Investigating Keratin as a Biomimetic Coating for Percutaneous Device Applications

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Introduction: Lack of integration of a percutaneous device with the host soft tissue often results in epithelial marsupialization and subsequent superficial infections. The observed marsupialization is often attributed to the body’s attempt to externalize the implant through migration (i.e., continuous wound healing). The current theory predicts that the epithelium can only stop its migration through an integrin mediated “contact inhibition” pathway [1,2]. Based on the current theory, it was realized that the epithelial down-growth could only be mitigated if the implant surfaces were to comprise of integrin binding sites. Thus, biomimetic coatings were considered. One of the candidates for such a coating is keratin. It is known that keratins have LDV amino acid sequences, which provide binding sites for cellular integrin [3]. In order to develop a keratin coating for percutaneous applications, it was necessary to understand its stabilization under in vitro conditions. It was therefore hypothesized that keratin, which was covalently attached to the implant, would possess a high hydrolytic stability and would also increase hydrophilicity of the implant surface to improve cell attachment and subsequent in vivo stability.

Materials and Methods:
Implant Manufacturing: A set of percutaneous implants (Ti6Al4V) was manufactured and porous coated (P 2 Ti coating) at Thortex Inc. (Portland, OR). The porous coated region was then further coated with a reduced form of keratin at the Wake Forest School of Medicine (Winston Salem, NC).

Contact Angle: A goniometer (KSV Instruments Cam 100) was used to measure the contact angle of a 5uL drop of ultrapure water placed on the surface of the titanium disk at five different locations (top, bottom, left, right, and center).

Stability Study: A 3-month hydrolytic stability study was performed on silane coated (n=2) and keratin coated disks (n=5). In a sterile environment, each disk was placed in 5 ml of sterile PBS and allowed to incubate at 37°C. At each time point, the disks were removed from the solution, rinsed in ultrapure water, air dried, and the surface chemistry determined through water contact angle measurements and XPS analysis.

Cell Adhesion: Dermal fibroblasts were seeded onto titanium disks and allowed to attach for 3 hours, after which non-adherent cells were washed off. Adherent cells were quantified using an MTS assay.

Animal Study: Using an approved IACUC protocol, coated and uncoated percutaneous implants were surgically placed in 3-6 month old Yucatan miniature pigs by an established method. Animals were euthanized at the end of 3 months. The implants and surrounding tissues were harvested and subjected to histological evaluation.

Results: In vitro data illustrated that keratin coatings on the titanium implants reduced the contact angle by approximately 40°. The stability study showed that the reduction in contact angle was maintained for over 90 days (Fig. 1). Keratin coated surfaces also showed a higher percentage of fibroblast attachment compared to uncoated substrates and similar to fibronectin (Fig. 2).

The in vivo study indicated that keratin coating did not produce any foreign body response, and hence, there was no fibrous capsule (FC) formed on the subdermal-barrier (Fig. 3(b)). However, the epithelial marsupialization was not completely prevented at the interface (Fig. 3(b)).

Discussion: In vitro analysis of covalently attached keratin, as a possible biomimetic coating, illustrated its longevity, hydrophilicity and cellular adhesive properties. The contact angle, as a measurement of coating integrity, suggested that this particular coating would, at least, be stable for at least 90 days, which was further validated in the in vivo study. The absence of FC indicated the biocompatibility of the coating. It also suggested the possible role of the integrin binding coating type in preventing the FC formation. However, this coating failed to prevent marsupialization completely. The bacterial mediated enzymatic degradation of keratin coating might have been responsible for the observed continuous epithelial marsupialization.

Conclusion: The covalent linkage of keratin using silane chemistry has been proven to be an effective method for attachment of keratin to the implant surface. Data indicated that such coating promotes cell-matrix attachment. However, further research is needed to improve degradation of the coating that was exposed to the external environment.

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References