CROSSLINKING OF COLLAGEN BY ULTRAVIOLET IRRADIATION AND GLUCOSE

*Ohan, M; +*Dunn, M (A-NIH-NIAMS)
+*UMDNJ - Robert Wood Johnson Medical School, Division of Orthopaedic Surgery, New Brunswick, NJ. P.O. Box 19, New Brunswick, NJ 08903, (732) 235-7972, Fax: (732) 235-6002, dunnmg@umdnj.edu

INTRODUCTION

Collagen as an implantable biomaterial is approved by the FDA for several applications and is currently under investigation for additional uses. Collagen in the form of films and fibers is being developed for soft tissue repair (i.e. ligament, meniscus, skin) or as a delivery vehicle for bone induction factors. The collagen must be crosslinked in order to increase the mechanical strength and resistance to proteolytic enzymes, properties especially crucial in load-bearing orthopaedic applications. Crosslinks are covalent bonds that stabilize and strengthen the triple-helical collagen molecule, both intermolecularly and intramolecularly.

Ultraviolet irradiation (UV) is known to rapidly induce crosslinks in collagen without introducing any potentially cytotoxic by-products.[1] However, an unfortunate consequence of UV crosslinking is partial denaturation or loss of collagen nativity via chain scission of the collagen polymer backbone.[2] Loss of nativity decreases strength and increases the susceptibility of the collagen to degradation by proteolytic enzymes.[3] Sugar (i.e. glucose) is capable of crosslinking collagen without inducing any potential cytotoxicity.[4] The purpose of this study was to investigate whether the induction of glucose-derived crosslinks to UV crosslinked collagen would limit the loss of nativity. We hypothesize that the concomitant generation of glucose-derived crosslinks during UV crosslinking will provide further structural integrity to the collagen, thus maintaining the native state of collagen. Glucose-incorporated collagen films were evaluated for the degree of crosslinking and for the relative nativity of the collagen.

METHODS

Glucose-incorporated (GI) collagen films, containing 0.1, 0.5, and 0.8% (w/v) glucose, were cast in polystyrene trays from 0.5% (w/v) acid insoluble bovine type I collagen dispersed in hydrochloric acid (pH 2.4). Control films contained no glucose. Four films were cast for each group. The films were allowed to dry in ambient air for 48 hours. Subsequently, the dried films were exposed to UV (254 nm) in a Stratalinker 2400 for 60 minutes, at a distance of 11 cm from the light source. After UV crosslinking, films were cut into 1.4 cm X 6 cm strips.

Degree of Crosslinking: Heat denaturation of collagen eliminates all non-covalent bonds in collagen, retaining only the covalent crosslinks. Four strips from each group were randomly selected and placed in boiling water for 10 seconds, causing denaturation. Strips were tensile tested to failure at a strain rate of 200%/minute on an Instron materials tester. The ultimate tensile load (UTL) was recorded; crosslink density is proportional to UTL.

Estimate of Collagen Nativity: Trypsin is a proteolytic enzyme capable of degrading denatured collagen and has little effect on intact, native collagen. Strips of films were incubated in a trypsin solution (1000 active enzyme units per ml PBS) at 37° C for 0, 120, and 240 minutes. For each time period, four strips were randomly selected from each group and tensile tested to failure at a strain rate of 200% on an Instron materials tester. The UTL was recorded.

Significant differences between films made with and without glucose were determined by a one-tailed Student *t*-test ($p < 0.05$) and are indicated by a '*' in Figures 1 and 2.

RESULTS

Degree of Crosslinking: The addition of sugar to the collagen increased the mean UTL values, indicating a higher degree of crosslinking (See Figure 1). The 0.1% GI films showed a significantly higher UTL compared to the control. At higher concentrations (0.5% and 0.8% glucose) there was no significant difference compared to the control.

Estimate of Collagen Nativity: Control films (0.0% glucose) showed marked decreases in UTL after 120 and 240 minutes trypsin exposure (See Figure 2). Conversely, the GI films, especially the 0.1% glucose, maintained their UTL out to 240 minutes. Without exposure (t=0 minutes) and after 120 and 240 minutes exposure to trypsin, 0.1% GI films had a significantly higher tensile load than the control. After 240 minutes, the 0.5 and 0.8% GI films maintained significantly higher load bearing capacity than controls.

Figure 1: Degree of Crosslinking :UTLof UV Crosslinked Films After Heat Denaturation (n=4/group)

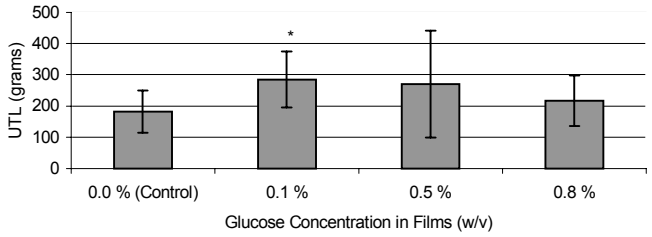
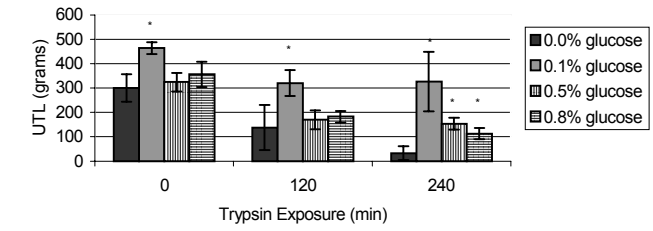


Figure 2: Estimate of Nativity :UTL of UV Crosslinked Collagen Films Exposed to Trypsin (n=4/group)



DISCUSSION

Collagen for orthopaedic applications requires crosslinking to increase the strength and durability of the implanted material. The maintenance of nativity during processing also provides resistance to proteolytic enzyme degradation. Both the degree of crosslinking and the nativity of the collagen influence the strength and the rate of resorption of the implant. In this study collagen crosslinked with glucose and UV was evaluated for its potential use as an orthopaedic biomaterial.

Results suggest that collagen films with a glucose concentration of 0.1% had the highest degree of crosslinking and the highest collagen nativity (the least denaturation). The UTL obtained for collagen after heat denaturation indicates that 0.1% GI films had a greater extent of crosslinking as compared to films without glucose. The decrease in UTL experienced by the control collagen films after 120 and 240 minutes of trypsin exposure indicates a loss of collagen nativity. Conversely, this decrease in load was not seen for the 0.1% glucose incorporated films, suggesting the preservation of nativity by the addition of glucose-derived crosslinks, consistent with our hypothesis. Glucose concentrations greater than 0.1% may have had an inhibitory effect, preventing collagen to collagen interactions.

Glucose incorporation into collagen materials generates crosslinks upon exposure to UV. The synergistic effect of UV and glucose crosslinking results in a material with increased strength and limited denaturation, without inducing any cytotoxic by-products. Future directions involve development of UV crosslinked glucose-incorporated collagen fibers potentially useful as scaffolds for musculoskeletal soft tissue reconstruction.

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