**Topical Delivery of Macromolecules to the Breast through Nipple-Areola Complex**

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**ABSTRACT SUMMARY**  
The study is aimed at exploring the feasibility of localized delivery of macromolecules to the breast through nipple-areola complex (NAC). Inulin (5 kDa), dextran (10 kDa), ovalbumin (43 kDa) and bovine serum albumin (BSA; 67 kDa) were used as model macromolecules in the study. In-vitro penetration of these macromolecules was studied using porcine and human NAC and breast skin. The influence of iontophoresis on the transport of BSA through the NAC was also studied. To visualize the transport through NAC, fluorescent micrographs were carried out with fluorescein-labeled macromolecules. The penetration of the macromolecules through NAC was several folds higher than the penetration through the breast skin. With increasing molecular weight the penetration of the macromolecules through the NAC decreased. The penetration through porcine NAC was comparable to the penetration in human NAC. Iontophoresis enhanced the penetration of BSA through NAC. The macromolecules were transported mainly through the ducts in the NAC. Overall the study demonstrated the feasibility of topical delivery of macromolecules to the breast through the NAC. The findings have implications for developing safe and effective localized therapy for breast cancer.

**INTRODUCTION**  
Breast cancer is the 2nd leading cause of cancer related deaths in women. Currently used chemotherapy and chemopreventive therapies are severely limited by systemic side effects, thus warranting alternative approaches. Several macromolecular drugs have been approved for treatment of cancer including trastuzumab, ado-trastuzumab, pertuzumab, lapatinib and bevacizumab. However the targeted delivery of these agents to the breast has been a challenge. Given that majority of breast cancers originate from the epithelial cells lining the ducts, localized drug delivery to the breast is a promising approach and could bring a safer therapy for breast cancer. To this end, NAC is a potential route for direct delivery of therapeutic agents to the breast. The epidermis is thinner in the NAC and more importantly has multiple duct openings. The feasibility of topical delivery of small molecules, through the NAC has been recently demonstrated. The main goal of this study is to explore the feasibility of delivering macromolecules to the breast through NAC.

**EXPERIMENTAL METHODS**  
Porcine breast tissue was procured from the local slaughterhouse and human breast tissue was obtained from Lions Eye and Tissue Bank, Sioux Falls, SD. The breast skin was used for comparison.

Drug transport studies were carried out in a vertical Franz diffusion cell. The donor compartment consisted of solutions of inulin, dextran, ovalbumin and BSA (10mg/ml spiked with corresponding 14C labeled compound) in ethanol: water (1:1). The receptor medium was phosphate buffer (pH 7.4). For iontophoretic studies, 10 mg/ml BSA solution in phosphate buffer (pH 7.4) was used. Cathodal iontophoresis was performed by placing the AgCl electrode in the donor compartment and the Ag electrode in the receptor compartment. The effect of current density (0.1-0.5 mA/sq.cm for 4 h), and current application time (2-6 h at 0.1 mA/sq.cm), on in-vitro penetration of BSA was studied. Samples were withdrawn from the receptor compartment at different time points. At the end of the study, the NAC was cut and was digested using tissue solubilizer. The drug concentrations in the samples were determined by liquid scintillation counting.

To visualize the transport through NAC 10 mg/ml of FITC-dextran and FITC-BSA in phosphate buffer (pH 7.4) were used. In case of FITC-BSA iontophoresis was applied (0.3 mA/cm²) for 4 h. Cryosections were prepared from the tip to the base of the nipple and studied by fluorescence microscopy.

**RESULTS AND DISCUSSION**  
All the macromolecules used in the study showed higher penetration through the porcine/human NAC, compared to the breast skin (Fig. 1). This signifies the advantage of topical delivery through the NAC. Interestingly the penetration profile through the porcine NAC was comparable to human NAC. Hence porcine NAC can be used as a suitable model to study the transport of macromolecules through human NAC.

![Fig. 1. In-vitro permeation profile of dextran via human (A) and porcine (B) NAC and breast skin. Each data point is represented as mean ± SEM, n=3-4.](image-url)
entire length of the nipple, there was a long lag-time (Table 1). At higher molecular weight (≥40kDa) the flux decreased significantly (Fig 2b); so iontophoresis was explored to increase the penetration of the macromolecules. The application of iontophoresis increased the penetration of BSA by more than 4-fold compared to hydroalcoholic and PBS solution of BSA (Fig 3A). The PBS solution did not show any measurable penetration of BSA through the NAC. An increase in current strength did not proportionally increase the permeation of BSA (Fig 3a). However 0.5mA/sq.cm showed higher permeation than the lower current strengths. Current application longer than 4 h did not cause significant increase in BSA penetration. The results indicate that the transport pathways in NAC may be saturable.

**Table 1. Permeation parameters of the macromolecules through porcine NAC after 48 h of treatment. (Mean ± SEM, n=4)**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Lag time (h)</th>
<th>Total permeation at 48 h (μg)</th>
<th>Total tissue retention at 48 h (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>12.0±0.36</td>
<td>81.4±1.07</td>
<td>107.5±1.53</td>
</tr>
<tr>
<td>Dextran</td>
<td>14.24±0.64</td>
<td>54.27±7.64</td>
<td>91.24±6.56</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>14.14±1.22</td>
<td>26.55±1.67</td>
<td>65.50±3.94</td>
</tr>
<tr>
<td>BSA</td>
<td>19.58±1.84</td>
<td>14.76±1.48</td>
<td>25.25±4.21</td>
</tr>
</tbody>
</table>

**Fig. 2.** (A) In-vitro permeation of macromolecules via porcine NAC; (B) flux values of macromolecules as a function of molecular weight. Each data point is represented as mean ± SEM, n= 4.

Microscopic studies showed that the macromolecules are mainly transported through the ducts and the surrounding connective tissue (Fig. 5a). In contrast, the iontophoretic transport of macromolecules was mainly through the ducts (Fig 5b). The results demonstrate that the ducts serve as a potential route for direct delivery of macromolecules to the breast.

**Fig. 4.** Fluorescence microscopic images of 7-8 μm thick cryosections of porcine NAC after 24 h treatment with FITC-dextran (upper row) and FITC-BSA (middle row). The lower row images are sections of porcine NAC after iontophoretic treatment (0.3mA/sq.cm for 4h) with FITC-BSA. Sections were taken at different depths from the tip to the base of the tissue, as shown in schematic diagram on left side. (Bar= 100 μm)

**CONCLUSION**

The findings from the study demonstrate that macromolecules can be delivered to the breast through the NAC and iontophoresis can be used to further enhance their penetration. The ducts play an important role in the transport of large molecules through NAC. Overall the findings from the study demonstrate the feasibility of developing localized macromolecular therapeutics for breast cancer.

**REFERENCES**

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