Materials Science and Engineering C 47 (2015) 298–312

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Efficacy of supermacroporous poly(ethylene glycol)–gelatin cryogel matrix for soft tissue engineering applications

Archana Sharma [a], Sumrita Bhat [b], Vijayashree Nayak [a,*], Ashok Kumar [b,*]

[a] Department of Biological Sciences, Birla Institute of Technology and Science, Pilani-K.K Birla Goa Campus, 403726 Goa, India
[b] Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, Kanpur, 208016 UP, India

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 11 October 2014
Accepted 8 November 2014
Available online 12 November 2014

Keywords:
Cryogels
PEG–gelatin
Tissue engineering
Biocompatibility
Biodegradability
Rheology

ABSTRACT

Three dimensional scaffolds synthesized using natural or synthetic polymers act as an artificial niche for cell adherence and proliferation. In this study, we have fabricated cryogels employing blend of poly (ethylene glycol) (PEG) and gelatin using two different crosslinkers like, glutaraldehyde and EDC-NHS by *cryogelation* technique. Synthesized matrices possessed interconnected porous structure in the range of 60–100 μm diameter and regained their original length after 90% compression without deformation. Visco-elastic behavior was studied by rheology and unconfined compression analysis, elastic modulus of these cryogels was observed to be $>10^5$ Pa which showed their elasticity and mechanical strength. TGA and DSC also showed the stability of these cryogels at different temperatures. *In vitro* degradation capacity was analyzed for 4 weeks at 37 °C. IMR-32, C2C12 and Cos-7 cells proliferation and ECM secretion on PEG–gelatin cryogels were observed by SEM and fluorescent analysis. *In vitro* biocompatibility was analyzed by MTT assay for the period of 15 days. Furthermore, cell proliferation efficiency, metabolic activity and functionality of IMR-32 cells were analyzed by neurotransmitter assay and DNA quantification. The cell–matrix interaction, elasticity, mechanical strength, stability at different temperatures, biocompatible, degradable nature showed the potentiality of these cryogels towards soft tissue engineering such as neural, cardiac and skin.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Regenerative tissue engineering is an emerging approach which regenerates patient's tissues and organs with the retention of biofunctionality, biocompatibility without eliciting any severe immune rejection [1]. To create functional cell grafts, the supporting matrix should be multi-component, porous, hydrophilic, biocompatible, biodegradable, structurally stable, elastic in nature, infection resistant, and mimic natural mammalian extracellular matrix (ECM) and have potential to repair severely damaged organs. An approach of using scaffold has further versatility such as an implantation of only scaffold or *in vitro* cultured cells over the scaffold, where supporting matrix prevents cell loss during the delivery and provides a microenvironment which allow cells to grow, proliferate and differentiate. Alternative approach is the incorporation of biomolecules into the matrix that could potentially enhance cell localization, efficiency of tissue repair and build a cell-friendly environment for better regeneration [2]. In order to achieve a more natural environment for *in vitro* and *in vivo* studies of cell behavior, we have been working on the fabrication of 3-D scaffolds by a novel technique called *cryogelation*. Different conventional scaffolding technologies [3] have been used but *cryogelation* technique has an advantage over other fabrication methods and provides a suitable engineered 3-D surface known as cryogels similar to the native extracellular matrix. These cryogel matrices posses ideal characteristics like large pores, high porosity, high mechanical strength and scale-up opportunities seem to offer a promising alternative towards the treatment of many diseases [4–12]. The aim of this study was to design 3-D cryogels by *cryogelation* technique which shows its potentiality for different soft tissue engineering applications such as neural, cardiac and skin.

Due to limited *in vivo* regeneration ability of neural and cardiac tissues, it is very difficult to get successful therapies for neural and cardiac defects. For neural defects, autologous, allogenic nerve grafts have been used but have some limitations such as shortage of nerve, mismatching with recipient, immune rejection, neuroma formation and no functional recovery [13–16]. To avoid immune rejection, acellular nerve grafts were preferred over allogenic but it delays the process of nerve regeneration and remodeling of extracellular matrix due to lack of viable cells [17]. In case of cardiac defects, to regenerate cardiac tissues *in vivo*, attempts are being made to restore the functionality of the heart affected from myocardial infarction by injecting myogenic cells into the affected myocardium [18]. Possible reasons which limit the survival of the delivered cells may be the harsh environment, low oxygen, or poor integration with the receiving tissue thus, restricting the growth and proliferation of

* Corresponding authors.
E-mail addresses: vijayashree@goa.bits-pilani.ac.in (V. Nayak), ashokkum@iitk.ac.in (A. Kumar).

http://dx.doi.org/10.1016/j.msec.2014.11.031
0928-4931/© 2014 Elsevier B.V. All rights reserved.

the cells [2]. The other organ which is exposed to the external environment and thus frequently subjected to injuries is skin. Xenografts, allografts and autografts as skin substitutes have been used but have drawbacks such as donor limitation and antigenicity. For acute wounds and full thickness burn injury, various acellular dermal constructs like integra, alloderm, etc., are available but are costly and require frequent dressing changes, which can make the patient prone to subsequent secondary bacterial infections [19–21]. Above limitations in all areas can be answered by employing the concepts of tissue engineering which aims to achieve three dimensional scaffolds for the repair of traumatic injuries of peripheral and central nervous systems, re-establish the structure and function of injured myocardium and skin. Various polymeric scaffolds have been used for nerve regeneration approaches, cardiac and skin tissue engineering which include natural polymers, synthetic non-degradable polymers, synthetic biodegradable polymers and conducting polymers [22–38].

In this research work, we have fabricated the polymeric 3-D scaffolds using the combination of synthetic polymer i.e., poly (ethylene glycol) (PEG) with two different molecular weights 2000 and 6000 and natural polymer i.e., gelatin. Gelatin is a non-immunogenic, biodegradable, biocompatible derived from thermal denaturation of collagen which is extracted from fish skin and bones. It increases the adherence of cells on the matrix due to the presence of RGD motifs which interacts with integrins of the cell. It has been used as a scaffolding material as an adhesive and absorbent pad for wound dressing and also for surgical use [39–41]. Another polymer i.e., PEG acts as an ideal material for mimicking microenvironment or acting similar to ECM and also reduces inflammation after implantation due to its inert surface and has shown promising results in various bioengineering applications such as in drug delivery, neural, cartilage, liver and cardiac [42–49]. PEG has low protein adsorption ability and this property is not beneficial for cell adhesion whereas, gelatin helps in cell adherence. Therefore, scaffolds were fabricated from the blend of natural–synthetic polymers using two different crosslinkers such as glutaraldehyde and 1-ethyl-3-[3 dimethylaminopropyl] carbodiimide (EDC-NHS). Glutaraldehyde is an important example of synthetic crosslinking and forms efficient crosslinking due to an advantage of biofunctionality i.e., the presence of both aldehyde and alcoholic group. EDC present in EDC-NHS crosslinker belongs to the class of zero-length crosslinker which modifies amino acid side-groups to permit crosslinking reaction but does not become a part of linkage where, NHS is used to improve the EDC crosslinking [50]. PEG–gelatin cryogel matrices have shown good mechanical properties and provided controlled cellular architecture or environment for cell adherence and their proliferation. These cryogels were characterized chemically, physically as well as mechanically in terms of porosity; cyclic swelling kinetics; flow characteristics; density; crystanallity; TGA; DSC; FTIR; *in vitro* degradation rate; unconfined compression and rheology. Efficiency of these scaffolds to support cell growth and proliferation was confirmed by growing different cell lineages such as human neuroblastoma cells (IMR-32), myoblast cells (C2C12) and fibroblasts (Cos-7) which validates the applicability of these scaffolds towards soft tissue engineering applications.

2. Materials and methods

2.1. Materials

Polyethylene glycol (M.W: 2,000 and M.W: 6,000) was purchased from Merck (Mumbai, India). Gelatin (from cold water fish skin; M.W: 60,000), 3-(4,5 dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT, 98%) reagent, 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDC), propidium iodide (PI), Dulbecco's modified eagle's medium (DMEM), antibiotic–antimycotic solution, 0.25% trypsin, phosphate buffered saline (PBS), Hoechst 33258 dye, papain, γ-amino butyric acid (GABA) and dopamine were purchased from Sigma chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Hyclone (UT, USA). *N*-Hydroxysuccinimide (NHS) and glutaraldehyde solution (25%) were bought from S.D. fine chemicals (Mumbai, India). Dimethyl sulfoxide (DMSO) was purchased from Qualigens fine chemicals (Mumbai, India). Ophthalaldehyde was purchased from Otto kemo (Mumbai, India). Cell lines i.e. IMR-32 (human neuroblastoma), C2C12 (mouse myoblast), and Cos-7 (fibroblast) cell lines were procured from National Centre for Cell Science (Pune, India). All other chemicals were of analytical grade.

2.2. Preparation of supermacroporous PEG–gelatin cryogels

Here, the blend of synthetic polymer (PEG) and natural polymer (gelatin) was used for the fabrication of cryogels with two different crosslinking agents by the *cryogelation* technique. Polyethylene glycol (PEG)–gelatin cryogels (PEG of different molecular weights 2000 and 6000) were synthesized by using glutaraldehyde and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride-N-Hydroxysuccinimide (EDC-NHS) as a crosslinking agent. For PEG–gelatin cryogels with glutaraldehyde as a crosslinker, blend solution of 1.5% PEG (150 mg/10 mL) of different molecular weights 2000 and 6000 and 5% gelatin (500 mg/10 mL) in a total volume of 10 mL deionized water was crosslinked by 300 μL of 25% (V/V) glutaraldehyde. For PEG–gelatin cryogels with EDC-NHS as a crosslinker, 1.5% of PEG (2000 and 6000) (150 mg/10 mL) and 4% of gelatin (400 mg/10 mL) were mixed properly and crosslinked by 23.4 mM EDC (45 mg/10 mL) with 13 mM NHS (15 mg/10 mL) in a total volume of 10 mL deionized water. PEG–gelatin solutions were then incubated in cryostat (Seelbach, Germany) at −12 °C for 16 h. After completion of incubation period gels were thawed in deionized water at room temperature and then kept for lyophilization at −57 °C overnight in lyophilizer (Martin Christ GmbH, Germany). Lyophilized cryogels were stored and used for further experiments. Cryogel monoliths were cut into disks of diameter, 8 mm and 13 mm, thickness in the range of 2 mm to 20 mm and were taken in triplicates (n = 3) for all the experiments.

2.3. Microstructure analysis (SEM)

Lyophilized PEG–gelatin (PEG of different molecular weights 2000 and 6000) cryogels synthesized with glutaraldehyde and EDC-NHS crosslinkers, respectively were analyzed for their microstructure. Cryogel sections of specific dimensions i.e. diameter: 8 mm and thickness: 3 mm were coated with gold using a sputter coater (Vacuum Tech, Bangalore, India) and their morphology was studied by using scanning electron microscope (SEM, FEI Quanta 200) at high vacuum at 20 kV. Cryogel monoliths were sliced into 100 μm thin sections using a microtome. To get a better understanding of the internal architecture of the scaffold these cryogel sections were stained with DAPI and observed under fluorescence microscope.

2.4. Gelation yield and flow rate of cryogels

Cryogels (PGG 2000, PGE 2000, PGG 6000 and PGE 6000) were synthesized with the help of two different crosslinkers such as glutaraldehyde and EDC-NHS. For the estimation of gelation yield, cryogel sections were first extensively washed with gradient concentrations of ethanol (20 to 100%) to remove the unreacted gel precursors [47]. After washing, these cryogel sections were lyophilized and weight of the dried cryogel (M_d) was recorded.

The gelation yield of PEG–gelatin cryogels was calculated using following formula

$$\text{Gelation yield } (\%) = \frac{M_d}{M_t} \times 100$$

where M_t is the total mass of the gel precursors in the reaction mixture and M_d is dried weight of cryogel.

Download English Version:

https://daneshyari.com/en/article/1428405

Download Persian Version:

https://daneshyari.com/article/1428405

Daneshyari.com