Silk-Based Biomaterial-Induced Fibrosis to Improve Integration of Transdermal Osseointegrated Prostheses

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INTRODUCTION: As of 2022, nearly two million individuals in the United States were living with limb loss, with over 185,000 new amputations annually. Above-the-knee amputations, often resulting from trauma or disease, are commonly reconstructed using socket-based prostheses. Despite improvements in socket customization, issues such as skin breakdown, swelling, uneven pressure distribution, and infection remain widespread, frequently impairing mobility and quality of life. Osseointegrated prostheses, implants directly anchored to bone, bypass the need for sockets and improve function, yet remain limited by high infection rates at the percutaneous interface where the implant traverses the skin. The soft tissue fails to securely integrate with the rigid implant, leaving a sinus tract that permits bacterial entry. Infection rates of up to 50% have been reported, often resulting in implant failure. Prior strategies to reduce infection, through implant surface coatings, geometry modifications, or anti-biofouling materials, have shown limited long-term efficacy. One proposed mechanism underlying failure is the mechanical mismatch between soft tissue and the stiff implant, which limits stable fibroblast adhesion and tissue integration. Silk-based biomaterials offer a promising solution due to their tunable mechanical properties, biodegradability, and biocompatibility. Previous studies have shown that layered silk scaffolds can support soft tissue regeneration in elastic organs. In this study, we examine the use of silk and plasticized-silk scaffolds designed to modulate tissue stiffness and promote subdermal tissue integration in a preclinical rabbit model (**Figure 1**). These biomaterials may help reduce sinus formation and infection risk at the skin-implant junction, improving the long-term viability of osseointegrated prostheses.

METHODS: Silk fibroin was extracted from Bombyx mori cocoons and processed into an aqueous solution following degumming, lithium bromide dissolution, and dialysis protocols. Bi-layered scaffolds were fabricated by casting silk or silk-glycerol films and layering with porous silk sponges formed via salt-leaching. Mechanical characterization of various formulations (2–6 wt% silk, 10–30 wt% glycerol) was performed under physiological conditions using uniaxial tensile testing to determine tensile strength, elastic modulus, and elongation to failure. Morphological features were assessed using scanning electron microscopy following fixation, freeze-drying, and gold coating. For in vitro testing, normal human dermal fibroblasts (NHDFs) were cultured on silk-based scaffolds or tissue culture plastic (**Figure 2**). To assess fibrotic activation, cells were treated with TGF-β1 and stained for α-smooth muscle actin (α-SMA) and F-actin. Confocal imaging quantified colocalization of fibrotic markers using Pearson correlation coefficients. Cytomorphology was further analyzed using Feret diameter and circularity metrics. A scratch assay was conducted to evaluate scaffold-supported wound healing. Fibroblasts were seeded on monolayered constructs, and scratch closure was tracked via serial imaging over 30 hours. Area quantification was performed in FIJI. In vivo analysis was performed using a subcutaneous implantation model in Sprague Dawley rats. Circular silk-based constructs, with or without central titanium rods, were implanted into dorsally created skin pockets. After 4 weeks, tissues surrounding the implants were harvested, fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Histological outcomes included scaffold width, residual scaffold area, and tissue morphology. This study was approved by IACUC. Statistical analysis employed Shapiro-Wilk tests for normality, followed by Mann-Whitney or Student's t-tests where appropriate. P-values < 0.05 were considered significant.

RESULTS: Mechanical testing demonstrated that both silk concentration and glycerol addition significantly influenced scaffold performance. Among six tested formulations, 4% silk-glycerol films exhibited the highest ultimate tensile strength (39.90 ± 8.65 MPa) and apparent modulus (141.78 ± 17.28 MPa), significantly outperforming plain silk controls at similar concentrations ($p < 0.05$). Stress-strain curves confirmed a more pronounced toe region in silk-glycerol films, suggesting improved viscoelastic behavior. Scanning electron microscopy confirmed that both silk and silk-glycerol constructs retained a distinct bi-layered architecture, with a ~20 µm-thick film overlying an open-pore sponge. Glycerol incorporation yielded smoother microstructures compared to the rougher silk-only interfaces, particularly in the sponge region. To assess fibrotic activation, immunofluorescent staining for F-actin and α-smooth muscle actin (α-SMA) was quantified in NHDF cultures. Cells seeded on silk-glycerol constructs showed significantly higher colocalization (Pearson coefficient = 0.363 ± 0.110) than those on plain silk (0.238 ± 0.097; $p < 0.0001$), indicating a pro-fibrotic shift in cellular phenotype (**Figure 3**). Cytomorphological analysis revealed no difference in Feret diameter between groups, but increased circularity was observed in silk-glycerol samples ($p = 0.02$). In scratch assays, both silk and silk-glycerol interfaces supported progressive wound closure over 30 hours. Although no significant differences in healing kinetics were observed between the two formulations, silk-glycerol scaffolds trended toward enhanced surface coverage by 24–30 hours. *In vivo*, subcutaneous implantation of scaffolds in a rat model revealed successful tissue integration and absence of inflammation across all groups after 4 weeks. Silk-glycerol constructs, especially when combined with titanium rods, showed significantly greater scaffold persistence (11.65% of tissue area) compared to silk + rod implants (9.38%; $p = 0.003$). Skin thickness was also significantly greater in silk-glycerol vs. silk-only implants ($p = 0.015$), suggesting enhanced scaffold-tissue remodeling.

DISCUSSION: Our findings demonstrate that a bi-layered silk-based scaffold, particularly when plasticized with glycerol, can modulate fibroblast behavior and promote localized fibrosis, addressing the mechanical mismatch at the skin-implant interface. In vitro, silk-glycerol constructs enhanced markers of fibroblast activation, while in vivo implantation showed successful integration with minimal inflammation and improved scaffold retention around titanium rods. These results suggest that silk-glycerol scaffolds may serve as a mechanically supportive, biologically compatible interface to stabilize transdermal implants and reduce infection-prone sinus formation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a novel silk-glycerol scaffold that promotes controlled fibrosis and soft tissue integration at the percutaneous interface, offering a promising strategy to reduce infection and improve the long-term success of osseointegrated prosthetic limbs. By modulating local mechanical properties and host response, this approach may overcome one of the most persistent barriers to widespread clinical adoption of transdermal implants.

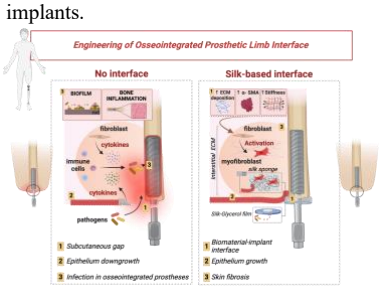

Figure 1. Engineering of osseointegrated prosthesis limb interface. Traditional osseointegrated implants suffer from infection and aseptic loosening issues resulting from insufficient subdermal attachment (left). Hypothesized benefits of subdermal implantation of engineered silk-based implant, including increased fibrotic induction and subsequent improved tissue stiffness at the transdermal interface, resulting in an improved seal between transdermal prosthetic and adjacent cicatrix tissue

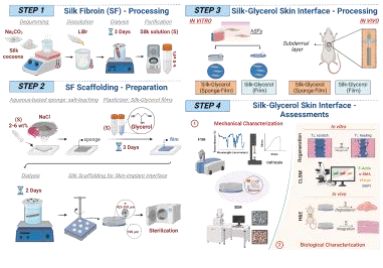

Figure 2. Manufacture and evaluation of silk-based interfaces. Silk fibroin sponges were produced by porogen leaching of sieved NaCl to form uniform pores, with or without glycerol, autoclave-sterilized, and tested with human dermal fibroblasts in vitro and as plasticized implants in rodents. Mechanical/structural characterization used FTIR, compressive modulus, ultimate tensile strength, and SEM. In vitro assays included ICC for αSMA and F-actin, quantitative cytomorphology, and scratch migration; in vivo assessment tracked scaffold degradation over time by IHC.

Figure 3. Quantification of NHDF fibrotic response to silk-based interfaces. A. Representative images of F-actin (green) and αSMA (red) in cells seeded on TCP, TCP with TGFβ, silk only constructs, and silk-glycerol constructs. B. Colocalization of αSMA and F-actin across constructs. Scale bar = 200 µm. C. Quantitative cytomorphology of normal human dermal fibroblasts seeded on silk and silk with glycerol constructs.