Polymer 54 (2013) 2088–2095

Contents lists available at SciVerse ScienceDirect

Polymer

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

Synthesis and characterization of biocompatible hydrogel using Pluronics-based block copolymers

Mahati Elluru [a], Hongyang Ma [a], Michael Hadjiargyrou [b,**], Benjamin S. Hsiao [a], Benjamin Chu [a,*]

[a] Department of Chemistry, Stony Brook University, Stony Brook, NY 11794, USA
[b] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

ARTICLE INFO

Article history:
Received 8 November 2012
Received in revised form
6 February 2013
Accepted 11 February 2013
Available online 19 February 2013

Keywords:
Lumpectomy
Hydrogel
Pluronics F-127

ABSTRACT

The tri-block copolymer $(PEO)_{99}$-$(PPO)_{69}$-$(PEO)_{99}$ (commercially known as F127, with PEO and PPO representing polyethylene oxide and polypropylene oxide, respectively), a member of Pluronics co-polymers, was chemically modified by introducing vinyl groups at the terminal ends of the polymer chain. With or without the addition of Pluronics or PEO chains, the modified copolymer could be cross-linked by UV irradiation to form a soft but tough gel-like scaffold. This cross-linked polymer has material properties, such as storage modulus (11–40 kPa), comparable to native human breast tissue. Swelling studies revealed that the strength of the cross-linked polymer networks could be adjusted to be comparable to human adipose tissue. Exposure of pre-adipocytes to the constituents of the Pluronics hydrogel and the cross-linked gel did not show any adverse effects on the cells, thus confirming the biocompatibility of the cross-linked hydrogel. Taken together, these findings suggest that cross-linking of modified Pluronics hydrogel can potentially yield a promising candidate scaffold for the regeneration of native tissue, such as breast tissue to repair defects resulting from lumpectomies.

1. Introduction

Breast conservation surgery (BCS), also known as lumpectomy, is a process that involves the removal of lump or tumor from the affected breast tissue and is often considered to result in minimal deformation of the breast. BCS patients sometimes undergo multiple excisions of their tumor along with radiation therapy [1]. These procedures often deform the shape of the breast and patients have been observed to report post-operative breast asymmetry. Post-surgery breast reconstruction is an option chosen by most women. With known psychological benefits, it has become a significant part of breast cancer management. Reconstruction using autologous tissue was one of the earliest explored options for the breast, but early attempts were unsuccessful, not reproducible and also resulted in significant donor-site morbidity [2]. These failures led to the development of an array of prosthetic options, with the most popular being silicone and saline implants. However, over time these implants have been associated with several safety concerns, including connective-tissue diseases, rheumatic disorders, risk of rupture and leakage, dislocation, capsular contracture, extrusion and ectopic mineralization [3]. Additionally, silicone implants are also known to pose difficulties with post-operative imaging techniques.

All prosthetic implants currently available are functional mainly for mastectomy patients, thus leaving autologous procedures as the only treatment of choice for lumpectomy patients. However, autologous techniques are very expensive and complex, often involving long and complicated surgical procedures. At present, no commercially viable products exist for lumpectomy patients. Thus, the development of a new material that (1) has the ability to be molded into the desired shape and size, (2) is able to withstand the radiation treatment without substantial decomposition, and (3) does not obscure X-ray examination on surrounding tissues, would greatly diminish the psychological impact of lumpectomies on patients and also become the key to encourage and assuage patients to receive the necessary treatment.

Hydrogels are known to be one of the substances that have the closest resemblance to natural living tissue than any other class of synthetic biomaterials [4]. They are three-dimensional networks of polymer chains that are hydrophilic and are capable of imbibing large amounts of water or biological fluids. Swollen hydrogel networks possess both the cohesive properties of solids and the diffusive transport characteristics of liquids. As a result of their high

* Corresponding author. Tel.: +1 631 632 7928; fax: +1 631 632 6518.
** Corresponding author.
E-mail addresses: Michael.Hadjiargyrou@stonybrook.edu (M. Hadjiargyrou), bhsiao@notes.cc.sunysb.edu (B.S. Hsiao), bchu@notes.cc.sunysb.edu (B. Chu).

0032-3861/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2013.02.017

water content and soft consistency, they very closely resemble the natural tissue [5,6].

Pluronics, also known as 'poloxamers' [7], are a family of tri-block copolymers consisting of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a basic X-Y-X structure, with X being EO chains and Y being PO chains. Owing to their amphiphilic properties, these copolymers exist in aqueous solutions in the form of single chains, micelles or physical gels. At concentrations above the critical micelle concentration (CMC) and at temperatures above the critical micelle temperature (CMT), the block copolymer molecules can self-assemble into micelles in aqueous solutions [7]. At higher polymer concentrations, the closed packing of micelles results in the formation of gel-like ordered structures. Above the 'critical gel concentration' (CGC), the micelles overlap to yield a gel-like medium with ordered quasi-lattice packing. Pluronics solutions are thus capable of exhibiting thermally reversible gelation behavior [8–10].

F-127 (EO_{99}-PO_{69}-EO_{99}) is one of the popular members in the family of Pluronics due to its excellent biocompatibility and FDA approval for use in pharmaceutical applications. Aqueous solutions of 20–30% F-127 exist in the liquid state at refrigerated temperatures, such as 4–5 °C, and transforms into a gel-like soft material upon warming to room temperature. This 'gelation' behavior is reversible due to the unique temperature dependent property of PO chains which are hydrophilic at low temperatures but become hydrophobic at higher temperatures [9,11].

Hydrogels fabricated from pure unmodified Pluronics can form a physical gel at body temperature. Unfortunately, the 'gels' formed may disintegrate in a relatively short time when exposed in an aqueous environment with continuous removal of the polymer in its micellar state. Thus, such a physical hydrogel needs to be modified in order to meet the implant needs for lumpectomy patients [12]. Dependent on the degree of cross-linking, partial chemical cross-linking of Pluronics can turn the physical 'gel' into a more stable, partially covalently bonded chemical gel. This chemically cross-linked gel will no longer be *completely* thermo-reversible. However, the timing on the slower disappearance of a chemically cross-linked gel can now be utilized to our advantage because the composite polymer network has the potential to act as a temporal scaffold for the natural regeneration of breast tissue. The experiments described here represent only the first step towards a future implant that will serve as a tissue engineered scaffold and degrade with time, while the endogenous cells regenerate the lost breast tissue resulting from lumpectomies. There are many parameters within our disposal, including, for example, the block length and block ratio of the copolymers, the degree of chemical cross-linking, the mixing of modified and unmodified Pluronics, and the use of charge groups on the tri-block copolymers to compensate the osmotic imbalance between the external fluid and the porous scaffold. The variation of those parameters should permit us to vary the temperature for thermal reversibility, the mechanical property of the gel network, and the decay rate of the scaffold. Thus, the current study merely focuses upon the feasibility of this concept.

2. Experimental

2.1. Materials

F-127 [$(PEO)_{99}$-$(PPO)_{69}$-$(PEO)_{99}$, with the weight-average molecular weight, MW: 1.26×10^4 Da was obtained courtesy of BASF corporation. Acryloyl Chloride, anhydrous N-Methyl-2-pyrrolidinone (NMP), and poly ethylene glycol (PEG, Molecular wt: 1500 Da) were purchased from Sigma Aldrich. The solvents were of analytical grade. All reagents were used as received without further purification. Irgacure 2959 (4-(2-hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone) was obtained from Ciba for use as a photo-initiator. Fisher-brand regenerated cellulose tubing (10k Molecular Weight Cut Off (MWCO), 32 mm flat-width) and dialysis tubing closures were purchased from Spectrum Laboratories. 3T3-L1 pre-adipocyte cell line used for cell studies was purchased from American Type Culture Collection (ATCC). Reagents for cell studies were obtained from multiple suppliers. Dulbecco's modified eagle's medium was purchased from Sigma Aldrich and Dulbecco's phosphate buffered saline was obtained from Gibco. Live/Dead cytotoxicity assay kit for cell based assays was obtained from Invitrogen.

2.2. Chemical modification of Pluronics F-127

Hydroxyl groups of F-127 were acrylated by reacting F-127 (10 g, 0.8 mmol) with ten-fold molar excess of acryloyl chloride. F-127 was completely dissolved in 200 mL NMP. The resultant solution was purged with nitrogen. Acryloyl chloride (8724 mg, dissolved in NMP) was added drop-wise into the Pluronics mixture with continuous stirring. The mixture was allowed to react for 48 h to complete the reaction. The resulting mixture was purified by dialysis (regenerated cellulose tubing, 10k MWCO), followed by freeze drying (Millrock bench-top freeze dryer) to obtain the final product, di-acrylated F-127 (DA-F127). The synthetic scheme for esterification reaction of Pluronic F127 with acryloyl chloride is illustrated in Fig. 1.

2.3. Gel composition and preparation

Gels containing a mixture of polymers, DA-F127 and poly ethylene glycol (PEG – 1500 Da), in 2:1 ratio were utilized to synthesize the hydrogels. The polymer mixture thus consisted of one cross-linkable polymer, DA-F127, and one non-cross-linkable polymer, PEG, that could be leached out to aid in the formation of a porous hydrogel. Gels were prepared by dissolving the required quantities of polymers and photo-initiator in deionized water to make 30% (w/v) solutions. The sol-state solution was placed at 4 °C overnight for homogenous mixing. All hydrogels used in this study were prepared in the same manner unless otherwise specified.

2.4. Gamma irradiation

5 ml 30% (w/v) gel samples of DA-F127 and Pluronic F-127 in plastic vials were exposed to varying gamma radiation dosages

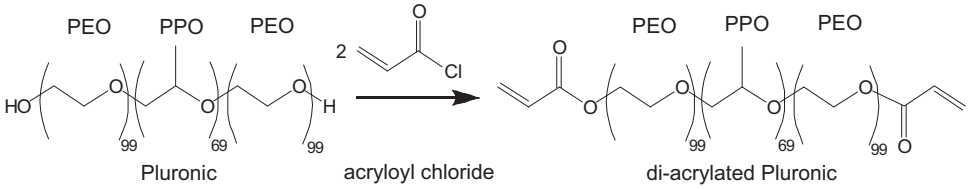

Fig. 1. Esterification reaction of Pluronic F127 with acryloyl chloride.