Healing of small wounds

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The mechanisms underlying wound healing are key features in numerous biological processes such as development, traumatic situations or even, in the broader context of collective cell motility, cancer dissemination. Usually, such processes are studied through a scratching technique consisting of the mechanical disruption (with a pipette tip, razor blade etc...) of a confluent epithelium in a traumatic and often uncontrolled fashion.

Here, we develop a novel method mixing microfabrication and tissue culture tehniques to manufacture a large number of circular model wounds in an epithelial tissue. Cells are constrained in their growth by cylindrical PDMS objects that, when removed, offer free surface for the epithelium to invade. The removal of a physical constraint is sufficient to trigger the healing process. This technique allows us to create a large number of small size wounds with well-defined geometries and sizes and in a non-traumatic situation best suited to understand the mechanisms underlying their healing.

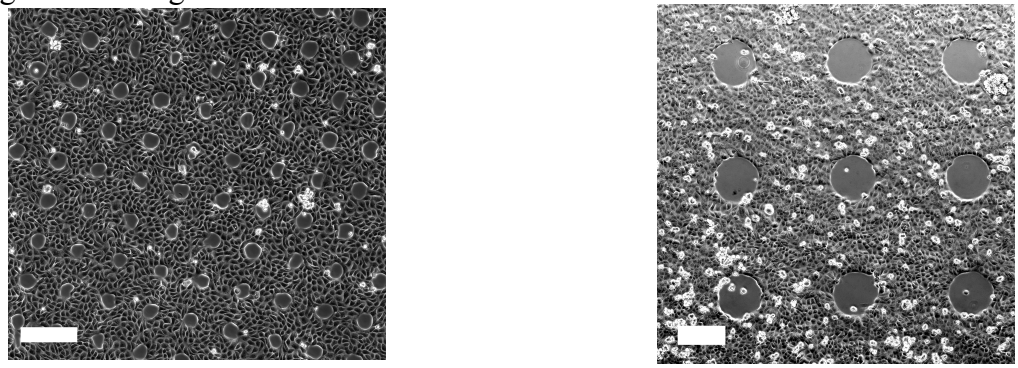

Fig1: Phase contrast imaging of initial situation showing two sizes of wounds (50µm and 200µm wide, scale bar:200µm).

This technique allows for a detailed analysis of the mechanics and dynamics of wound healing from both a biological and physical point of view. We show that two main mechanisms are responsible for the closing of the wound: an acto-myosin ring involved in a purse string mechanism and small protrusions that actively drive the closing.

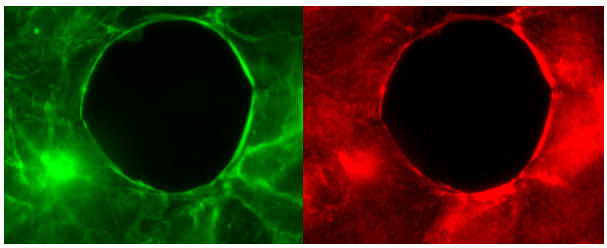

Fig2: Immunostaining of F-actin (green) and Phospho-Myosin Light Chain (red) in a 50µm wide wound showing the acto-myosin ring.

We have also developed a theoretical model of the closing of the tissue taking into account all of these ingredients. We get orders of magnitude of the parameters through different types of experiments (inhibition of critical pathways, photo-ablation of the actin cable) and find good agreement between the model and the experiments.

Our new approach allows for a systematic and quantitative understanding of the critical processes involved in wound healing and could prove useful in the future for the study of epithelial architecture and collective mechanisms in tissues and cellular invasion.