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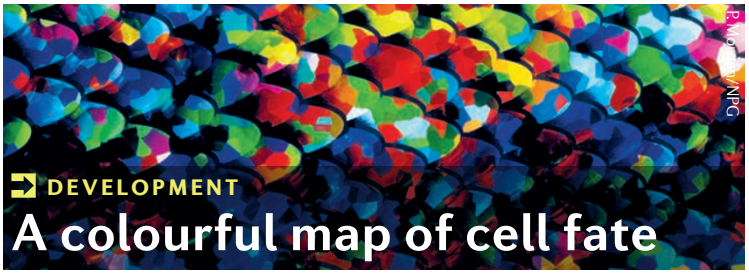

Researchers have engineered a multicoloured zebrafish that produces skin cells in over 70 distinguishable colours, enabling tracking of the behavioural dynamics of hundreds of individual epithelial cells during skin regeneration *in vivo*.

The transgenic zebrafish system called skinbow is based on a previously published technique termed Brainbow, in which neurons were engineered to randomly express different ratios of coloured fluorescent proteins, resulting in distinct colours between cells. In the skinbow study, the researchers introduced constructs containing Brainbow expression cassettes into zebrafish embryos and identified one transgenic line in which the entire body surface, including the fins, was fluorescently labelled. Importantly, labelling was restricted to the superficial layer of epithelial cells and was not present in the underlying dermal tissues.

Quantitative PCR (qPCR) analysis of superficial epithelial cells (SECs) revealed that each cell contained more than 100 copies of the Brainbow expression cassette, and these were integrated at a single genomic site. Each gene copy randomly expresses a red, blue or green fluorescent protein, resulting in an extensive colour palette comprising thousands of possible hues. The number of distinguishable colours was limited to around 70 by the resolution of the confocal microscope used to image the cells. Nonetheless, this resolution was sufficient to distinguish individual SECs from their neighbouring cells by colour.

Live-imaging experiments, which examined the caudal fins of transgenic fish at 12-hour intervals over the duration of 20 days, revealed that individual cell colours were stable, and fluorescence intensity increased with duration at the epithelial surface. In a single imaging experiment, the team were able to simultaneously track several hundred individual cells from their first appearance on the surface of the fin until their loss an average of 8.4 days later.

The team carried out a series of imaging experiments to monitor and quantify the behaviour of individual cells under homeostatic conditions, during the repair of abrasive injuries and during post-amputation tissue regeneration. Under homeostatic conditions, they observed the rearrangement of neighbouring SECs to fill the gaps left by cell shedding, the replacement of lost cells with new SECs, and *de novo* SEC 'birth'. Following minor abrasive injury, surviving cells increased in surface area and mobilized to the site of injury to reduce tissue exposure. After fin amputation, there was a rapid mobilization of SECs over large distances to cover the wound, followed by fin regeneration involving the integration of *de novo* SECs with pre-existing SECs.

Finally, to assess the role of reactive oxygen species (ROS) in fin regeneration, the team combined skinbow-based imaging with a redox-sensitive fluorogenic probe and manipulated ROS levels by treating animals with antioxidants. The results of this experiment suggest that epidermal surface exfoliation results in a burst of ROS that promotes tissue regeneration.

In summary, by combining multicolour cell barcoding, real-time *in vivo* imaging and large-scale cell tracking software, the skinbow platform can provide valuable insights into the mechanisms underlying epithelial repair and regeneration. Furthermore, the ability to combine skinbow with other reporters and inducible activators will allow the actions of molecular factors to be linked to cellular behaviour.

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ORIGINAL ARTICLE Chen, C.-H. *et al.* Multicolor cell barcoding technology for long-term surveillance of epithelial regeneration in zebrafish. *Dev. Cell* **36**, 668–680 (2016)