UNDERSTANDING THE PERIPROSTHETIC MICROENVIRONMENT SURROUNDING PERCUTANEOUS DEVICES: USE OF SIMPLE IMMUNOHISTOCHEMICAL TECHNIQUES TO PREDICT HEALING OUTCOMES

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Percutaneous devices (PDs) constitute foreign materials that penetrate through the protective skin barrier to provide connection between internal and external environments. It has been previously shown that the periprosthetic tissue at the percutaneous device skin interface is under a continuous state of wound healing, which often results in epidermal downgrowth. This continuous downgrowth is detrimental to the long-term survival of these devices. Such downgrowth can contribute to device loosening or infection. To date, there are no effective methodologies available to either prevent or quantify the degree of epidermal downgrowth indicating a need to find effective markers to document the healing response around these devices. In this study, periprosthetic tissues from a previous pig-back study were subjected to two different evaluations: (1) standard histology (hemotoxylin and eosin staining) and (2) immunohistochemical staining (IHC). Healing responses around percutaneous devices made with different material types (silicon, porous titanium, smooth titanium, and titanium oxynitrite) were examined using cytokeratin 6 and collagen 4 to determine the degree of wound healing and granulation tissue maturity. Specifically, cytokeratin 6 is expressed by migrating keratinocytes found in the wound edges. Briefly, skin tissue samples from each implant type were collected at necropsy, embedded in optimized cutting media, flash frozen, and then cut into 10-micron thick sagittal sections. Each staining procedure was then optimized. The immunohistochemical staining procedure was optimized by varying the exposure time of primary and secondary antibodies. The hemotoxylin and eosin staining protocol was optimized by varying the amount of alcohol used in Weigert's working solution preparation for faster and uniform penetration of the nuclear stain Hematoxylin. The interfacial tissues were then analyzed using either a photo or a confocal microscope. Preliminary data (Figure 1) indicated that there were noticeable differences in the periprosthetic regions between the material types used. The immunohistochemical staining data confirmed that the periprosthetic tissue is a hyper cellular region with a high density of blood vessels (collagen 4) and migrating keratinocytes. This data further confirmed the morphological differences observed between implant types using standard histology. Continued analysis will quantify the amount of collagen 4 within the periprosthetic tissue and the amount of keratin 6 present in the epidermis. This data will then be examined using imageJ software. Semi-quantitative data from each implant type will then be compared to predict biocompatibility. This research successfully demonstrated that immunohistochemical staining could be a potential tool for understanding the healing cascades around the percutaneous device. Further investigation of immunohistochemical staining may provide novel methods to test biocompatibility of percutaneous devices. Such research may even further the development of percutaneous prosthetics and other invasive medical devices.
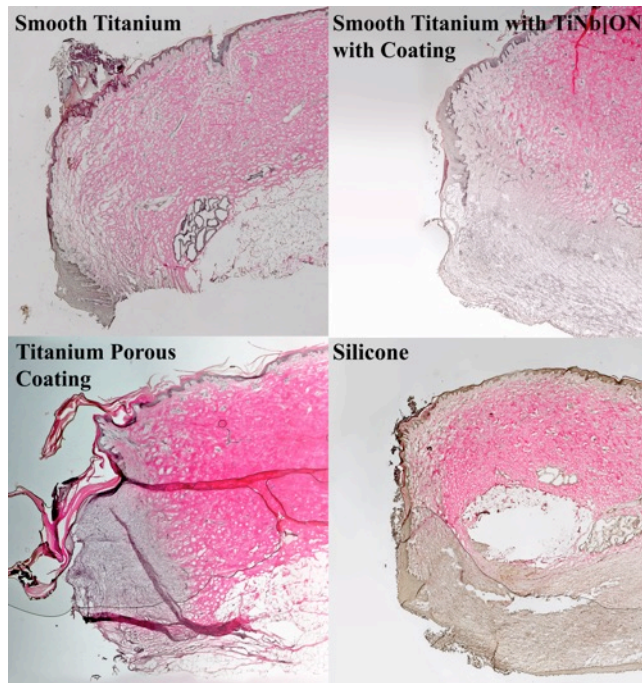
Figure 1: A representative set of photomicrographs of pig skin sections take from the skin-implant interfaces of percutaneous devices, showing more cellular matrix within the granulation tissue.
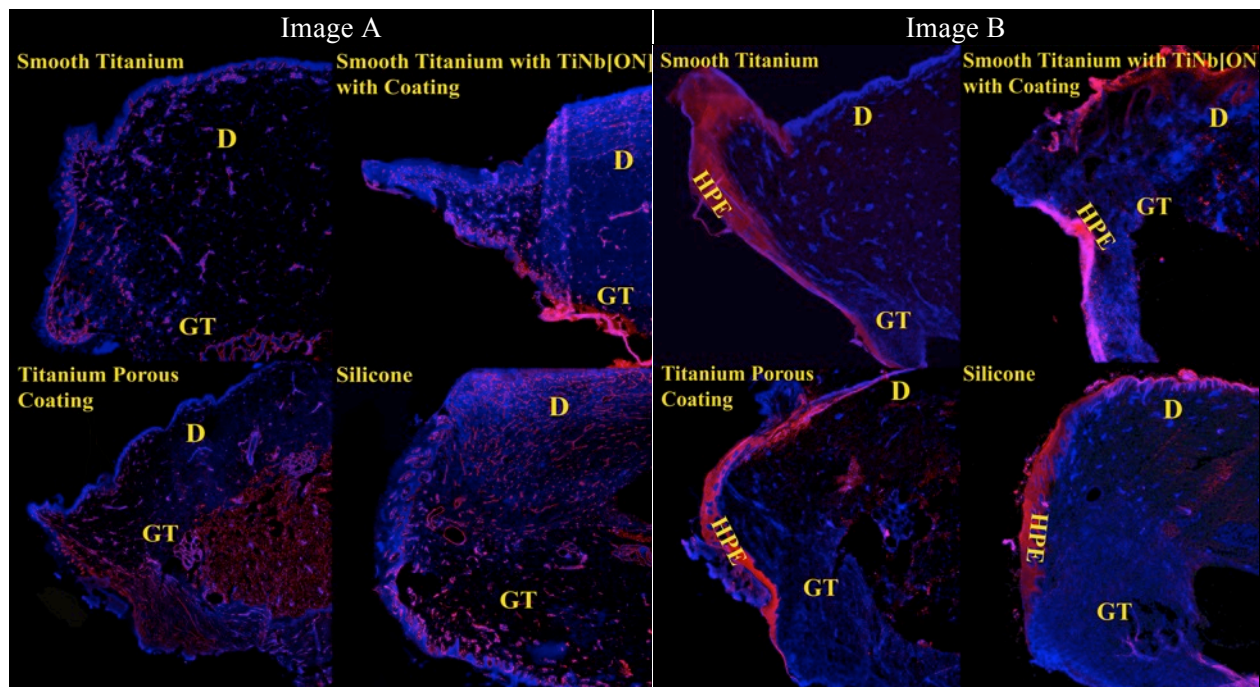

Figure 2: A Representative set of confocal images of skin-implant interface. Image A was stained for collagen IV (red) and nuclear stain (blue). Image B shows the presence of cytokeratin 6 (red) within the migrating epidermis and nuclear stain (DAPI, blue). (D – Dermis; GT – Granulation Tissue; HPE – Hyperplastic Region)