PROJECT SYNOPSIS, School of Life Sciences
Director of studies: Director of studies: Professor Mark Eastwood

Project title: The critical fibroblast - keratinocyte interactions in scarring and fibrosis
Background to research and synopsis:
Scleroderma (SSc) is a connective tissue disease of unknown aetiology that causes considerable morbidity and mortality in affected individuals. As excessive scarring leading to fibrosis is the most characteristic pathological hallmark of SSc, elucidating pathogenesis of such fibrosis is likely to be beneficial in understanding the nature of fibrotic disease in general. In the very early stages of wound healing, regulation is due in part to direct contact between keratinocytes and underlying fibroblasts, or to release of cytokines from one cell to another, or indeed, a combination of both. It has been shown that keratinocytes can induce dermal fibroblasts to differentiate into myofibroblasts, a hallmark of tissue fibrosis, via endogenous transforming growth factor β (TGFβ) activity which is further antagonized by keratinocyte-derived or exogenous interlukin-1 (IL-1). Interaction between fibroblasts and keratinocytes has also been found to modulate levels of matrix metalloproteinase. Fibroblasts in three dimensional collagen matrices can generate contractile forces, similar to those found in fibrotic lesions. Our laboratory has developed a tethered 3-D fibroblast populated collagen lattice system, the tensioning-culture force monitor (t-CFM). Application of external mechanical forces promotes cells to secrete cytokines and other growth factors which may feed back to modulate cell behaviour during fibroblast-collagen matrix remodelling. Several studies have investigated the mechanisms on how fibroblasts become myofibroblasts, and it seems that the two most important stimuli are mechanical tension and TGFβ activity. Therefore mechanical tension can be regarded as an additional important regulatory factor in a study of fibrosis. So far most of the studies with SSc fibroblasts have been in monoculture with cells grown on plastic substratum. The proposed study here, represents a more physiological model with keratinocytes and fibroblasts growing together in a 3 dimensional collagen matrix. **The hypothesis of this proposal** is that interactions between fibroblasts and epithelial cells have a profound influence on pathogenesis of SSc. In particular over-expression of pro-fibrotic proteins and enhanced contractility, characterised by SSc lesional fibroblasts, results from failure to correctly recognise and respond to the cellular environment due to aberrant interactions with the ECM and/or de-regulated intracellular communication between fibroblasts and epithelium. In this study we propose to develop a novel 3-D co-culture system using dermal fibroblasts and epithelial keratinocytes which is physiologically more relevant to the *in vivo* environment observed in SSc. **The specific aims of this project are to**: 1. Develop and utilise the t-CFM to examine keratinocyte-fibroblast interaction within a 3-D collagen matrix, as a model to study SSc, and to determine effects of externally applied mechanical load on gene and pro-contractile matrix protein expression. 2. Investigate the multi-cellular endogenous response to an externally applied force in the t-CFM system, by evaluating cell proliferation, migration, gene profiling, differentiation, and contractile characteristics of SSc fibroblasts, when co-cultured with keratinocytes. 3. Study cell-cell interaction and characterize contribution of cytokines (TGFβ, CCN2 and IL-1) co-proteins Syndecan-4, TSP-1 on cell contraction, and tensional homeostasis using the t-CFM.
Training: The successful candidate will receive training in human cell culture techniques, advanced biomaterials and use of specialised equipment developed in this laboratory, in addition to ad-hoc courses as required. During the first year of the PhD programme all students in the School of Life Sciences attend an induction programme which enables them to make full use of all facilities.
Supervisory Team and Research Environment:

Supervisor Name	Role (DoS, 2nd Supervisor,)	No. of successful PhD supervisions	Current student load For 20010/11 (FTE)	School
Mark Eastwood	DoS	2	0	Life Sciences
David Abraham	2nd	8	3	Royal Free/UCL

Recent publications by supervisors relevant to the project: (£15,000 consumables provided)
Leask A, Shi-Wen X, Khan K, Chen Y, Holmes A, **Eastwood M**, Denton CP, Black CM, **Abraham DJ**. Loss of protein kinase Cepsilon results in impaired cutaneous wound closure and myofibroblast function ". *J Cell Sci* 2008 15;121:3459-67.
Chen Y, Leask A, **Abraham DJ**, Pala D, Xu S, Khan K, Carter DE, Wilcox-Adelman S, Goetinck P, Denton CP, Black CM, Pitsillides AA, Sarraf CE and **Eastwood M**. "Heparan sulphate-dependent ERK activation contributes to the over-expression of fibrotic proteins and the enhanced contraction by lesional dermal scleroderma fibroblasts of their extracellular matrix". *Arthritis Rheum* 2008;58(2):577-585
Informal enquiries: eastwood@westminster.ac.uk
http://www.westminster.ac.uk/schools/science/research/research-groups/cell-communication