Materials Science and Engineering C 80 (2017) 263–273

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Comparison of glutaraldehyde and procyanidin cross-linked scaffolds for soft tissue engineering

Yang Yang, Alastair Campbell Ritchie *, Nicola M. Everitt *

Bioengineering Research Group, Faculty of Engineering, University of Nottingham, Nottingham NG7 2RD, United Kingdom

ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form 24 May 2017
Accepted 28 May 2017
Available online 29 May 2017

Keywords:
Procyanidin
Cross-linker
Gelatin/chitosan porous scaffold
Mechanical properties
Biocompatibility
Soft tissue engineering

ABSTRACT

Soft tissue injuries are among the most difficult orthopaedic conditions to treat, and regenerative medicine holds the promise of better treatments of these injuries. There is therefore a requirement for substrates and porous scaffolds which provide an appropriate chemical and mechanical environment for cell attachment, growth, proliferation and differentiation. In this study, cross-linked porous gelatin-chitosan (Gel/Chi) scaffolds with high porosity (>90%) were fabricated and their internal morphology, pore sizes and porosities were characterized using scanning electron microscopy (SEM), micro computed tomography (micro-CT) and mercury intrusion porosimetry. The cross-linking agents chosen for this study were Procyanidin (PA), chosen for its biocompatibility, and glutaraldehyde (GA), chosen for comparison as a highly effective cross-linker. Concentrations of these cross-linkers varied from 0.1% to 1% (w/v) and controls had the same gelatin-chitosan blend but were untreated. It was found that the water absorption of cross-linked scaffolds decreased as the cross-linker concentration increased and *in vitro* collagenase degradation test showed both cross-linkers increased the biostability of the scaffolds. Scaffolds were also tested under compressive load to investigate their resistance to deformation. The results indicated that both cross-linkers increase the stiffness of the scaffolds both initially and at higher strains, but GA cross-linked scaffolds had a higher compressive stiffness than scaffolds cross-linked with PA for a given concentration. Results from cyclic compression and stress relaxation tests showed that PA cross-linked scaffolds recover more rapidly after deformation. 3T3 fibroblasts were cultured on the scaffolds to assess cytotoxicity and biocompatibility. The results indicated that PA was non-cytotoxic and promoted the attachment and proliferation of the seeded cells, while fewer cells were seen on GA cross-linked scaffolds, indicating that the GA had conferred some cytotoxicity. PA cross-linked Gel/Chi porous scaffolds show promise as three dimentional porous scaffolds in tissue engineering, as porous substrates for biomimetic culture environments, and for regenerative medicine applications, due to their excellent biocompatibility and easily adaptable mechanical properties, as well as their lower cost compared to collagen and fibrin based substrates.

1. Introduction

Scaffolds and wound dressings that mimic the mechanical properties and chemical nature of native soft tissues have been fabricated with the aim of supporting tissue regeneration when injuries or failures occur [1–3]. Generally, scaffolds with porous structure, suitable mechanical properties and acceptable biocompatibility are some of the design requirements to fulfil this aim [3]. Suitable pore size and porosity can support cell migration as well as water absorption, while adequate mechanical properties can prevent failure in service and provide sufficient strength to be handled for implantation [4,5]. The biocompatibility of the fabricated scaffold is another concern: no toxic residuals should remain in the scaffolds after cross-linking, hence non-toxic cross-linking agents are highly advantageous [6].

Chitosan, a low cost natural biopolymer prepared by deacetylation of chitin is widely used in tissue engineering as it has a similar structure to glycosaminoglycans (GAG) which is the main component of extracellular matrix (ECM) [7]. Chitosan derived materials have been used to make membranes, sponges, fibres, hydrogels and micro-particles that may be used in wound dressings, drug & growth factor carriers, and scaffolds for tissue engineering due primarily to their biocompatibility [8–11]. Chitosan has also been used as a copolymer or cross-linking bridge to enhance the mechanical properties of the biopolymer blends in tissue engineering applications [1,12–14]. Gelatin is a thermally denatured product mainly obtained from type I collagen in animal bone and skin. In contrast to collagen, gelatin has low immunogenicity and lower cost, these attributes make it widely investigated in tissue

* Corresponding authors.
E-mail addresses: Yang.Yang@nottingham.ac.uk (Y. Yang), epzac2@exmail.nottingham.ac.uk (A.C. Ritchie), emznme@exmail.nottingham.ac.uk (N.M. Everitt).

http://dx.doi.org/10.1016/j.msec.2017.05.141
0928-4931/

engineering [15,16]. It has also been reported that the carboxyl groups of gelatin are able to link with chitosan through hydrogen bonding and can also act as a bridge between chitosan and a cross-linker [17,18].

Several cross-linking reagents have been used in scaffold fabrication to obtain desirable mechanical properties. These cross-linkers can be divided into two categories: natural and synthetic cross-linking agents. Carbodiimide (1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC)) and glutaraldehyde (GA) are two of the frequently used synthetic cross-linkers while genipin (GP) is a natural cross-linker and is becoming popular in tissue engineering research due to its efficacy and low cytotoxicity [15,19–22]. It should be noted that cell proliferation on substrates may be restricted by certain cross-linking agents. For instance GA, a commonly used cross-linker, may cause local incompatibility, inflammation or calcification that limits cell ingrowth and cytotoxicity (even at concentrations as low as 3.0 ppm) after being released into the host as a result of cross-linked polymer degradation [23,24].

In this study, the cross-linking agents used are GA and procyanidin (PA). PA is a polyphenol that is found in fruits and vegetables and has been isolated for use as cross-linker in tissue engineering recently [25,26]. Studies have shown that procyanidins not only have the ability to stabilize the extracellular matrix-derived scaffolds that primarily rely on hydrogen bonding, but also add antioxidant and pharmacological activity to these biomaterials due to its capacity to absorb free radicals [26–30]. Cross-linking and stabilising biologically derived collagen can significantly increase the mechanical properties of the material: in one

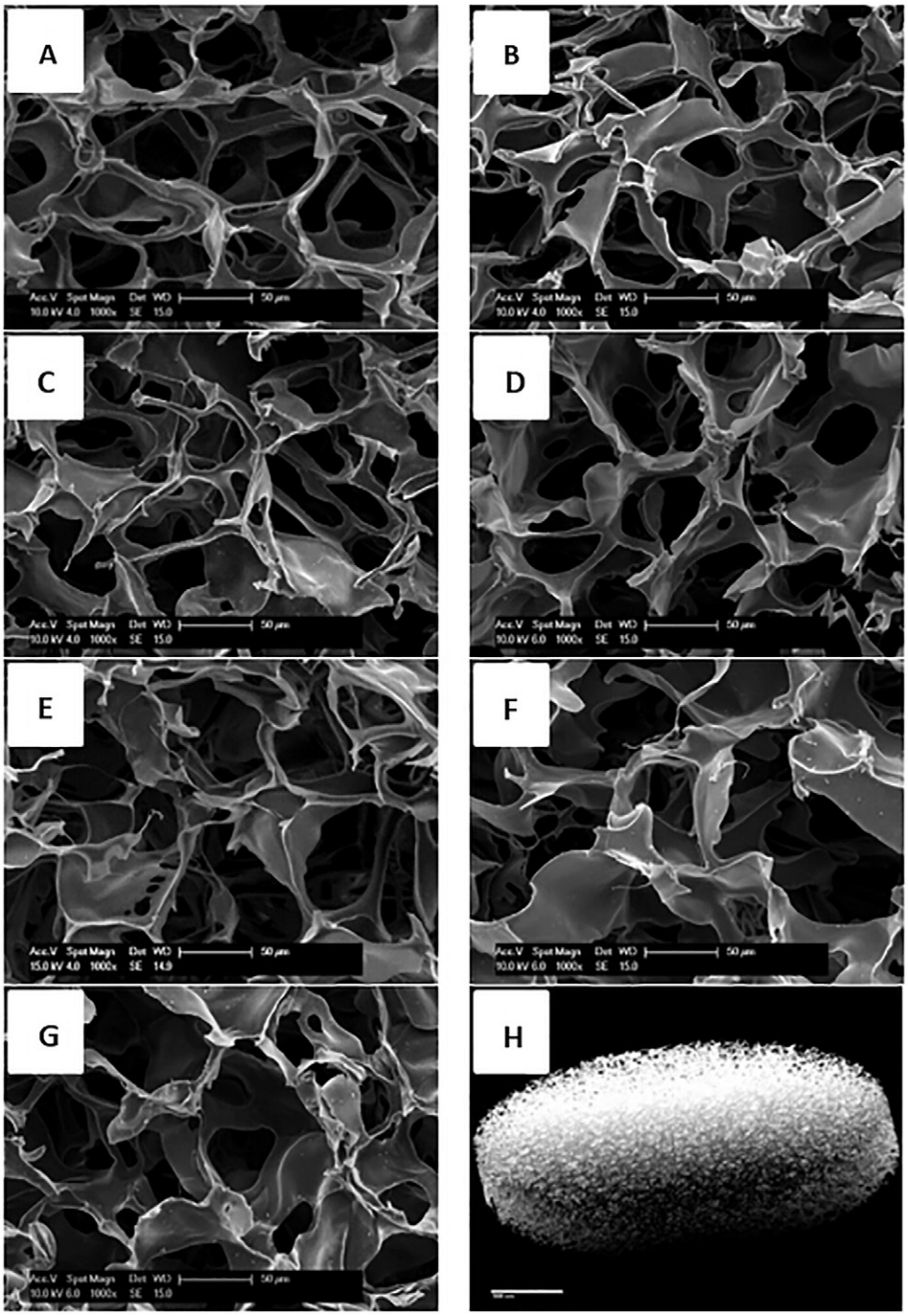

Fig. 1. (A) SEM image of cross-section of uncross-linked scaffold, scale bar = 50 μm; (B–D) SEM images of cross-section of PA 0.1, 0.5 and 1.0 scaffold, scale bar = 50 μm; (*E*–G) SEM images of cross-section of GA 0.1, 0.5 and 1.0 scaffold, scale bar = 50 μm; (H) Reconstructed 3-D view of the uncross-linked scaffold, white colour represented solid part of scaffold, scale bar = 500 μm.