In Situ Fibrillizing Collagen Solutions for Soft Tissue Augmentation

Dale DeVore[1], Melissa Collins[1], Dave Grant[2], and Sheila Grant[2]
[1]EternoGen, LLC, Columbia, MO; [2]Dept. of Bioengineering, University of Missouri, Columbia, MO

Statement of Purpose: Collagen, the principal protein constituent of fibrous connective tissues, is a semi-rigid rod-like macromolecule with an axial ratio of 200 to 1. The molecules can self assemble into axially ordered fibrils similar to those produced in vivo in connective tissue. Injectable collagen preparations are prepared from animal hides following enzyme digestion to dissociate intact fibers into molecular units. The dissociated molecules have the capability to spontaneously re-associate into fibrillar units (fibrillogenesis) similar to native fibrils. Most injectable collagen-based products are composed of reconstituted collagen fibrils that have limited durability following injection into tissues due to degradation by matrix metalloproteinases (MMPs). The authors have prepared clear, viscous molecular collagen compositions that undergo spontaneous fibrillogenesis when exposed to tissue fluids and exhibits enhanced resistance to degradation by MMPs.

Methods: In situ fibrillizing collagen was prepared from atelopeptide porcine collagen powder sourced from Sewon CelloTech, Inc (Seoul, Korea). Collagen was solubilized in 0.2M acetic acid and filtered through a 0.22µM filter. The filtered collagen was precipitated, the precipitate having a concentration of approximately 30mg/mL recovered by centrifugation, resolubilized in 0.5M acetic acid, and adjusted to neutral pH by stepwise dialysis in EDTA solutions with increasing pH. The final collagen solution was clear and transparent, neutral pH solution containing residual EDTA. The following characterization studies were performed:

Release and retention of EDTA: Release and retention of EDTA is an important parameter to measure since EDTA is the stabilizing element in the collagen solutions. Two hundred milliliters (200µL) of RPC Pure-Collagen was slowly injected into 2mL of D.I.U.F. 37°F water in glass vials using a 27G needle. The samples for each release time set were immediately transferred to the 37ºC temperature controlled chamber. The sample sets were removed from the controlled temperature chamber at 2, 5, 15, and 30 minutes and 4 hrs, 18 hrs, 4, 7, and 14 days. All of the D.I.U.F solution was removed from the vials using sterile, disposable pipettes and placed in a labeled storage vials. Fibrillized collagen fibril pellets and D.I.U.F supernatants were stored at 4°C until ready for analysis. All samples were analyzed for EDTA using standard HPLC methods.

Collagenase Resistance Test: To demonstrate the collagen resistance to degradation, a collagen degradation test was performed. Test collagen fibrils (in situ fibrillizing collagen) or control collagen fibrils (Sewon collagen) were incubated with 50U/ml of collagenase for 3 hours. Incubation time was 3 hours. After incubation, samples of each preparation were centrifuged and the supernatants analyzed for hydroxyproline content to calculate the amount of degraded collagen.

Evaluation of In situ Fibrillizing Collagen Solutions Injected Subcutaneously in Rabbit Ear Tissue: To determine how fast the collagen fibrilizes in situ, a terminal animal study was performed. Four rabbits were used in this study to evaluate the fibrillogenesis (or polymerizing) times and appearance of subcutaneous implantation. Each animal was injected with 0.1ml of the collagen solution subcutaneously in each ear (3sites/ear). Implants were removed at 2.5, 5, 10, 15, 20, 30, 45, and 60 minutes after implantation, placed in fixative and examined by transmission electron microscopy.

Results: EDTA Release and Retention: The appearance of fibrillized collagen was observed within 2 minutes of injection into D.I.U.F water. Results from HPLC analysis demonstrated rapid release of EDTA from the collagen solution injected into D.I.U.F water within this initial 2 minute test sample. After 15 minutes approximately 10mM of EDTA remained bound to the fibrillized collagen.

Collagenase Resistance: The samples of in situ fibrillized samples demonstrated a very low digestion rate. In the 1 hour incubation time, less than 1% collagen content of the in situ fibrillized collagen was digested by collagenase, while greater than 32% collagen content of control collagen fibrils were digested.

TEM Structure of In Situ Fibrillized Collagen Fibrils: TEM images from each sample explanted from 2.5 to 60 minutes following subcutaneous implantation in rabbit ear tissue showed well banded collagen fibrils demonstrating the rapid fibril formation of the viscous, soluble collagen composition.

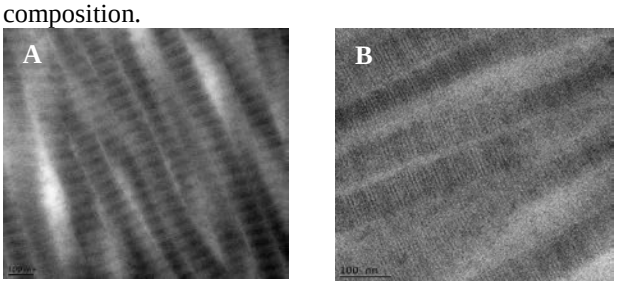

Figure 1. TEM images of in situ fibrillization of collagen. A) fibrillization 2.5 minutes after injection; B) fibrillization 60 minutes after injection.

Conclusions: The authors developed collagen molecular compositions that are injected as clear, viscous solutions. These solutions undergo spontaneous fibrillogenesis when exposed to tissue fluids. Complete fibrillogenesis occurs in approximately 2 minutes resulting in a pliable and porous collagen matrix. These compositions have been successfully evaluated in preliminary clinical studies and offer a collagen product for soft tissue augmentation with enhanced ease of injection, increased clinical durability, and evidence of tissue bio-restoration [1].

References: [1] DeVore D., Zhu J., Brooks R., Rone-McCrate R., Grant DA., Grant SA. Journal of Biomedical Materials Research Part A, 2016 104A:758-767.