Pigmentation of the nipple-areolar complex and its reconstitution in breast reconstruction

Thesis for the Degree of Doctor of Philosophy

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ABSTRACT

Reconstruction of the nipple-areolar complex is the final step in the recovery of women who have undergone mastectomy for breast cancer. Reconstruction of breast shape has been an area of major developments in surgical techniques in recent times. Nipple-areolar reconstruction, however, has not reached the same stage of evolution and is not as widely practiced. Clinicians that do perform nipple-areolar reconstruction as an integral part of a breast reconstruction program, indicate that it is difficult to match the colour of the normal nipple-areola and that the colour of reconstructions fades over time.

The purpose of this research was to assess the quality of current methods of nipple-areolar reconstruction, with special reference to pigmentation and, if they were found lacking, to investigate the feasibility of producing an engineered pigmented skin substitute that could be used in this clinical context. The research falls into three main parts - a clinical study of patients who have undergone breast reconstruction, a histological study of normal areolar skin and a cell culture study.

Patients who had undergone nipple-areolar reconstruction (n=63) were found to be less happy with the colour of their nipple-areolar reconstruction than with the general attributes of the breast reconstruction as a whole. There was found to be a measurable colour mismatch between normal and reconstructed nipple-areolar complexes and a reduction of colour saturation (i.e. fading) of the reconstructed nipple-areolar complex over time.

Pigmentation of the nipple-areolar complex at a histological level has not previously been reported and was a logical area to study if improvements in the colour of nipple-areolar reconstructions are to be achieved. Melanin and melanocyte complements in breast and areolar skin of twenty patients were measured using
conventional histochemical staining, immunohistochemistry and image analysis. The melanin content of areolar skin was about twice that of breast skin. This could mainly be accounted for by the higher number of melanocytes in areolar skin but basement membrane convolution and higher amount of melanin per melanocyte also contributed.

Detection of all normal melanocytes present in sections of skin was not straightforward. Preliminary experiments were carried out with several different primary antibodies for immunohistochemical labelling. The antibody clone TA99 (also known as Mel-5) against Pigment Associated Antigen (PAA) was found to be the most sensitive and was used in the quantitative study of melanocytes in breast and areolar skin.

Production of a tissue-engineered skin construct with the pigmentedary characteristics of nipple-areolar skin is theoretically possible and could be used as an adjunct to current methods of nipple-areolar reconstruction. Production of such a construct for clinical use would have to be in an environment free of the toxins and potentially infective serum that are often used for *in vitro* cell culture.

It was possible to grow keratinocytes and melanocytes *in vitro* from adult surgical discard skin from the trunk (breast and abdomen). Initial cultures of melanocytes from this source in a serum-free medium were unsuccessful and culture of keratinocytes in serum-free medium was very slow. A subsequent series of experiments showed that melanocytes and keratinocytes can be grown successfully in the presence of autologous human serum and that the commonly used additives phorbol 12-myristate 13-acetate (PMA) and cholera toxin can be omitted from melanocyte culture medium if autologous human serum is used. Cells cultured in such a medium were used to produce new skin constructs by seeding them onto freeze-thawed human dermis.
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