

Maggots and Wound Healing: The Effects of *Lucilia sericata* Larval Secretions upon Human Dermal Fibroblasts

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INTRODUCTION: The development of dressings capable of debriding, cleaning and closing recalcitrant wounds, remains as yet, an unmet challenge. Tissue engineering may play a role in meeting this challenge, as demonstrated by recent developments including the use of growth factor delivery systems and skin replacement technology. In conjunction with elucidating how *Lucilia sericata* larvae can often stimulate healing when applied to wounds^{1,2}, tissue engineering has the potential to provide the total wound care package. Here, the effects of larval secretions upon interactions between human, dermal fibroblasts and extracellular matrix proteins, including the use of 2D and 3D wound assays were investigated.

METHODS: Larval excretion/secretion (ES) collection:

One-day-old sterile *L. sericata* larvae were bathed in phosphate buffered saline (PBS). ES/PBS mix was extracted and sterile filtered. Protein concentration and protease activity was estimated using Bio-Rad's protein assay or fluorescein isothiocyanate (FITC)-Casein assay respectively. Heat-treated ES was heated at 100°C for 30 min, yielding insignificant protease activity.

Cell adhesion: Human, dermal, neonatal fibroblasts were plated into wells pre-coated with fibronectin. An ES blank (PBS) or whole ES was immediately added. Following incubation and aspiration to leave adhered cells for assaying, ATP concentrations were estimated using Packard's ATPLite™-M kit.

Modification of fibronectin by ES: Samples of fibronectin incubated with ES, were tested for evidence of proteolytic degradation products using 12% SDS-PAGE.

Cell spreading: Cells were seeded into fibronectin pre-coated wells, with or without ES. After 4h incubation, images were taken using Leica DMIRB inverted microscope and analysed with QUIPS software.

Cell migration: Cells were seeded upon fibronectin surfaces in 2D *in vitro* wound assays or within 3D gel-based (1.5 mg/ml collagen, 30 µg/ml fibronectin) *in vitro* wound assays, with or without ES. Their migration over 48h was observed and quantitated using still images, time-lapse digital photography and Leica QUIPS software.

Additionally, within the 3D assays, cell distribution and morphology was observed using confocal microscopy.

RESULTS: ES reduced fibroblast cell adhesion upon fibronectin³. It also modified fibroblast spreading upon the substrate, by reducing cell surface area and increasing roundness³. Heat-treatment significantly decreased the modulatory activity of ES, indicating a role for ES proteases³. Pre-exposure of fibronectin to ES before addition of fibroblasts, also resulted in modified cell adhesion and the presence of fibronectin proteolytic fragments³. The *in vitro* wound assays revealed ES to promote fibroblast migration, both in 2D (Fig. 1) and within the 3D collagen gel environment. Differences in cell morphology were also observed.

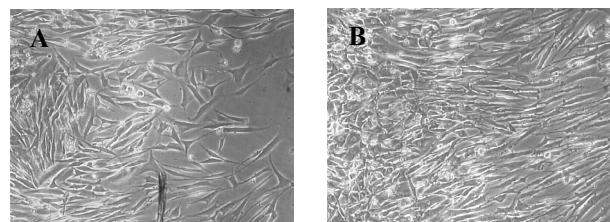


Fig. 1. Migration of fibroblasts across a fibronectin surface, into free space. At 0h, cell boundary positioned along left hand edge of image. Progression seen after 48h in A. ES blank; B. 0.1 µg/ml ES.

DISCUSSION & CONCLUSIONS: Interactions between fibroblasts and fibronectin surfaces were shown to be modified by *L. sericata* ES and in particular, by the proteases present within the ES. The observed reduction in cell adhesion and spreading upon fibronectin, may enhance fibroblast migration, as indeed, was seen in the 2D and 3D *in vitro* wound assays. Alteration of fibronectin by the activity of ES may also exert an influence over fibroblast behaviour, as there is evidence that its proteolytic degradation products bind to fibroblasts to elicit different responses⁴. In the clinical setting, ES released by larvae placed onto the wound, may induce fibroblast migration into the wound space, facilitating tissue regeneration.

REFERENCES: ¹ R.A. Sherman, M.J.R. Hall and S. Thomas (2000) *Annu. Re. Entomol.* 45,55-81. ² D. Bonn (2000) *Lancet* 356, 1174. ³ A.J. Horobin, K.M. Shakesheff, S. Woodrow et al (2003) *Br. J. Dermatol.* 148, 923-933. ⁴ P. Huhtala, M.J. Humphries, J.B. McCarthy et al (1995) *J. Cell. Biol.* 129, 867-879.