Crosslinking and composition influence the surface properties, mechanical stiffness and cell reactivity of collagen-based films

Chloe N. Grover, Jessica H. Gwynne, Nicholas Pugh, Samir Hamai, Richard W. Farndale, Serena M. Best, Ruth E. Cameron

Keywords: Films, Crosslinking, Cell adhesion, Electron microscopy, Atomic force microscopy

1. Introduction

Research on biomaterial scaffolds for use in soft tissue engineering reflects their ability to deliver cells whilst acting as a mechanical support [1–5]. Despite the widespread use of scaffolds composed of extracellular matrix proteins [4,6–9], detailed analysis of their properties at a cellular lengthscale has not been carried out; in particular the effect of composition and crosslinking on stiffness, surface roughness and ability to support cell activity. Previous studies have concentrated on assessing the individual effect of different compositions and methods of crosslinking on cell activity [10,11] or on physical properties [12–15]. However, comparison of the physical properties and cell reactivity of films of different compositions before and after crosslinking has not yet been performed. The prior research has indicated that crosslinking, using a carbodiimide system, is an effective way of increasing the mechanical stiffness and degradation resistance of biomaterials without being cytotoxic. However, carbodiimide treatment of biomaterials – the formation of crosslinks between free amine groups, typically on lysine residues, and free carboxylate anions, typically on glutamate (E) or aspartate (D) residues – will reduce the availability of cell-binding sites. Hence, although crosslinking can be used to enhance the mechanical stiffness and reduce the roughness of films, it reduces their capacity to support cell activity and could potentially limit the effectiveness of the collagen-based films and scaffolds.

This study focuses on determining the effect of varying the composition and crosslinking of collagen-based films on their physical properties and interaction with myoblasts. Films composed of collagen or gelatin and crosslinked with a carbodiimide were assessed for their surface roughness and stiffness. These samples are significant because they allow variation of physical properties as well as offering different recognition motifs for cell binding. Cell reactivity was determined by the ability of myoblastic C2C12 and C2C12-α2+ cell lines (with different integrin expression) to adhere to and spread on the films. Significantly, crosslinking reduced the cell reactivity of all films, irrespective of their initial composition, stiffness or roughness. Crosslinking resulted in a dramatic increase in the stiffness of the collagen film and also tended to reduce the roughness of the films ($R_q = 0.417 \pm 0.035 \mu m$, $E = 31 \pm 4.4$ MPa). Gelatin films were generally smoother and more compliant than comparable collagen films ($R_q = 7.9 \pm 1.5$ nm, $E = 15 \pm 3.1$ MPa). The adhesion of α2-positive cells was enhanced relative to the parental C2C12 cells on collagen compared with gelatin films. These results indicate that the detrimental effect of crosslinking on cell response may be due to the altered physical properties of the films as well as a reduction in the number of available cell binding sites. Hence, although crosslinking can be used to enhance the mechanical stiffness and reduce the roughness of films, it reduces their capacity to support cell activity and could potentially limit the effectiveness of the collagen-based films and scaffolds.

Corresponding author. Tel.: +44 1223 362966; fax: +44 1223 334366. E-mail address: cng21@cam.ac.uk (C.N. Grover).

Available online 12 May 2012

Article history:
Received 16 January 2012
Received in revised form 22 March 2012
Accepted 7 May 2012

Abstract

This study focuses on determining the effect of varying the composition and crosslinking of collagen-based films on their physical properties and interaction with myoblasts. Films composed of collagen or gelatin and crosslinked with a carbodiimide were assessed for their surface roughness and stiffness. These samples are significant because they allow variation of physical properties as well as offering different recognition motifs for cell binding. Cell reactivity was determined by the ability of myoblastic C2C12 and C2C12-α2+ cell lines (with different integrin expression) to adhere to and spread on the films. Significantly, crosslinking reduced the cell reactivity of all films, irrespective of their initial composition, stiffness or roughness. Crosslinking resulted in a dramatic increase in the stiffness of the collagen film and also tended to reduce the roughness of the films ($R_q = 0.417 \pm 0.035 \mu m$, $E = 31 \pm 4.4$ MPa). Gelatin films were generally smoother and more compliant than comparable collagen films ($R_q = 7.9 \pm 1.5$ nm, $E = 15 \pm 3.1$ MPa). The adhesion of α2-positive cells was enhanced relative to the parental C2C12 cells on collagen compared with gelatin films. These results indicate that the detrimental effect of crosslinking on cell response may be due to the altered physical properties of the films as well as a reduction in the number of available cell binding sites. Hence, although crosslinking can be used to enhance the mechanical stiffness and reduce the roughness of films, it reduces their capacity to support cell activity and could potentially limit the effectiveness of the collagen-based films and scaffolds.