Fibrin Based Hydrogels to Promote Healing and Limit Infection in Wound Therapy

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Introduction: Silver sulfadiazine (SSD) is a widely used broad-spectrum antimicrobial agent to treat burn wound infections. We have designed a fibrin-based wound dressing using chitosan for the controlled release of silver sulfadiazine (SSD). This dressing is intended to control infection and may further aid in neovascularization when applied to topical wounds. In this study, we entrapped SSD into chitosan microspheres (CSM) to produce a controlled release of SSD. The SSD-loaded CSM (SSD-CSM) was then impregnated into a PEGylated fibrin gel (SSD-CSM-PEGylated fibrin gel) and used as a wound dressing. We provide evidence that SSD-CSM-PEGylated fibrin gel supports both antimicrobial activity and tube formation by adipose-derived stem cells (ASC) *in vitro* and blood vessel in-growth *in vivo*.

Materials and Methods: CSM were prepared by a novel water-in-oil emulsification process with simultaneous ionic coacervation as previously described (1). (Figure 1A) PEGylated fibrinogen was also prepared as previously described (2). *In vitro* release kinetics of SSD from SSD-CSM and SSD-CSM-PEGylated fibrin gels were determined by using a Franz diffusion apparatus. SSD-CSM-(ASC)-PEGylated fibrin gels were prepared using passage two human ASC (50,000 cells/ml of gel). Blood vessel in-growth was observed using these gels applied over a deep partial thickness burn wound in Lewis rats.

Results: SSD-CSM prepared with an initial drug concentration of 10 mg w/w with respect to chitosan resulted in the formation of microspheres 125-180µm in size and 76.50 ± 2.8% SSD entrapment. We observed that SSD-CSM-PEGylated fibrin gels, showed a slight initial burst release of 73.23 µg/ml, after which the concentration decreased to 67.5 µg/ml by 12 h and maintained an equilibrium state for 72 h. (Figure 2) Assessment of antibacterial activity showed that SSD-CSM-PEGylated fibrin gel was able to exhibit microbicidal activity at 125 and 100 µg/ml against *S. aureus* and *P. aeruginosa,* respectively. When ASC were added to the SSD-CSM-PEGylated fibrin gels they were viable and exhibited formation of characteristic tube-like structures *in vitro* (2). (Figure 1B) The SSD-CSM-PEGylated fibrin gels applied over a deep partial thickness burn wound in rats were found to be well vascularized by day 7.

Discussion and Conclusions: The SSD-CSM delivers SSD in a controlled manner for a period of 3 days. SSD-CSM-PEGylated fibrin wound dressing provides antimicrobial activity and a matrix that induces angiogenesis to hasten the healing process.

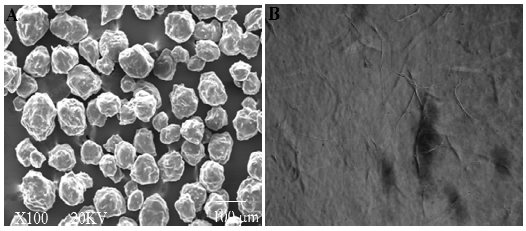

Figure 1: (A) SEM image of SSD-CSM shows irregular surface characteristic of SSD and (B) LM shows the tube-like network structures.

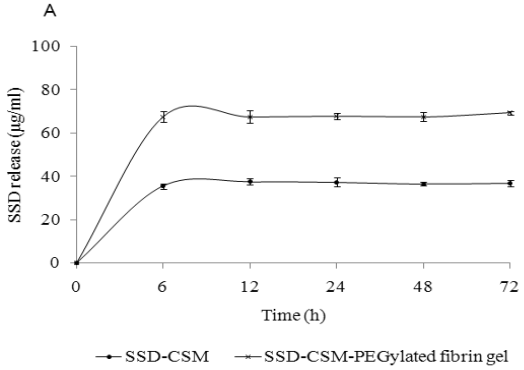

Figure 2: *In vitro* SSD release profiles in SSES, pH 7.4, at 37°C shows controlled release for 72 h.

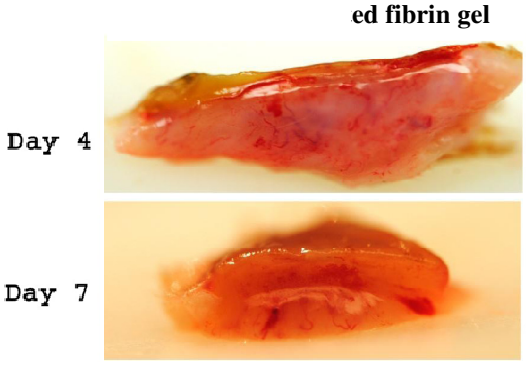

Figure 3: *In vivo* explanted PEGylated fibrin gels loaded with SSD-CSM beads exhibit robust blood vessel in-growth at 4 and 7 days after burn injury.

References

1. Shanmuganathan S, Shanmugasundaram N, *et al.* Carbohydrate Polymers.73, 201, 2008.

2. Zhang G, Wang X, *et al.* Tissue Eng. 12, 9, 2006.