Microbial penetration across microneedle arrays treated piglet skin – an *ex vivo* study

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Introduction

Microneedle (MN) arrays typically ranging between 100µm to 1mm in height, are designed to penetrate the external skin barrier, the *stratum corneum (SC),* permitting transdermal delivery of therapeutic agents. These MNs (Fig1A), when pressed against skin, create transport pathways of micron dimensions (Fig1B) and, therefore, increase the flux of molecules across the *SC*.

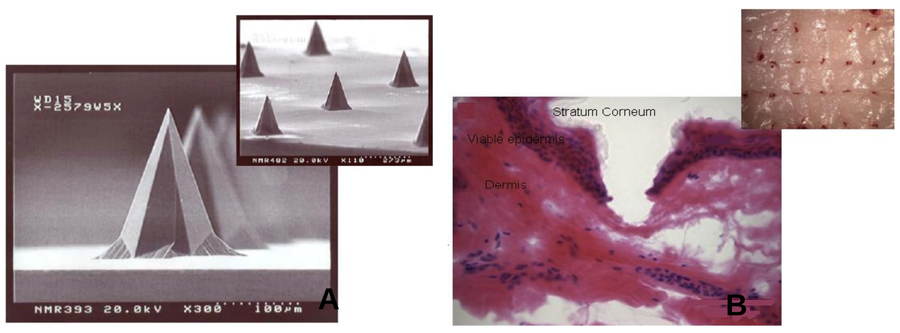


Figure 1. (A) Silicon MN array[1] (B) Holes created after MN Insertion into pig skin[2]

Removal of the MN device prior to the application of a drug leaves open the possible influx of unwanted agents, including bacteria and viruses to the holes or flow paths produced (Fig1B), which may stay open for some time after removal.

MN puncture has never been associated with skin infection. However, to date, no study has investigated the ability of microorganisms (MOs) to traverse MN-induced holes in the *stratum corneum.* In the present study, the penetration of radiolabeled-bacterial (*Pseudomonas aeruginosa or Staphylococcus epidermis*) or fungal (*Candida albicans*) MOs through MN-treated piglet skin was investigated.

Methods

Preparation of MO suspension:

Three MOs (*P. aeruginosa, S. epidermis and C. albicans*) isolates were inoculated into 25 ml of Mueller-Hinton broth (MHB) containing 100 µl of [^{3}H] thymidine (1mCi/ml) & incubated at 37°C for 18h. Suspensions of each MO were centrifuged, drained and resuspended in sterile PBS. A colorimetric absorbance of 0.1 (*P. aeruginos & S. epidermis)* and 1.0 (*C. albicans*) set at OD of 550nm was carried out, to give a concentration of 10^7 c.f.u(colony forming unit)/ml.

Ex vivo permeation studies:

The penetration of MOs from ≈400µm thick piglet skin was investigated using a sterile Franz cell apparatus at 37°C. Receptor compartments (12 ml) were filled with sterile PBS and the skin was sandwiched between donor and receptor compartments of Franz cells (Fig. 2).

1.0 ml of radiolabled-suspension of MOs (10^7 cfu/ml) was placed in the donor compartment and left for 24hrs for biofilm formation. After 24hrs, excess MO suspension was removed from the donor compartment and the skin was punctured in one of three different ways,

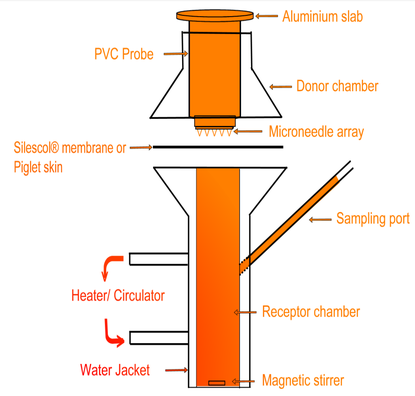
- MN punctured and left in place (MN P&L),
- MN punctured and removed (MN P&R) &
- Hypodermic needle (21G) punctured and removed.

After 24 h, the receptor solution, tape-stripped skin (representing *stratum corneum, SC*) and viable tissue samples were assayed for radioactivity, using a liquid scintillation counter. Then, the Mann-Whitney nonparametric test was used for comparison, where $P < 0.05$ was considered statistically significant.

MN arrays (6x5) used in the present study were conical in shape with 280µm in height and 250µm in base width[1].

Results

Figure 2. Franz-Cell apparatus setup



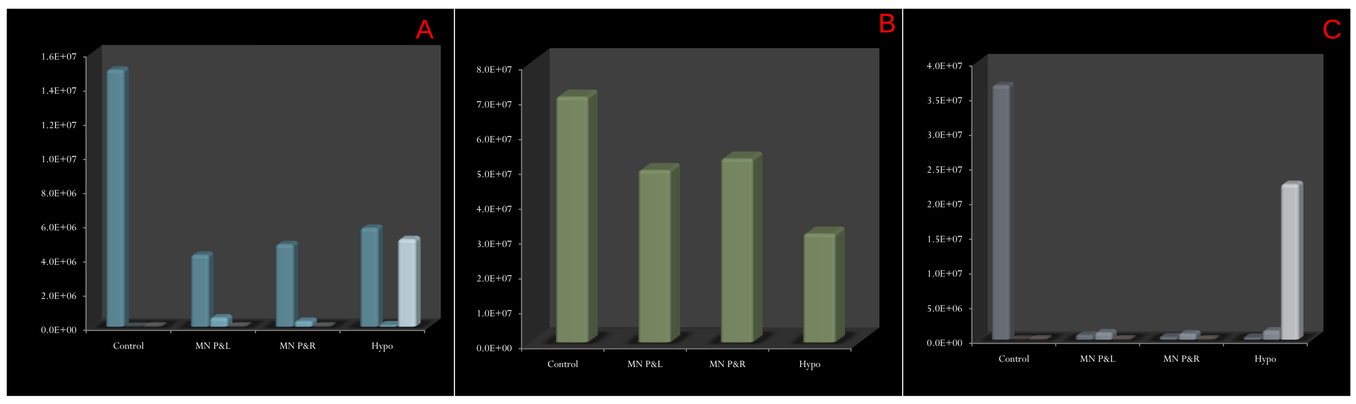


Figure 3: Cumulative amount of MOs penetration across MN or Hypodermic needle-punctured piglet skin (A) *S. epidermis* (B) *P. aeruginosa* (C) *C. albicans*. N=3.

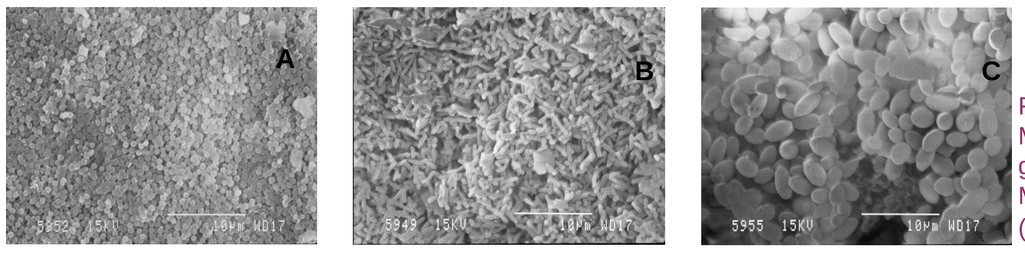


Figure 4: Scanning Electron Microscopy (SEM) showing the general morphology of Microorganisms (A) *S. epidermis* (B) *P. aeruginosa* (C) *C. albicans*

The penetration of radiolabeled-MOs across the treated skin is shown in Figure 3. Hypodermic needle treated skin samples showed higher number of MOs in the receptor solution and *SC*, followed by MN P&R and MN-P&L in place. However, control membranes without puncture showed significantly ($P < 0.05$) higher MOs on surface (i.e. *SC*).

Discussion

Differences in permeation of MO could be attributable to size of holes created in the skin , dimensions of MOs and its motility across punctured membrane;

- 21G hypodermic needle puncture created a hole of cross sectional area 2.06mm^2, while that associated with MN array puncture is 0.994mm^2 in total.
- Holes created by the hypodermic needles remained opened for more than 24 h, whereas the holes created by the MNs were not visible after 24h.
- *C. albicans* is oval shaped 4.0 x 2.5µm, *P. aeruginosa* is rod shaped of 0.7 x 2.0µm and *S. epidermidis* is a cocci of diameter 1.0 µm (Fig.4).

Conclusion

This *ex vivo* study illustrated the possibility of MOs penetration across punctured skin. The hypodermic needles-punctured skin created holes of larger diameter and remains open for longer period; therefore, higher number of MOs was penetrated into the receptor solution. On the contrary, the holes created by the MNs are only few microns wide and typically contracted following withdrawal of the MN. In addition, MN P&L caused blockage of the holes by MNs and therefore allowed lower penetration of MO into the viable tissue and the receptor solution.

In conclusion, precautions should be taken in the manufacturing and maintenance of the sterilely of MNs, to prevent infections associated with the application of MN devices.

References

1. Wilke, N., Morrissey, A. (2007) J Micromech Microeng. **17:** 238-244.

2. Jeong W.Lee, Jung-Hwan Park, Mark R. Prausnitz. *Biomaterials.* 2008; 29(13): 2113-24.