Effective Release of a Broad Spectrum Antibiotic From Elastin-like Polypeptide-Collagen Composite

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Statement of Purpose: Collagen hydrogels have been extensively used for encapsulation of cells and bioactive molecules because of their biocompatibility. However, collagen hydrogels show poor mechanical properties. Elastin-like polypeptide (ELP)-collagen composites demonstrated better mechanical properties and equivalent biocompatibility compared to collagen scaffolds [1]. This research investigated the release of a commonly used broad spectrum antibiotic (doxycycline hyclate) from the collagen and ELP-collagen composite hydrogels. We then tested the efficacy of the released doxycycline against four bacterial strains commonly encountered in clinical settings. These strains included: *E. coli* (Gram-negative, facultative), *P. aeruginosa* (Gram-negative, aerobic), *S. sanguis* (Gram-positive, facultative), and *methicillin-resistant S. aureus* (Gram-positive, facultative).

Methods: *Preparation of hydrogels:* ELP with a sequence of $[VPGVG]_{120}$, where G = glycine, P = proline, and V = valine, was produced as described [1]. To prepare ELP-collagen hydrogels, 25 mg ELP, DI water (160 µL), 10 X PBS (200 µL), 1 N NaOH (40 µL), and type I collagen (rat tail, 4 mg in 1.6 mL) were gently mixed and incubated at 37°C in a humidified environment for 24 h.

Antibiotic release: Doxycycline (0, 1, 2, or 5% w/w) was added to above solution and the gelation procedure was followed. The amount of doxycycline released at 37°C in PBS was determined by measuring the absorbance of the supernatants at 345 nm by xMark spectrophotometer.

Bioactivity of the released doxycycline: Bioassays were performed using the disk diffusion method [2]. *E. coli* (BLR-DE3), *P. aeruginosa*, *S. sanguis*, and *S. aureus* were swabbed onto petri dishes containing Luria Bertani agar, tryptic soy agar, sheep's blood agar, and tryptic soy agar, respectively. The hydrogels with or without doxycycline were placed on the surfaces of the agar plates and incubated at 37°C for 18 h. The inhibition of bacterial growth was observed by comparing the zones of inhibition created around the hydrogels.

Statistical analysis: All experiments (n = 6) were reported as mean ± 95% confidence interval. Statistical evaluation was done using ANOVA with Bonferroni and Games-Howell post hoc tests for equal and unequal variances. Values with $p \leq 0.05$ were deemed significantly different.

Results: Fig. 1 shows that both the collagen and the ELP-collagen hydrogels rapidly released doxycycline in the initial 48 h followed by a gradual release phase over the next 72 h. The ELP-collagen hydrogel seemed to release the doxycycline more gradually compared to the collagen hydrogel. For example, after 72 h, the ELP-collagen hydrogel loaded with 5% w/w doxycycline had released 4.5 ± 0.1 µg doxycycline per mg of scaffold, while corresponding collagen hydrogel had released 5.0 ± 0.1 µg doxycycline per mg of scaffold ($p < 0.05$). The bioactivity assays (Fig. 2) revealed that the doxycycline released from all hydrogels was effective against all the four strains of bacteria tested. The zones of inhibition created around the hydrogels were dependent on the doxycycline loading ($p < 0.05$; data not shown).

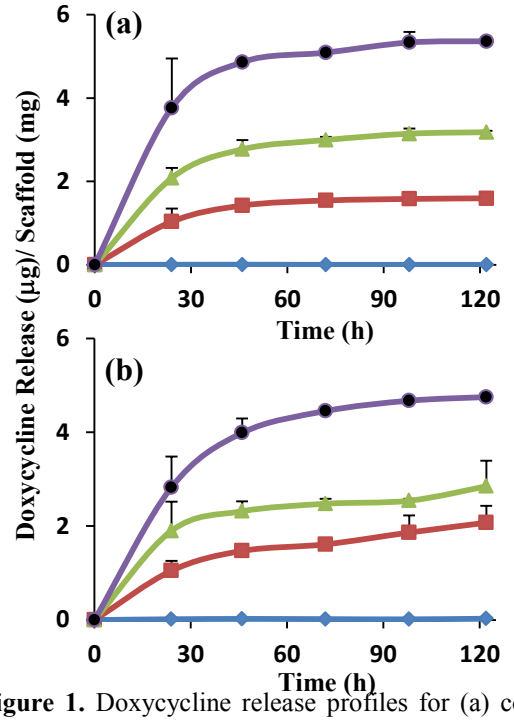

Figure 1. Doxycycline release profiles for (a) collagen and (b) ELP-collagen hydrogels. Doxycycline dosage: ◆: 1%w/w; ■: 1%w/w; ▲: 1%w/w; ●: 1%w/w

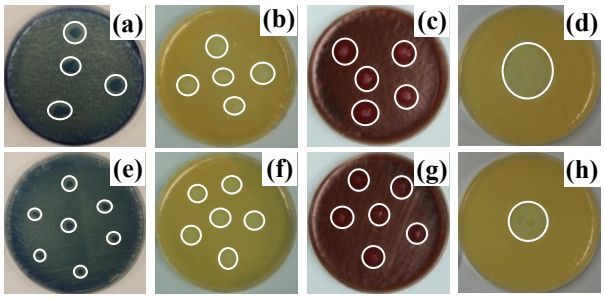

Figure 2. Bioactivity assays for (a-d) collagen and (e-h) ELP-collagen gels containing 5% w/w doxycycline against (a,e) *E. coli*, (b,f) *P. aeruginosa*, (c,g) *S. sanguis*, and (d,h) *S. aureus*. Circles indicate zones of inhibition.

Conclusions: Combined with their improved mechanical properties, the gradual and effective drug release from the biocompatible ELP-collagen hydrogels shown here may be beneficial for drug delivery and tissue engineering.

References: 1. Amruthwar, et al. *Dent Mater.* 2013:29,211. 2. Barry et al. *J Clinical Microbiol.* 1979:10,885.