Researcher (s):	Prof Desmond Tobin (PI) Co-Researcher: Dr Fiona Browne
Research Institution:	University College Dublin
Project Title:	The scalp hair follicle as a source of collagen VII-positive
Research Area:	Wound Healing
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Lay Summary

Epidermolysis bullosa (EB) is a group of inherited and incurable skin disorders, where blistering/erosions can occur spontaneously or after minimal physical trauma. Chronic wounds are then complicated by infections, prolonged inflammation, and in some types, skin cancer. Currently management of EB is limited to control of pain and itch, minimisation of infection, and surgical management of complications. There is an urgent need to improve patients' life opportunities.

In this project, we will focus on patients with Recessive Dystrophic EB (RDEB), whose skin splits in the upper dermis, and ask whether their skin can benefit from harnessing regenerative power of their (often) unaffected scalp hair follicles (HF). Patients with RDEB carry a mutated COL7A1 gene, making them unable to produce much, if any, functioning type VII collagen. This collagen helps to hold skin together when placed under even minimal strain.

Skin sites that are often protected from RDEB blistering include scalp and underarm; areas with large HF with functioning type VII collagen. The rich regenerative capacity of HF makes them excellent potential repair 'tool-kits' in RDEB. Our project team has expertise in HF biology and clinical care of EB patients that is unique in Ireland. We propose in this proof-of-concept project to examine if cell-based approaches based on type VII collagen-expressing HF cells could support RDEB blisters and blister-prone skin that lacks this key collagen. Using an array of biomolecular tools, we will compare type VII collagen status in normal healthy scalp skin and HF, and compare this with type VII collagen status in ethically-obtained scalp HF from RDEB patients. We will examine skin and HF tissues as well as different cell types isolated from skin and HF, for insights into how best to exploit the remaining RDEB type VII collagen-expressing cells to aid skin integrity in this vulnerable patient group.

Project Abstract

While the human integument contains life-long cycling hair follicles (HF) that produce either tiny invisible vellus hairs or large coarse terminal hairs, their role in skin homeostasis remains unclear. This is very relevant for (R)DEB skin, where HF may 'escape' the type VII collagen deficiency that blights much of the interfollicular RDEB skin.

Thus, this project seeks to:
Investigate the potential to leverage a greater understanding of the residual type VII collagen expression in RDEB terminal hair follicles in order to support blister-healing and stabilization of blister-prone inter-follicular skin in RDEB.
1) Define type VII collagen expression (at gene/protein levels) in normal skin tissues and cells from body sites that produce vellus-hair follicles and terminal hair follicles (in situ/in vitro).
Question: How does type VII collagen expression (gene and protein) compare between interfollicular skin (vellus-haired) and terminal hair follicles (plucked and intact) in normal healthy skin? w?

2) Define type VII collagen/COL7A1 expression (gene & protein levels) in RDEB patient hair follicles (HF) from terminal-haired sites (scalp, axilla, via punch biopsy) and plucked HF in situ/in vitro.
Question: How does type VII collagen expression compare between intact RDEB terminalhaired scalp and plucked (RDEB) HF from scalp/axilla?

3) Define how the cell biology of keratinocytes & fibroblasts cultured from RDEB inter-follicular scalp compares with keratinocytes & fibroblasts cultured from normal inter-follicular scalp.
Question: How is the function of RDEB inter-follicular scalp skin keratinocytes and fibroblasts modulated by co-culture with similar cells cultured from normal scalp?

4) Define how keratinocytes and fibroblasts cultured from RDEB scalp terminal hair follicles interact with keratinocytes and fibroblasts cultured from normal scalp terminal hair follicles (intact/plucked).
Question: How is the function of RDEB scalp hair follicle outer root sheath (ORS) keratinocytes and dermal sheath (DS) fibroblasts modulated by co-culture with similar cells cultured from normal scalp hair follicles?

5) Investigations of alopecic and scarred RDEB scalp skin that previously grew hair.

Question: Why does scarred scalp skin exhibit a lower tendency to blister that do other body skin on the same patient. Methods: Using transmission electron microscopy (EM) analysis to assess status of AFs in RDEB HFs, we will extend to an assessment of HF-lacking scared skin to see if altered, reduced in number, or absent. We will additionally assess expression of validated stemness and differentiation cell markers (keratinocytes/ fibroblasts) between normal and RDEB-derived cells/intact and plucked HFs, and scarred alopecic skin. We will assess changes in HF-associated stromal microenvironment (beyond COL7A1 protein/gene expression) in RDEB patients that may be transitioning toward alopecia/hair loss. We will additionally include assessment of expression/activity (via zymography) of MMPs that turnover COL7A1 (e.g., MMP-1,-2,-8,-9). Furthermore, we additionally include assessment of the expression of anti-inflammatory POMC-derived molecules (a-MSH & ACTH) in RDEB versus normal haired tissue

Blog post written about project for website

*Not blog but part of project application: 'How this project will make a difference to the lives of those with EB and their families'

RDEB markedly affects quality-of-life (QoL) in physical, emotional and social terms. *Frew et al, (2009)* developed/validated a 'Quality of Life in Epidermolysis Bullosa' score. The is a significant correlation between worsening RDEB skin disease severity and QoL score, and generalized severe EB had greater QOL impairment than any disease previously assessed.

The Co-applicant is a member of an international multidisciplinary panel of social and health care professionals and people living with EB, which addressed whether psychosocial support can help people cope with EB. The panel published recommendations (*Martin et al, 2019*) and showed that most existing research is not focused on interventions that help. While the burden of RDEB on Irish health system is unstudied, the UK PEBLES study reported annual costs of £98K/adult and £25K/child for dressings alone (*EB World Congress 2020*). Many adult EB patients are not employed. Those that are, struggle to juggle leave for medical care, illness, and hospital visits.

Project Impact: There is no cure for EB and current treatments are inadequate. Poor understanding of how to leverage the regenerative capacity in non-blistered EB skin, in particular terminal-haired skin (focus of project), has limited development of new therapies. This study aims to provide within the 3-year life of this project a rationale for a novel therapeutic approach to stimulate wound-healing in blistered RDEB skin by exploiting the regenerative cellularity of remaining terminal hair follicles that retain

type VII collagen expression. We expect to see that hair follicle in RDEB patients from sites like scalp and axilla have keratinocytes and fibroblasts that are relatively 'normal' in terms of their proliferation and differentiation capacity.

Thus, it may be possible to shift regenerative capacity of the hair follicle to the damaged interfollicular skin via discovery of early targets (potentially hair follicle BMZ components (glassy membrane).

However, as alopecia is still a common feature in patients with RDEB, we need to understand the possibility for early intervention to help retain/save terminal hair growth, as this provide functions to the underlying skin for greater patient comfort. Another benefit would be to retain the cosmetic benefit that scalp hair provides, and the psychosocial impact of this, especially to female patients.

Quotes we have from Researchers

None

Researcher (s) Bio

Desmond Tobin is a Full Professor of Dermatological Science at UCD School of Medicine and Director of the Charles Institute of Dermatology, University College Dublin (since Sept 2018). His H-Index is currently 65 with 12778 citations (Scopus, Summer 2022). and 77 with 20,000 citations (Google Scholar, Summer 2022). Previously he was Professor of Cell Biology and Director of the Centre for Skin Sciences (2009-2018) at University of Bradford, where he holds an Honorary Visiting Research Professorship. Desmond was awarded a BSc from the National University of Ireland (Maynooth), a PhD from the University of London (St. John's Institute of Dermatology) and has held post-doctoral training and Junior Faculty positions at New York University Medical School's Department of Dermatology. Over the past 25+ years he has researched in basic and applied skin/hair sciences, with a particular focus on the regulation of hair growth in health and disease especially those conditions with an immune-medicated basis, and on the biology of human melanocytes/pigmentation in health and disease. His lab was the first to identify antibodies to hair follicle-specific antigens in patients with acute alopecia areata and is working to identify targeted (auto)antigens in this common condition. His lab was the first to establish melanocytes from the human hair follicles in long-term culture. His lab also identified a 'self-similarity' between the POMC peptide system in the human hair follicle pigmentary unit and that of the central stress axis. Specifically, he examined how pigmentation can be regulated by CRF and POMC peptides (incl. beta endorphin), and more recently by bone morphogenetic proteins. His lab revealed an unexpected role for filopodia in melanin transfer under the influence of Myoxin-X and CDC42. His lab

report a highly unusual asymmetric organelle distribution post mitosis in human skin epidermis progenitor cells, as the basis of human skin pigmentation. Des Tobin holds fellowships of: Royal College of Pathologists (UK) Royal Society of Biology (UK) Institute of Biomedical Sciences (UK), Institute of Trichologists (UK) (Vice-President 2000-2021). Elected member of the Royal Irish Academy (2021) Chair of the British Society for Investigative Research 2018-2020 Panel member of the REF 2021 (UK) (Sub-panel 3 (Allied Health Professions, Dentistry, Nursing and Pharmacy). Des serves(ed) on several editorial boards including Chief Editor of the 'Skin Physiology' section of Frontiers in Physiology. He has published approx. 180 publications, incl. 3 books.