Effect of Processing Temperature on the Morphology and Drug-Release Characteristics of Elastin-Like Polypeptide - Collagen Composite Scaffolds

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Statement of Purpose: Recently, we prepared elastin-like polypeptides (ELP) - collagen composites with significantly better mechanical properties and equivalent biocompatibility compared to neat collagen scaffolds.[1] Here, we report on the effect of processing temperature on the release characteristics of the ELP-collagen hydrogels using a model protein (bovine serum albumin, BSA) and a commonly used antibiotic (doxycycline hyclate). ELP exhibits an inverse phase transition behavior in response to changes in its environment. We hypothesized that processing the composites at temperatures higher than the inverse phase transition temperature of ELP will affect their microstructure and subsequently achieve tunable release of bioactive agents from the composites.

Methods: *Hydrogel Preparation*. ELP-collagen gels were formed by mixing ELP and collagen (ELP:collagen = 2.5:1; 8 mg collagen) in PBS and incubating at 37°C.[1] The hydrogels were further incubated either at 37, 45, or 55°C for 2 h followed by air-drying at 37°C.

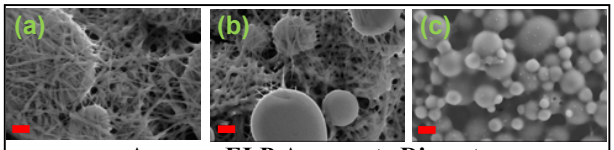
Morphological Characterization. Freeze-dried scaffolds (n = 3) were imaged using a SUPRA 40 scanning electron microscope (SEM, Carl Zeiss). ELP aggregate dimensions were measured with ImageJ digital analysis software.

Bioactive Agent Loading and Release. ELP-collagen solutions were mixed with 3.0% w/w BSA or doxycycline and processed as above. The amounts of BSA and doxycycline released into 100 µl PBS at 37°C were analyzed by measuring absorbance at 280 and 345 nm using a ND-1000 Nanodrop spectrophotometer.

Statistical Analysis. ANOVA and Games-Howell post hoc test for unequal variances were performed. Results reported as mean ± 95% confidence intervals. $p \leq 0.05$ against hydrogels processed at 37°C denoted by *.

Results: Processing conditions affected both the ELP and collagen phases. When processed at 37°C, the ELP-collagen composite scaffold showed fibrils of collagen interspersed with ELP aggregates (Fig. 1a). The processing conditions affected the ELP aggregate size (Fig. 1a-c). Collagen phase appeared to lose its fibrillar structure partially or completely when processed at 45 or 55°C (Fig. 1b,c), respectively and became an afibrillar coating on ELP aggregates. These microstructures impacted the release characteristics resulting in a bi-phasic BSA release profile with the initial fast release and the later linear release (Fig. 2a) and a linear doxycycline release profile (Fig. 2b). Interestingly, the linear release rate appeared to depend on the ELP aggregate size and collagen structure (both governed by processing) and showed opposing trends for BSA and doxycycline.

In case of BSA, higher levels of chain entanglements are likely between BSA and collagen-coated ELP aggregates. This may explain the progressively slower BSA release observed from the hydrogels processed at 45 and 55°C, where the afibrillar collagen content appeared progressively higher compared to those processed at 37°C. In contrast, a progressively faster doxycycline release may be expected from the hydrogels processed at 45 and 55°C, where progressively smaller ELP aggregates with an overall increased surface area were available for diffusion compared to those processed at 37°C.



Average ELP Aggregate Diameter

4.41 ± 0.27 µm	3.25 ± 0.19 µm*	0.96 ± 0.05 µm*

Fig 1. Effect of processing temperature on scaffold morphology and ELP aggregation. (a,b,c) SEM images of scaffolds processed at 37, 45, and 55°C, respectively. Scale bar = 1 µm.

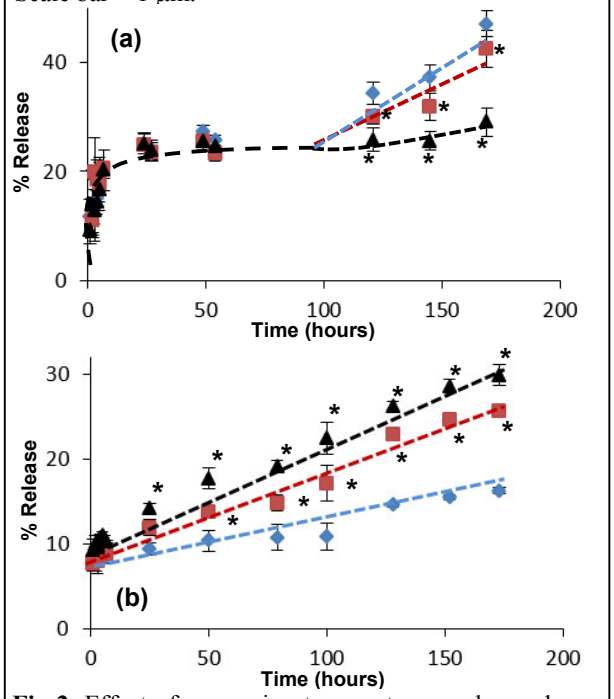


Fig 2. Effect of processing temperature on drug release. Release profiles at 37°C for ELP-collagen (2.5:1) gels loaded with 3.0 % w/w (a) BSA and (b) doxycycline. Gels were processed at (◆) 37, (■) 45, and (▲) 55°C.

Conclusions: These results demonstrated that a variety of controlled microstructures could be produced via the incorporation of ELP into collagen matrix while varying scaffold formation process. These findings suggest that our composites offer a versatile *in vitro* release, which can be tailored using ELP incorporation, temperature, and molecular size of the bioactive agents.

Reference: [1] Amruthwar S, et al. JBMR-A (In press).