based hydrogels (A); Synthesis of PAAc (B) and PNIPAM (C) by UV polymerization from monomers of acrylic acid and NIPAM, respectively, in the presence of cross-linker and photoinitiator.