Biomaterials 31 (2010) 1655–1665

Contents lists available at ScienceDirect

Biomaterials

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

Cross-linked open-pore elastic hydrogels based on tropoelastin, elastin and high pressure CO_2

Nasim Annabi [a], Suzanne M. Mithieux [b], Anthony S. Weiss [b], Fariba Dehghani [a,*]

[a] School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW 2006, Australia
[b] School of Molecular and Microbial Biosciences, The University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 16 October 2009
Accepted 17 November 2009
Available online 6 December 2009

Keywords:
Tropoelastin
α-Elastin
Hydrogel
Glutaraldehyde
High pressure CO_2
Fibroblast

ABSTRACT

In this study the effect of high pressure CO_2 on the synthesis and characteristics of elastin-based hybrid hydrogels was investigated. Tropoelastin/α-elastin hybrid hydrogels were fabricated by chemically cross-linking tropoelastin/α-elastin solutions with glutaraldehyde at high pressure CO_2. Dense gas CO_2 had a significant impact on the characteristics of the fabricated hydrogels including porosity, swelling ratio, compressive properties, and modulus of elasticity. Compared to fabrication at atmospheric pressure high pressure CO_2 based construction eliminated the skin-like formation on the top surfaces of hydrogels and generated larger pores with an average pore size of 78 ± 17 μm. The swelling ratios of composite hydrogels fabricated at high pressure CO_2 were lower than the gels produced at atmospheric pressure as a result of a higher degree of cross-linking. Dense gas CO_2 substantially increased the mechanical properties of fabricated hydrogels. The compressive and tensile modulus of 50/50 weight ratio tropoelastin/α-elastin composite hydrogels were enhanced 2 and 2.5 fold, respectively, when the pressure was increased from 1 to 60 bar. *In vitro* studies show that the presence of large pores throughout the hydrogel matrix fabricated at high pressure CO_2 enabled the migration of human skin fibroblast cells 300 μm into the construct.

1. Introduction

Tissue engineering offers the potential to create functional and viable tissue constructs for patients requiring organ replacement [1]. A three dimensional (3D) scaffold is necessary to serve as a template to guide cell growth and tissue development. The chemical composition, mechanical properties, porosity, pore interconnectivity, and 3D structures of the scaffold greatly influence the formation of the new tissue [2]. Hydrogels have emerged as leading candidates for engineered tissue scaffolds as much of the native extracellular matrix (ECM) in the body forms hydrated polymeric networks that closely resemble the mechanical, biological and physical properties of hydrogels [3]. Hydrogels composed of natural polymers, such as collagen, hyaluronan (HA), fibrin, alginate, agarose, dextran, chitosan, and elastin-like polypeptides (ELPs), are desirable for tissue engineering due to their similarities with the extracellular matrix, high chemical versatility, typically good biological performance and inherent cellular interaction [4].

Elastin is an insoluble, polymeric, ECM protein that provides various tissues in the body with the properties of extensibility and elastic recoil [5]. Elastin-based biomaterials are increasingly investigated due to their remarkable properties such as elasticity, self-assembly, long-term stability, and biological activity [6]. Elastin is highly insoluble as a consequence of extensive lysine mediated cross-linking and therefore difficult to process into new biomaterials. Consequently, soluble forms of elastin including α-elastin [7–9], recombinant tropoelastin (rTE) [10,11], and engineered recombinant elastin-like polypeptides (ELPs) [12] are frequently used to form cross-linked hydrogels. Tropoelastin is the soluble precursor of elastin, and α-elastin is an oxalic acid-solubilised derivation of elastin [13]. rTE has been chemically cross-linked to form synthetic elastin (SE) hydrogels [11]. The fabricated SE hydrogels had strength, elasticity, and biocompatibility properties similar to those of naturally occurring human elastic tissue. *In vitro* and *in vivo* experiments revealed that these SE hydrogels could support cellular growth; however, the non-homogenous and limited porosity of these constructs prohibited cellular migration deep into the hydrogels. Generally, this lack of cellular migration into the 3D structures due to the existence of a discrete discontinuous pore network is an issue associated with the current approaches used for SE hydrogel fabrication. Electrospinnig techniques [14] and dense gas carbon dioxide (CO_2) [7,9] have been used to facilitate greater cell penetration and infiltration into the 3D structure of elastin-based biomaterials.

* Corresponding author.
E-mail address: fdehghani@usyd.edu.au (F. Dehghani).

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.11.051

Dense gas CO_2 has been used widely as an attractive means of producing porous biomaterials for tissue engineering applications [15,16]. It has been exploited as a foaming agent to induce porosity in the structure of several common hydrophobic polymers such as poly (lactic acid) (PLA), poly (lactic acid-co-glycolic acid) (PLGA), and polycaprolactone (PCL) [15–18]. However, dense gas CO_2 generally has low solubility in hydrophilic polymers. Various techniques such as CO_2-water emulsion templating [19–23] or the use of a co-solvent system have been developed to improve the ability of a dense gas to diffuse into a hydrophilic polymer and produce porosity [24,25]. Hydrogels fabricated using these techniques generally contain small pores with the average pore size less than 26 μm and display limited porosity [20,21].

The results of our previous studies demonstrate that highly interconnected pores with thin walled structures resembling natural elastin can be produced by cross-linking α-elastin using glutaraldehyde (GA) under high pressure CO_2 [9]. The cell infiltration throughout the hydrogels was considerably enhanced compared with the samples produced at atmospheric conditions. This improvement resulted from CO_2 induced channels within the structure of the α-elastin hydrogels [9]. However, the low number of lysine residues (less than 1%) in α-elastin resulted in limited cross-linking with GA and consequently poor mechanical integrity [26]. The use of a cross-linking agent such as hexamethylene diisocyanate (HMDI) that can react with other amino acids available in the α-elastin structure increased the cross-linking density and mechanical properties of fabricated hydrogels [7]. The reaction was undertaken in a CO_2 expanded dimethyl sulfoxide (DMSO) solution and required solvent residue removal after the process. *In vitro* studies demonstrated that the fabricated constructs promoted cellular migration and growth throughout the 3D matrix [7].

The objective of this study was to fabricate a composite recombinant rTE/α-elastin hydrogel in an aqueous based system with desirable properties for tissue engineering applications. The addition of rTE containing 35 lysine residues per molecule to the protein solution was expected to increase the cross-linking density and promote the mechanical properties of elastin-based hydrogels. The effect of pressure, rTE and GA concentrations on the characteristics of hybrid hydrogels were investigated. *In vitro* studies were conducted to assess the cellular growth and proliferation in the 3D structures of fabricated hydrogels.

2. Materials and methods

2.1. Materials

rTE isoform SHELΔ26A (Synthetic Human Elastin without domain 26A) corresponding to amino acid residues 27–724 of GenBank entry AAC98394 (gi 182020) was purified from bacteria on a multi-gram scale as previously described [27]. α-elastin extracted from bovine ligament was obtained from Elastin Products Co. (Missouri USA). GA was purchased from Sigma. Food grade carbon dioxide (99.99% purity) was supplied by BOC. GM3348 fibroblast cell line was obtained from the Coriell Cell Repository. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), penicillin and streptomycin. All tissue culture reagents were obtained from Sigma.

2.2. Hydrogel formation

2.2.1. Hydrogel fabrication at atmospheric pressure

In each experiment a 100 mg/ml of rTE/α-elastin in PBS (150 mM NaCl) was mixed with GA at 4 °C and the solution was immediately pipetted into a Lab-Tek chamber slide. The slide was then placed at 37 °C for 24 h to fabricate a hydrogel. The cross-linked hydrogel was removed from the slide, washed repeatedly with PBS, and stored in PBS for characterisation.

A preliminary set of experiments were conducted to determine the required ratio of GA and rTE/α-elastin solution for the hydrogel fabrication. A 50/50 weight ratio rTE/α-elastin was used to optimise the concentration of GA and protein solution. The concentration of rTE/α-elastin (50/50) solution was varied between 5 mg/ml and 100 mg/ml, and GA between 0.05 and 0.5% (v/v). The solutions were pipetted into a Lab-Tek chamber slide and then placed at 37 °C for 24 h. The cross-linked hydrogels were washed in PBS, then placed in 100 mM Tris ($(HOCH_2)_3CNH_2$) in PBS for 1 h to inhibit further cross-linking and stored in PBS for characterisation.

2.2.2. Dense gas in hydrogel formation

The experimental procedure used to fabricate rTE/α-elastin hydrogels was similar to our previous study for the fabrication of cross-linked α-elastin hydrogel by the dense gas CO_2 [9]. Briefly, rTE/α-elastin solution containing GA was injected into a custom-made Teflon mould placed inside the high pressure vessel. After the vessel was sealed and approached thermal equilibrium at 37 °C, the system was pressurised with CO_2 to 60 bar, isolated and maintained at these conditions for a set period of time. The system was then depressurised and the sample was collected. Cross-linked structures were immediately washed repeatedly in PBS, and then placed in 100 mM Tris in PBS for 1 h. After Tris treatment, the hydrogels were washed twice and stored in PBS for further analysis.

The effects of reaction time, cross-linker concentration, depressurisation rate, and rTE concentration on the characteristics of hydrogels were assessed. Different concentrations of GA (i.e. 0.25 and 0.5%(v/v)) were mixed with 100 mg/ml of rTE/α-elastin (50/50) and the solutions were pipetted into the mould, containing two individual wells and placed inside the high pressure vessel. The system was then pressurised to 60 bar for a certain period of time. Different rates of depressurisation were used in order to investigate the effect of depressurisation rate on the properties of fabricated hydrogels. The effect of addition of rTE on the characteristics of the hydrogels was investigated by using weight ratios of 25/75, 50/50, 75/25, and 100/0 rTE/α-elastin. All samples were prepared over a 1 h reaction time as our preliminary results demonstrated that the rigidity of fabricated hydrogels at high pressure was not significantly improved by keep increasing the reaction time.

2.3. Swelling properties

The swelling behaviour of the GA cross-linked rTE/α-elastin hydrogels produced at high pressure and atmospheric conditions was evaluated at two different temperatures (37 °C and 4 °C) in PBS. The hydrogels were lyophilised prior to use and were weighed dry. The samples were then swelled in 10 ml PBS for 24 h. For each temperature, at least three samples were tested. The excess liquid was removed from the swelled samples and the swelling ratio was calculated based on a ratio of the increase in mass to that of the dry sample.

2.4. Scanning electron microscopy (SEM)

The SEM images of samples were obtained using a Philips XL30 scanning electron microscope (15 KV) to determine the pore characteristics of the fabricated hydrogels and to examine cellular infiltration and adhesion. Lyophilised α-elastin hydrogels were mounted on aluminium stubs using conductive carbon paint, then gold coated prior to SEM analysis.

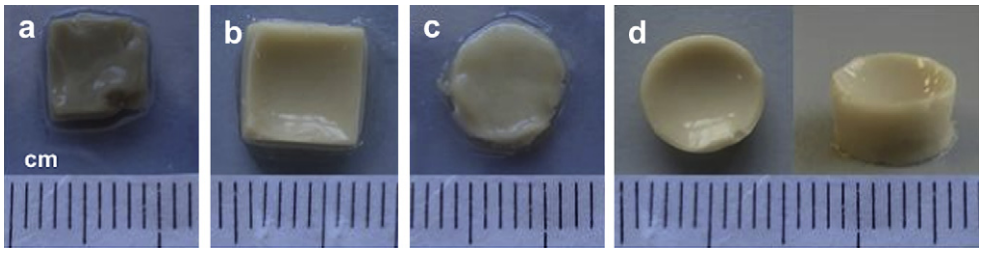

Fig. 1. GA cross-linked rTE/α-elastin hydrogels produced at (a, b) atmospheric condition, and (c, d) high pressure CO_2 (0.25% (v/v) GA was used in a, c and 0.5% (v/v) GA in b and d).