Comparative *In Vivo* Evaluation of a Novel Silk Hydrogel Injectable for Drug Delivery

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Statement of Purpose

Inherent shortcomings in widely available mammalian-derived biomaterials continue to fuel a need for non-mammalian derived, functional and controllable biomaterials. The present study characterizes *in vivo* a new alternative injectable biomaterial, a silk fibroin hydrogel (SFH) containing an arginine-glycine-aspartic acid (RGD) peptide. The RGD sequence offers enhanced material biocompatibility, serving as a point for cellular attachment. SFH presents unique opportunities for the coupling of therapeutic agents, in terms of agent compatibility, binding mode, and tailored release profile, as silk fibroin is a hydrophobic protein, atypical of hydrogel constituents and has already been shown capable of releasing drugs *in vitro* (Hofmann, S., J Control Release, 2006. 111(1-2): p. 219-27). This work is the first evidence of a truly injectable and biocompatible SFH as evaluated in the guinea pig intradermis, this locale being particularly relevant as a potential route of therapeutic delivery to the immune system.

Methods

A Hartley guinea pig intradermal model was used for evaluation of the SFH, which was compared to the current "gold-standard" injectable biomaterial, bovine collagen-derived Zyplast (Inamed Aesthetics, Santa Barbara CA). Briefly, SFH was injected through a 30g needle as a 50µl bleb shape into the dorsal intradermis to the left of the midline at a frequency of 6 sites per animal with Zyplast injected contralaterally in the same fashion in 3 animals which were incubated for 4 weeks (N = 18 sites per group). An additional 2 animals were injected in the same fashion with 5 SFH sites and 2 Zyplast sites each for a 13 week harvest point. During the course of incubation, sample injection sites were monitored for hair loss, discoloration, and palpability by a "blind" observer and pictures of the sites were collected.

Animals were sacrificed and the implanted material harvested in a full-thickness skin sample. Histological evaluation of both H&E and Trichrome stained cross-sections of the tissue was performed by a "blind" pathologist and graded in a series of relevant categories.

Results/Discussion

One of 18 SFH implants in the 4 week sample group induced ulceration; the Zyplast samples were free from ulcers. By 13 weeks, none of the 10 SFH or 4 Zyplast injections had induced ulceration. Both implant materials displayed similar palpability over the course of 4 and 13 weeks.

At week 4 both implant materials exhibited minimal gross pathological reactions, with 1 Zyplast sample exhibiting implant mineralization and 1 SFH sample exhibiting epithelioid cyst formation. The host tissue responses to each material at this time point were characterized as being fibrotic, but consistent with typical wound healing (**Figure 1**).

At 13 weeks, SFH and Zyplast showed similar rates of persistence with 75% of sites exhibiting presence of residual implant material. All sites exhibited minimal and decreased residual cellularity with respect to histological samples at the 4 week time point (**Figure 1**). Further evidence of biocompatibility and host tissue ingrowth was provided by the deposition of new, less-organized collagenous material in the reticular dermis.

Conclusions

In this study SFH exhibited comparable biocompatibility to a widely used mammalian-derived biomaterial, Zyplast as evidenced by cellular infiltration and implant remodeling. This high degree of biocompatibility is believed to stem from a combination of minimally immunogenic and relatively inert silk fibroin with the unique properties of the RGD sequence. This data sets precedent for further evaluation of SFH as an agent for drug delivery, cell delivery, tissue repair, and reconstructive surgery. There exists great potential for tailoring of gel properties such as implant persistence, viscosity and cellular ingrowth profiles to meet the needs of specific repair targets *in vivo* including cartilage, bone, and dermal tissue.

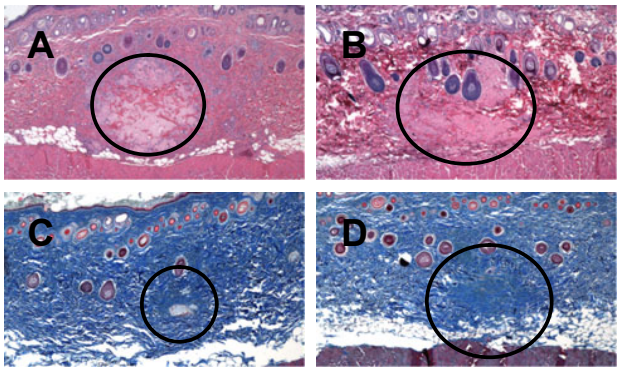

Figure 1. H&E images of SFH (A) and Zyplast (B) day 28 with Trichrome images of SFH (C) and Zyplast (D) at day 92. Implant material area of interest is circled, all images taken at 4X.