

Bioartificial mucosa: delivery of biomaterials to the skin-percutaneous device interface in *in vitro* human organotypic skin cultures

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ABSTRACT

We have developed an *in vitro* culture system composed of organotypic human skin explants interfaced with titanium rods or stainless steel fixator pins. The use of this interface provides a model to evaluate strategies for creating a stable, long-term connection with living skin and chronic percutaneous devices. Our hypothesis is that the delivery of specific biomaterials at this interface will create a dynamic, slowly flowing matrix for skin biointegration, local administration of drugs or antimicrobials. We define this concept as the generation of an artificial mucosa, because it mimics the situation of several epithelial tissues (like the periodontal junction between the tooth and the junctional epithelium [1]) where antimicrobial peptides and mucins are constantly extruded

Keywords: medical device, skin biomaterial, implant interface, osseointegration, port access devices

1 INTRODUCTION

The use of medical devices passing through the skin has continued to rise. In conjunction with an increased use of catheterization and a general tendency toward minimally invasive procedures in medicine, the need for rapid and reproducible testing methods to evaluate the interfaces between devices and internal tissues and organs has dramatically increased. In the particular case of permanently implanted percutaneous devices, complications ranging from break-down of the skin to serious infections have substantially limited their success, mainly because they do not permit the formation of a stable dermal and epidermal sealing around the implant [2-5].

We have previously presented a system that permits the *in vitro* analysis of the interface between skin and external devices [6,7] and that we have termed "Peramo's chamber". That system was used to observe changes in tissue morphology of the skin in contact with pins in the presence or absence of a variety of materials in solution, delivered discontinuously. The results indicated that

during short culture periods (up to five days), the skin specimens had a deteriorating basal lamina and a disappearing stratum spinosum and that during longer culture periods (fifteen days), the presence of the pins induced apoptosis and hyperproliferation in the areas of the skin close to the pins. The discontinuous delivery of a mixture of hyaluronic acid and dermatan sulfate resulted in a moderate reduction of apoptosis and proliferation and consequently in an improved preservation of the soft tissue around the pins.

In these previous works the material was delivered discontinuously at the skin-device interface. In this regard, it was logical to question whether a continuous delivery of the solutions would be better than the discontinuous delivery and under what conditions. It is not known whether a delivered material may be more effective in helping avoid infection (and possibly helping in skin regeneration) when continuously pumped at the interface. This fact maybe important in the effectiveness of products if and when used in long term implanted devices.

In this report we present observed changes in tissue morphology of the skin in contact with the rods or pins in the presence or absence of different materials and at different pumping rates, including physiological saline, PBS, sodium lauryl sulfate (SLS) and a mixture of hyaluronic acid and dermatan sulfate (HA+DS). This type of treatment may be applicable to tissue areas in contact with fixator pins and other percutaneous devices *in vivo* with the expectation that this bioartificial mucosa may help maintain a better epidermal architecture and tissue integration with the percutaneous device.

2 EXPERIMENTAL METHODS

Full thickness human breast skin explants from discarded material from surgeries performed at the University of Michigan Health System were used. After removal of subcutaneous fat, the tissue was rinsed abundantly with PBS 1X containing 125 µg/ml of Gentamicin (Invitrogen/GIBCO, Carlsbad, CA) and 1.87 µg/ml of Amphotericin B (Sigma-Aldrich, Milwaukee, WI) and placed in aliquots of the same medium in an incubator at 37°C for 2 hours with change of medium

every hour. The culture medium used was EpiLife (Cascade Biologics, Portland, OR). In addition, the medium was supplemented with 75 µg/ml of Gentamicin and 1.125 µg/ml of Amphotericin B. The final concentration of calcium used in the culture medium was 1.2mM.

After preparation, skin specimens of approximately 1.5 cm² were cut using a scalpel and cultured for up to five days at 37° C and 5% CO₂ atmosphere, epidermal side up at the air-liquid interface, in a Transwell system consisting of 6-well Transwell carriers (Organogenesis, Canton, MA) and six Corning Costar supports (Fisher Scientific, Pittsburgh, PA). Culture medium was changed every 24 hours and the stratum corneum remained constantly exposed to the air. Figure 1A shows a conceptual representation of the system. Figure 1B shows the device used for discontinuous delivery and Figure 1C shows the device for pumping. The glass lids that covered the culture plates were produced in-house. The body of the lid itself is solid, created from a non-corrosive material such as glass that permits visualization of the biological tissue underneath. The lid has been designed to work with up to six reservoirs containing biological tissues and had up to six apertures for the insertion of the pins or rods. The apertures formed air-tight seals to prevent contaminants entering the reservoirs. For experiments, skin specimens were punctured with either 3mm or 6 mm diameter sterile biopsy punches. Medium changes on wells were performed using the small feeding opening on the lid located next to each aperture, which were otherwise permanently closed. All materials used were autoclaved before use on each experiment. Fixator pins, (Stainless Steel 316, ISO 5832-9 4mm OD and 10 cm long, McMaster-Carr) were identical, with same OD of 4mm. Titanium rods, (1.0 cm OD and 10 cm long) were hollow, with 0.8 cm ID. The hollow pins had six orifices machined at the bottom of the pin for delivery of the biomaterial, while the hollow rods had four orifices. For cleaning and elimination of particulates or extraneous materials the rods were sonicated for 30' with Liquinox and Citranox (VWR, Westchester, PA) and autoclaved before use on each experiment.

3 RESULTS AND DISCUSSION

The concept of slowly pumping a material at the skin-external device interface derives from the idea of replicating the slow growth and movement of the epidermal layer but also from the fact that epithelial tissues and junctions in the body slowly release materials that help prevent infection.

Our *in vitro* culture chambers were used to observe changes in tissue morphology of the skin in contact with pins or rods in the presence of different materials. The skin specimens were punctured to fit at the bottom of hollow titanium rods or stainless steel pins. The materials were delivered to the specimens through the rods continuously by using an attached fluid pump or discontinuously using the pins device, as indicated in Figure 1. After culturing histological analysis of the skin explants was then performed.

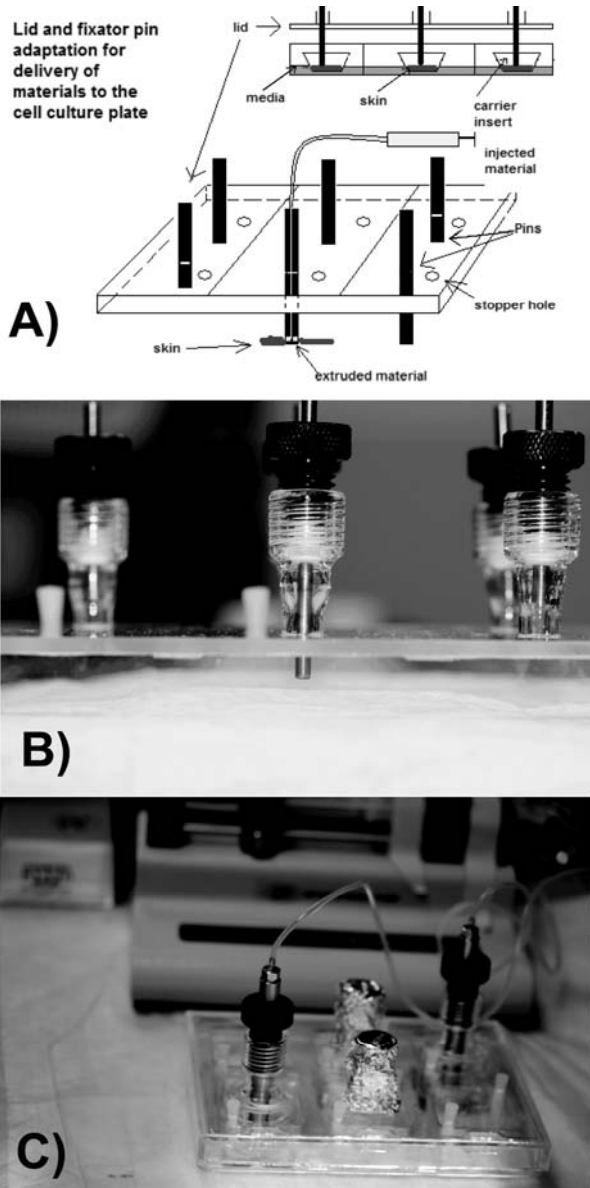


Figure 1. A) Conceptual *in vitro* representation of the system developed for the delivery of materials to the skin-percutaneous device interface. B) The chamber is composed of a culture plate to place skin specimens, covered with a specially designed lid where pins are inserted and used for discontinuous delivery. The culture system in C) is connected to a fluid pump and used for continuous delivery.

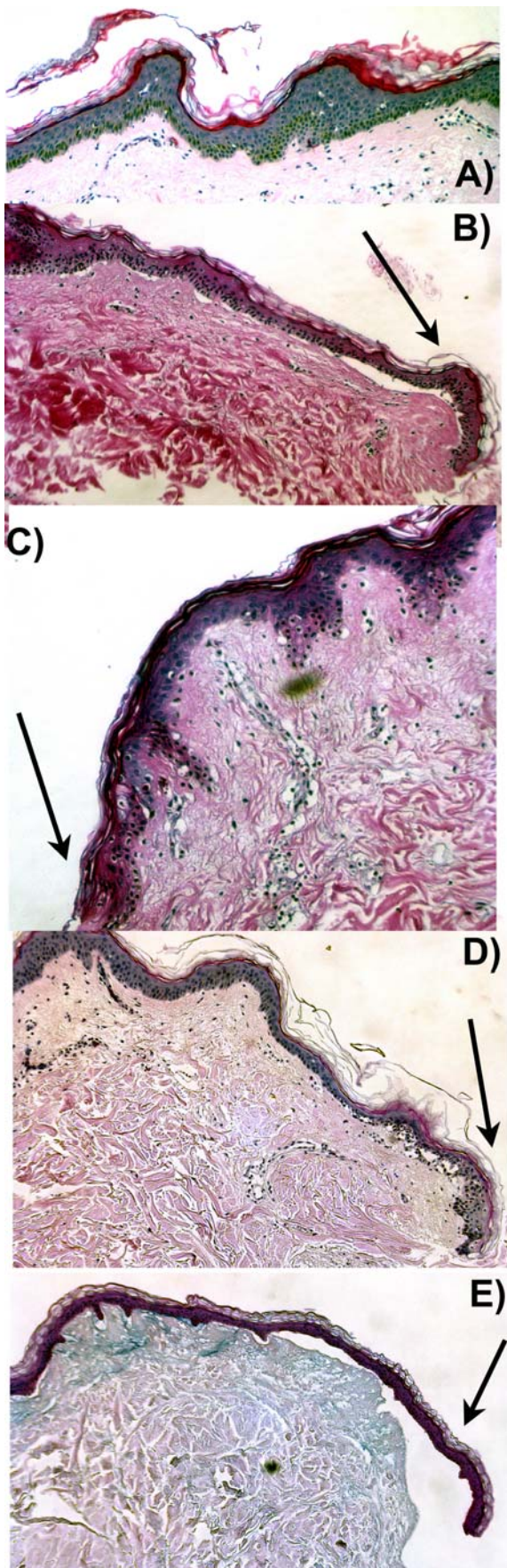


Figure 2 (left). H&E staining pictures of skin specimens cultured using our system. B) to C) correspond to specimens with pins experiments while D) to E) correspond to specimens with rods experiments. Arrows indicate the location of the pins or rods. The delivery of the materials was performed as described in the Experimental Methods section. Description: A) 5 day control, unpunctured specimen (cultured without pin or rod). B) 5 day pin-cultured specimen with PBS 1X. C) 5 day pin-cultured specimen with HA+DS. D) 3 day rod-cultured specimen. E) 3 day rod cultured specimen with SLS.

Figure 2 presents a composite panel of images of tissue morphology of the skin in contact with the rods or pins and in the presence or absence of different materials. This panel is not intended to provide a comparison of tissue quality between skin interfaced with pins or rods, but to give a general view of the possibilities of this bioartificial mucosa to study tissue changes *in vitro* in a broad range of conditions and using a variety of sample materials. Figure 2A show the regular histology of a specimen that was not interfaced with pin or rods but cultured using the Transwell system. It appears only slightly deteriorated after the five day culture. In the rest of the pictures the arrows indicate the position of the pins or rods. Figure 2B and 2C show specimens interfaced with pins for 5 days where a single 100 μ l shot of PBS 1X (Figure 2B) or hyaluronic acid and dermatan sulfate (Figure 2C) was delivered daily. General observations appear to indicate that during these short culture periods (five days), specimens interfaced with fixator pins treated with HA+DS had a slightly better epidermal architecture and dermal structure than control specimens or specimens treated with PBS or physiological saline. Figures 2D and 2E show specimens in rod experiments. As can be seen, when a surfactant like SLS is continuously delivered at a rate of 0.1 μ l/min during three days the specimen becomes very necrotic, as shown in 2E.

4 CONCLUSIONS

The results presented indicate that this type of treatment may be applicable to tissue areas in contact with fixator pins and other percutaneous devices *in vivo* with the expectation that the delivery of the materials replicating the situation found in tissue mucosas may help in preventing infection or in the regeneration process. On the other hand, the *in vitro* model system makes it possible to perform rapid, repeatable studies of living skin response to chronically implanted percutaneous medical devices.

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