How epithelial cell extrusion is regulated by cell density

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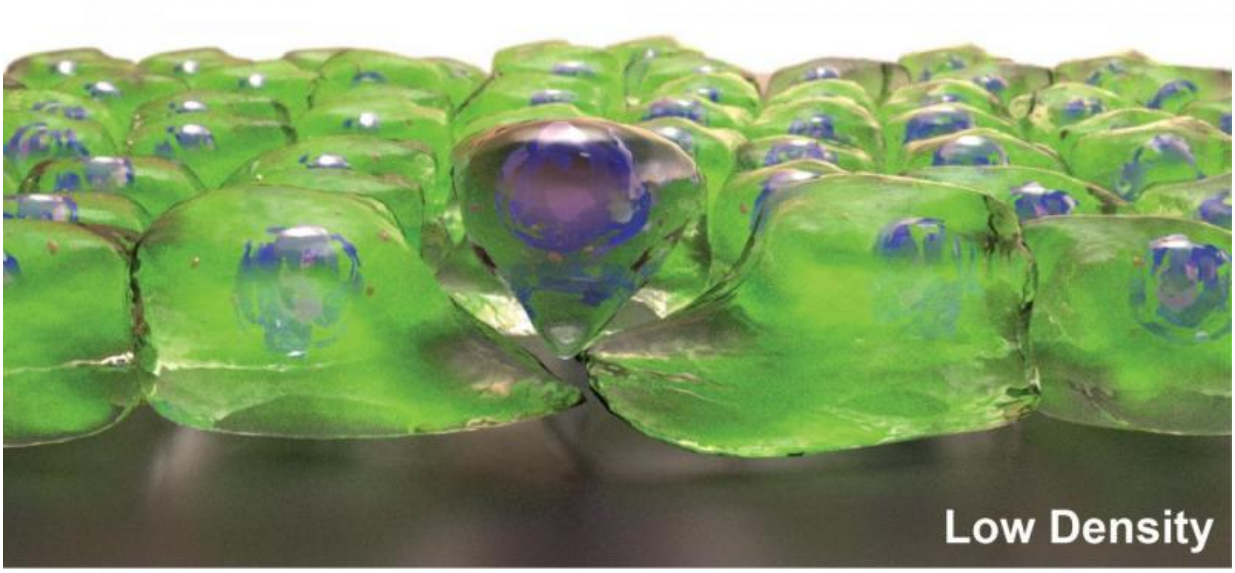
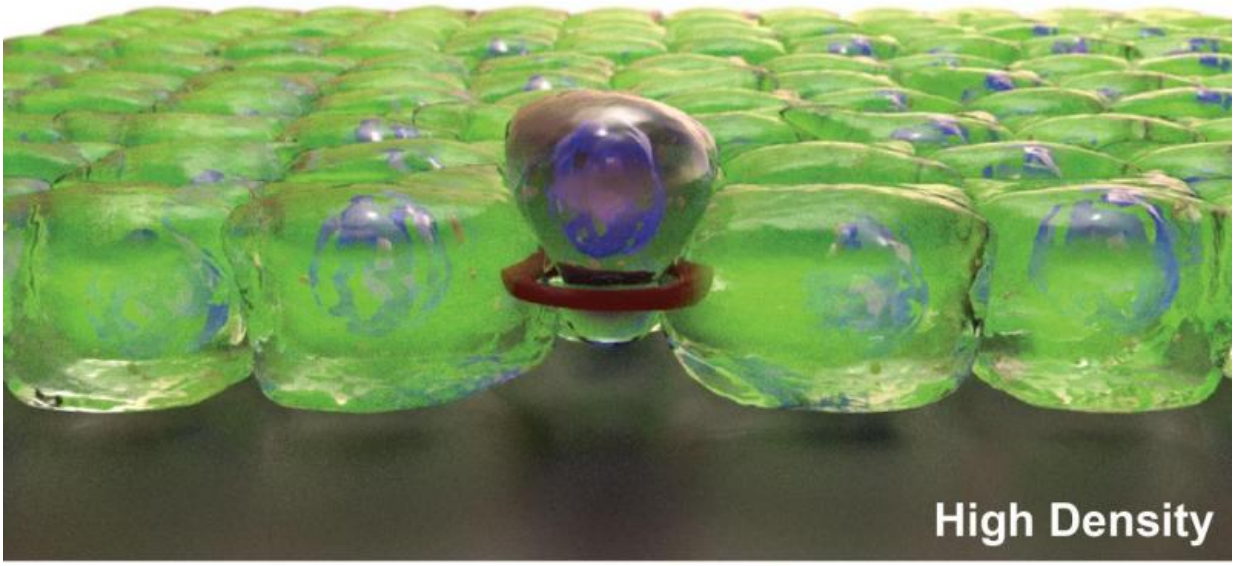

Low Density


High Density

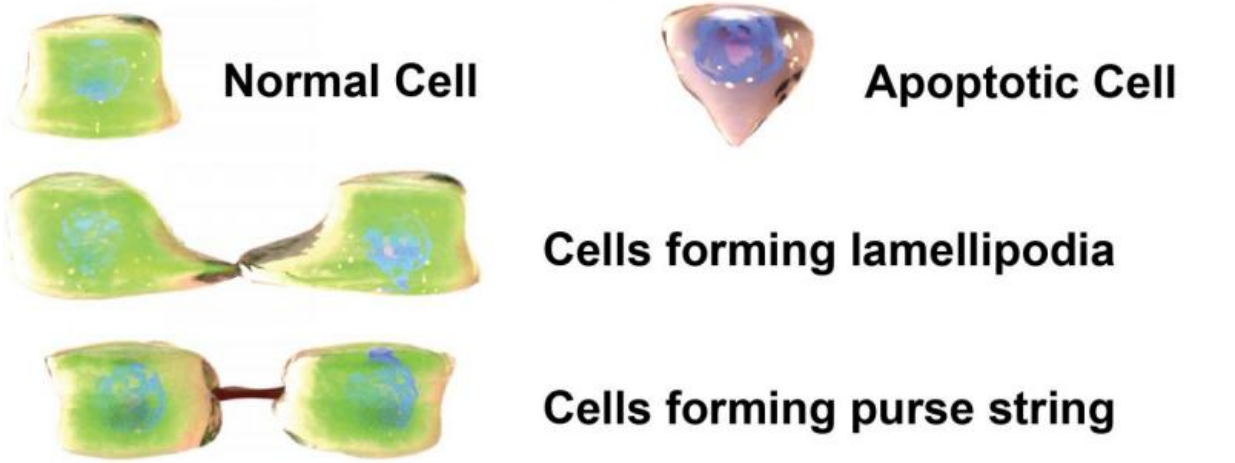

Normal Cell
Apoptotic Cell
Cells forming lamellipodia
Cells forming purse string

Figure: Cell extrusion mechanisms at different cell density. At low density, apoptotic cells are extruded by cell crawling and lamellipodia, while purse-string contraction drives extrusion at high density. Credit: National University of Singapore

An international collaboration has revealed how epithelial cell extrusion is regulated by cell density. The study was published in the scientific journal *Current Biology* on 5 October 2016.

How does cell density affect extrusion?

The external and internal surfaces of the body are covered by a layer of cells known as epithelial cell sheets. The classic example of an epithelial cell sheet is skin, but epithelial layers also line internal cavities such as blood vessels, the stomach, and the mouth. The primary role of these cell sheets is to provide a protective barrier against physical damage and infection. In order to perform these functions, the integrity of the epithelial cell sheet must be maintained by balancing cell renewal and removal. For example, the layer of cells lining the intestine is renewed every five days. Deteriorating, damaged, or unnecessary cells are targeted for elimination by apoptosis – the process of programmed cell death – allowing them to be eliminated without causing damage to the neighboring healthy cells, as would occur during inflammation.

Removal of these apoptotic cells from the epithelial cell sheet to maintain an intact barrier layer takes place by the process of cell extrusion. To date, studies have shown that epithelial cell extrusion occurs via formation of a contractile ring made up of protein based cables and motors in the cells surrounding the cell targeted for extrusion. The contractile ring tightens around the base of the extruding cell,

pushing it out of the epithelial sheet and bringing the surrounding cells together. Although this 'purse-string' mechanism of contraction is commonly seen in epithelial cell sheets, many of these observations have been based on the assumption that the epithelial layer is a collection of individual cells. However, in reality, these multi-cellular sheets are highly complex structures, with large variations in cell dynamics and cell density.

In order to account for this level of complexity, an interdisciplinary team of biologists, engineers, and biophysicists was assembled by Professor Benoit Ladoux from MBI and Institut Jacques Monod, and Assistant Professor Yusuke Toyama from MBI. The scientists used microfabrication to create circular micro-patterns surfaces that enabled control of the growth and density of epithelial cell sheets. By observing cell extrusion events in cell sheets grown on these patterns, with time-lapse and traction-force microscopy, they discovered that cell density led to two distinct modes of cell extrusion. At a low cell density, the cells in a tissue are dynamic and mobile. As these cells are moving freely, occasionally cell density becomes high in a small patch in the tissue. Cells at this dense region undergo apoptosis, and the cells surrounding the apoptotic cell selected for extrusion collectively crawl towards the targeted cell, and extend large, flat protrusions called lamellipodia underneath it. This action levers the apoptotic cell out of the sheet, causing its extrusion. However, at high density, cells are too tightly packed to move, preventing collective cell migration and lamellipodia-based extrusion. Under these conditions, the cells surrounding the apoptotic cell form a contractile ring, and use purse-string contraction to squeeze out and extrude the cell.

This study revealed, for the first time, that two distinct mechanisms exist to expel apoptotic cells from epithelial cell sheets. Selection between cell extrusion mechanisms is defined by cell density – cell crawling and lamellipodia extension is the predominant mechanism at low density, but

purse-string contraction is favoured at high density. The existence of these complementary mechanisms could be important for ensuring the removal of unnecessary cells (e.g. apoptotic cells) in different circumstances to maintain the integrity of the epithelial cell sheet.

More information: Leyla Kocgozlu et al. Epithelial Cell Packing Induces Distinct Modes of Cell Extrusions, *Current Biology* (2016). DOI: 10.1016/j.cub.2016.08.057

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