# Towards Understanding the Skin-Percutaneous Implant Integration with the host soft tissue

Daniel H. Betz; Taylor A. Ford; Brian M. Holt; Mark Van Dyke; James P Beck; Roy D Bloebaum; Sujee Jeyapalina

[1]Department of Orthopaedics, University of Utah, Salt Lake City, UT; [2]Wake Forest School of Biomedical Engineering and Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA

**Introduction:** In spite of von Recum's publication of the theoretical causalities of soft-tissue-implant interface failure in 1984 (1), much of the underlying mechanisms of those failure modes remain largely unexplored. The lack of specialized binding sites on the surface of an implant is believed to prevent epithelial cellular adhesion to the implant surface. Adherens mediated cell spreading and cyto-physiological feedback from these bindings sites often dictates cell behavior (e.g. proliferation, differentiation, and protein expression). We hypothesized that implant surfaces coated with known, skin-specific pro-adhesion complexes (focal adhesion points) would facilitate improved cellar attachment when compared to untreated/uncoated implants. Furthermore, once established, these cell surface interactions would limit epithelial migration, facilitate the epithelial differentiation, and prevent fibrous tissue formation. To test the hypothesis, two biomimetic coating types were investigated in an *in vivo* pig model. The two types of coating were: 1) keratin (intended to promote cell-to-matrix junctions) and 2) hydroxyl appetite (intended to promote cell-to-cell (surface) type junctions).

**Materials and Methods:**

*Implant Manufacturing:* Implants were manufactured and porous coated at Thortex Inc., Portland, OR. One third of the implants were further coated with keratin with a proprietary technique at Wake Forest School of Medicine (Salam, NC) and another one third of the implants were coated with a medical grade HA at APS Material Inc. (Dayton, OH).

*In vivo Study:* Using an approved U of U IACUC protocol, coated and uncoated percutaneous implants were surgically placed in 3-6 month old Yucatan miniature pigs by an established method. Implant exit sites were dressed and protected for 2 weeks and animals were allowed to ambulate for 3 months. A strict weekly cleaning regime of mild soap and water was also implemented. Animals were euthanized at the end of 3-months, and the implants and surrounding tissues were harvested.

*Histology and Immunohistochemistry (IHC) and Enzyme-Linked Immunosorbent Assay (ELISA):* A strip of tissue was taken at the interface and frozen immediately for immunohistochemical studies. From this tissue, 5-10 µm thin samples were generated and treated with antibodies against K6 and Collagen lV and analyzed using a confocal microscope. Periprosthetic granulation tissues from the 50µm thick sections were identified and meticulously excised from the rest of the tissue and then digested in pepsin (1:100 ratio). Digested samples were subjected to ELISA analysis and collagen I/III ratios were computed. The rest of the tissue was processed for hard plastic embedment and stained with H&E (2).

**Results:** The data from this study indicated that both coatings had prevented the formation of fibrous capsule (FC). However, skin marsupialization was still evident in all samples (HA = ~786**,** Keratin = ~1025 and control = ~1182 µm/month**),** but at a reduced rate in the HA coated implants (Fig.1). Collagen content analyses showed that the collagenous matrix of periprosthetic tissue had more collagen I (matured dermis) around the HA (collagen l/111 =5.1:1) and keratin coated (collagen l/111 =1.4:1) implants than that of the uncoated control (collagen l/111 =0.6:1). As shown in Fig. 2, IHC data indicated the presence of migrating epithelium at the interface in all samples.

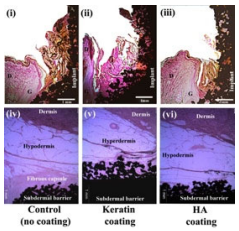


Figure 1: Photomicrographs showing the skin-implant interfaces of coated ((ii) and (iii)) and uncoated ((i); control) implants, and presence/absence of FC on the subdermal barrier (iv) – (vi).

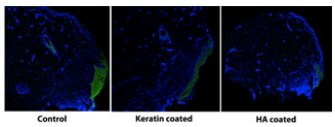


Figure 2: Expression of K6 in terminal epithelium at the 3-phase junction: green-K6 and blue nuclear stain (DAPI).

**Discussion:** The collagen content analyses (i.e. ELISA for collagen I and III) of periprosthetic granulation tissue indicated that the collagen type I: III ratio (~5:1) was very similar to that of a healthy skin (~4-5.5:1) (3) in HA coated implants. This is promising data, but continuing marsupialization remained a concern. The reasons for the continued but reduced marsupialization rate could be attributed to micro-mechanical forces at the interface or coating instabilities. For example, the HA coating used in this study was plasma coated. It is known that under physiological conditions, HA would dissolve or release particles of HA. Although the stability of the coatings may not have been ideal, the data clearly suggests that incorporation of coatings that promote adherens-type, cell-cell or cell-matrix adhesion may prevent the formation of FC.

**Conclusion:** The surfaces that promote both types (cell-to-cell and cell-matrix) of interactions did indeed prevent fibrous capsule formation, These findings suggest a skin layer with limited migratory capacity and the beginnings of a stable skin-implant interface. In summation, our hypothesis was partially supported.

**Acknowledgement:** This research study is supported by the National Institutes of Health (Grant # 5RC1AR058356), Office of Rehabilitation R&D Service, DVA SLC HCS, Salt Lake City, UT.

**References**

1. von Recum AF. J Biomed Mater Res. 1984 Apr;18(4):323-36
2. Emmanual J, Hornbeck C, Bloebaum RD. Stain Technol. 1987;62(6):401-10
3. Riekki R, Parikka M, Jukkola A, Salo T, Risteli J, Oikarinen A. Archives of Dermatological Research. 2002:294(4):178-184


**©2013 Society For Biomaterials**