Improvement of Hydrogel Stability by *O,N* Acyl Rearrangement: A New Approach for Securing Ocular Wounds

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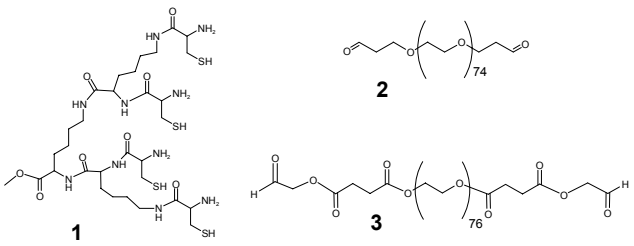
Statement of Purpose: In the field of tissue engineering and wound repair, there is a growing interest in developing materials that are suitable for simultaneous stabilization of a wound site and repair of the native tissue. There are a number of widely accepted properties that a biomaterial must have to succeed in tissue engineering applications. Among them, the scaffold must be biocompatible, has its mechanical properties matching that of the native tissue, and must be biodegradable at a rate that closely matches the rate of new tissue formation within the construct.[1,2]
Currently, sutures do not meet all of these requirements and do not provide the best solution for ocular wound repair. Recently, we have described new injectable hydrogels derived from a peptide dendrimer for repairing corneal lacerations.[3,4] Nevertheless, even though these materials form hydrogels and seal small wounds, their stability were too short to match the rate of new tissue formation for larger wounds. Thus, we are exploring alternative chemistry approaches to stabilize the network using a C-terminal glycol aldehyde ester allowing an *O,N*-acyl migration to afford a irreversible pseudoproline linkage and a more stable hydrogel for repairing large ocular wounds.

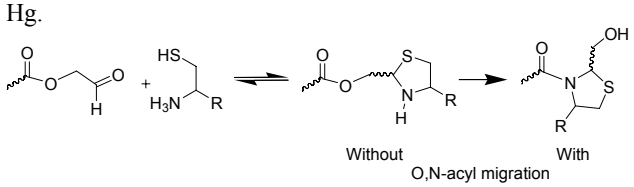
Methods: The cysteine dendron **1** and PEG-DA **2** were synthesized as previously reported.[3] Poly(ethylene glycol) di-ester-aldehyde (PEG-ester-Ald) **3** was prepared and fully characterized by NMR and MALDI-TOF.



Scheme 1: Structure of Dendron **1,** PEG-DA **2**, and PEG-ester-Ald **3.**

To prepare the hydrogels, the dendron **1** was reacted with either PEG-DA **2** (former system) or PEG-ester-Ald **3** in HEPES buffer at pH = 7.4. A hydrogel formed spontaneously within several minutes upon mixing the two aqueous solutions. Hydrogel time degradations were taken as 90% of the original reological properties after equilibrium. The rheological measurements were obtained on a TA Instruments AR 1000. For the first set of experiment the hydrogels were placed in a humidity chamber. For the second set of experiments, the samples were placed in a six well plate with distilled water. The hydrogel reological properties were then measured at appropriate time intervals until a 10% lost of reological properties was observed or for up to 40 weeks. The eye surgery was performed as we have previously reported.[3]

Results/Discussion: The new hydrogel system using PEG-ester-Ald **3** that undergoes a O,N-acyl rearrangement, gave better stability (40 weeks vs. 43 days) than the hydrogel prepared with PEG-DA **2**.[3,4] This case is due to a molecular rearrangement in the hydrogel crosslinking system (Figure 1). The irreversible formation of the amide linkage with the PEG-ester-Ald **3,** increased the stability of the hydrogel network when compared to the network formed through reversible thiazolidine linkages. These new hydrogels show better rheological properties than the former system (E = 950 vs. 220 kPa and G* = 67 vs. 12 kPa). This slower degrading hydrogel was then assessed for securing a 4.1 mm linear corneal laceration and compared with nylon suture and lower time stability system.[3] The linear corneal leaking pressure (LP) for the suture treated eyes was 80 ± 23 mm Hg. The LP for the eyes repaired with hydrogel sealant was 112 ± 18 mm Hg.



Degradation Time	Without *O,N*-acyl migration	With *O,N*-acyl migration
Unswelled	43 days	12 hours
Swelled	>40 weeks	24 weeks

Figure 1. Improvement of the degradation characteristics of hydrogel with vs. without *O,N*-acyl migration formulations (50% w/w).

Conclusions: The PEG-ester-Ald was obtained in high yield and high purity. The new hydrogels formed from this material show longer stability than previous systems as a consequence of this irreversible O,N-acyl rearrangement. The encouraging results obtained in this linear laceration study demonstrate that this hydrogel maybe of utility for closing large ocular wounds.

References:
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[4] Wathier M. *J. Cataract Refract. Surg.* 2005; 31: 1208-1212

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