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# Photomediated crosslinking of C6-cinnamate derivatized type I collagen

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**Abstract**

Synthesis and characterization of cinnamated Type I collagen and its related mechanical properties after photomediated crosslinking were investigated in detail. Using an EDC/NHS conjugation method, collagen was chemically modified to incorporate a photosensitive cinnamate moiety. The cinnamated collagen was fully characterized by $^{1}$H NMR, UV–vis, and circular dichroism (CD) spectroscopy, as well as by rheological and mechanical analyses. Cinnamated collagens with varying degrees of derivatization retained collagen triple helical structure. The rheological spectra of collagen solutions demonstrate that the storage modulus decreases with increasing cinnamate content, owing to a decrease in physical crosslinking. The kinetics of the crosslinking process in both hydrated gels and dry films were monitored by UV–vis spectroscopy and confirmed that crosslinking was complete within 60 min of irradiation. The uniaxial stress–strain behavior of crosslinked collagen films, including Young's modulus and ultimate tensile strength, was comparable to values reported for glutaraldehyde-crosslinked monomeric collagen films. These data demonstrate that derivatization of collagen with photosensitive cinnamate moieties provides a facile route for solid-state crosslinking, thereby improving the mechanical properties of collagen and enhancing the potential applicability of collagen-based materials in tissue engineering and drug delivery.


*Keywords:* Collagen; Crosslinking; Photocrosslinkable; Photodimerizable; Cinnamate; Blood vessel

## 1. Introduction

Collagen has been processed into a variety of physical forms, including mini-pellets, tablets and nanoparticles for use as drug and gene delivery vehicles, as an injectable suspension for soft tissue augmentation, as matrices for use as wound dressings, and as constructs for engineered blood vessel, heart valve, tendon, and skin substitutes [1–4]. As materials for tissue engineering applications, collagen matrices are intended to provide mechanical support and potentially act as a scaffold to facilitate cellular repopulation of the engineered tissue [3,4]. Characteristically, crosslinking of collagen is required in order to optimize construct stability and mechanical behavior [5–7].

Chemical crosslinking of collagen has most often involved the reaction of a bifunctional reagent with amine groups of lysine or hydroxylysine or by initial activation of residue bearing carboxyl groups followed by direct reaction with available amine groups [8]. Examples of the former approach include use of

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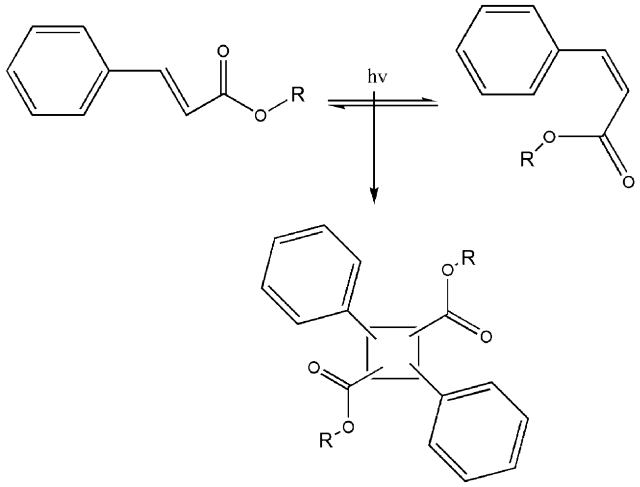
glutaraldehyde [9–11], diisocyanates [12,13], or diimidoesters [14], and polyepoxides [15–17] as crosslinking reagents. Glutaraldehyde can be used under aqueous conditions and rapidly forms a high degree of collagen crosslinks. Significantly, due to its potential to undergo condensation polymerization, glutaraldehyde can link residues that are spaced far apart and thereby enhance the extent of crosslink formation. However, glutaraldehyde crosslinking has been associated with late collagen calcification and despite extensive post-treatment washes residual glutaraldehyde, which is a known cytotoxin, often remains [18,19]. In contrast, diisocyanates are easily removed, but low water solubility and toxicity are acknowledged limitations. As an alternative approach, acyl azides [20–23] and the water-soluble carbodiimide, EDC/NHS [24–26], provide examples of amide bond formation via initial carboxyl group activation followed by reaction with either collagen bearing amines or bifunctional (amino) poly(ethylene oxide) (PEO) or poly(propylene oxide)-*b*-PEO-poly (propylene oxide) (PPO–PEO–PPO) as synthetic intermolecular linkers [27–31].

A significant limitation for all current chemical crosslinking schemes is the loss of both *spatial* and *temporal* control over the reaction process. Specifically, within more complex multicomponent collagen-based constructs, these reagents will inevitably react with all amine or carboxyl bearing components, including, for example, non-collagen structural proteins, glycosaminoglycans, growth factors and other bioactive compounds, or cells. Thus, the application of most standard reagents is limited in the case of multicomponent systems or structures. Moreover, the majority of these methods require that the collagen-based material come in contact with a solvent(s) and, therefore are ineffective under solid-state conditions. In an effort to devise a crosslinking strategy that is efficient in solid state, achieves precise control over the nature and the degree of crosslinking, and facilitates spatial and temporal control over the reaction process, we describe herein a versatile strategy, which exploits the known photosensitivity of cinnamate groups [32–36]. This reactivity is primarily based on the $\pi$ electron density of the photoactive chromophore (–CH═CH–), with dimerization of cinnamate groups presumably as a result of [2+2] $\pi$ electron cycloaddition [36] (Scheme 1). Significantly, this reaction does not require the addition of a light sensitive initiator, which is typically required for crosslinking reactions based on photosensitive acrylate or acrylamide moieties [5,37]. As a consequence, unanticipated side reactions due to the presence of free radical initiators are minimized. Although cinnamate chemistry has been widely applied in photolithography and has potential applications in liquid crystal display technology, its application in the crosslinking of biopolymers has been somewhat more limited. Significantly, Matsuda and colleagues [38–40] have reported the synthesis and characterization of a variety of photocrosslinkable hydrophilic polymers, including chondroitin sulfate, hyaluronan, and gelatin, using cinnamate and other photodimerizable groups. However, to our knowledge this is the first report, which describes the synthesis of monomeric collagen–cinnamate derivatives with preservation of triple helical structure. In addition, related mechanical and rheological properties of both photocrosslinked gels and films are characterized.




Scheme 1. . Photomediated crosslinking of cinnamate derivatized compounds.

## 2. Experimental section

### 2.1. Materials

Cinnamoyl chloride, *t*-butoxylcarbonyl-6-amino-hexanol, triethylamine, 4-dimethylaminopyridine (DMAP), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxy-succinimide (NHS), and all solvents were obtained from commercial sources and used as received, unless otherwise noted. Dialysis was conducted in a 4 °C cool room using Spectra/por membrane (MWCO 3500) obtained from VWR Scientific (West Chester, PA).

### 2.2. Instrumentation

All $^1$H NMR spectra were recorded at room temperature on an INOVA 600 or INOVA 400 spectrometer (Varian, Palo Alto, CA) operating at a $^1$H resonance frequency of 600 or 400 MHz. Thirty-two scans were acquired for signal-to-noise averaging and a recycle delay of 30 s was used to ensure quantitative spectra. In all cases, the deuterated solvent was used as