Cell movement patterns

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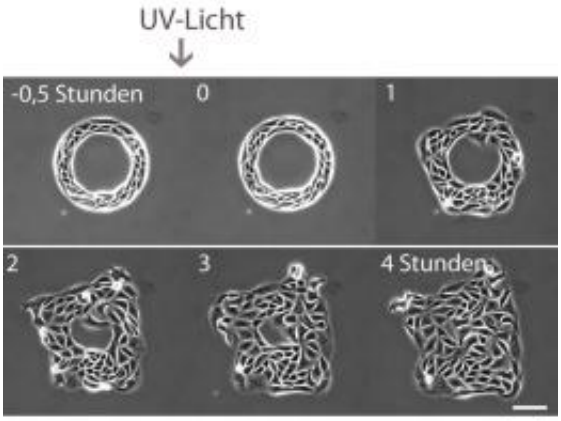

Cells on expansion course: the Stuttgart scientists initially only released a ring-shaped area of substrate, on which cells can grow, by irradiating a polymer layer with UV light through a corresponding ring-shaped photomask. Consequently, the cells initially formed a ring with a diameter of almost 300 micrometres. When the researchers released the remaining substrate from the polymer layer using a second UV pulse - indicated by the arrow - the cells spread in just a few hours. Credit: MPI for Intelligent Systems

(PhysOrg.com) -- Whereas a cut knee often reduces children to tears, adults are more likely to be distressed by the fear of cancer. In both cases, that is wound healing and the growth and spread of tumours, a particular characteristic of the body's cells plays a crucial role: their capacity to move in their tissue environment. Together with colleagues from Japan, scientists from the Max Planck Institute for Intelligent Systems in Stuttgart and the University of Heidelberg have developed a

very promising method for the study of cell movement. The new method enables the examination of the collective behavior of small groups of cells in an environment that imitates living tissue. Using this new method, the Stuttgart cooperative project was able to study the collective spreading behaviour of epithelial cells in the early stages of healing processes. The information gained from this study confirms the potential offered by the new method in generating new insights into cell migration, a process that has been under investigation for decades.

“Cell movement is interesting from a variety of perspectives,” says Ralf Kemkemer, “it plays a role in wound healing, of course, but also in the behavior of tumor and immune cells which often travel long distances to reach the lymph nodes.” The biophysicist is Group Leader at the department of Joachim Spatz, Director of the Max Planck Institute for Intelligent Systems. As part of an international cooperative project, doctoral student Claudio Rolli and Kemkemer have developed an amazingly simple method at the institute in Stuttgart. This new method enables researchers to study the collective movement behaviour of cell clusters in far greater detail than before, and provides scientists with a key to gaining a better understanding of these processes, which play a role in both healing and disease. As a consequence, medical research will also benefit from this development.

The first object studied by the Stuttgart scientists using the new method comprised small clusters of a few dozen epithelial cells. “These cells form the boundary surfaces in our body,” explains Kemkemer. They are located in the body’s many membranes, for example in our mucous membranes, in the very sensitive external surface of the eye, and they also line our organs. In case of injury, the epithelial cells, along with other cells, assume the task of closing wound openings. As part of this process, they start to move collectively and then reproduce through cell division.

The substrate for growing cells can be structured using light

The question as to how exactly this migration unfolds in the course of wound closure has long been the subject of research. However, it is still far from being completely understood, mainly because the methods available to investigate it up to now tended to be rather approximate. For decades now, scientists have been making cell clusters grow on a substrate that is supposed to resemble the natural environment. At the beginning of the experiment, they scratch a hole in the cell cluster using a sharp tip and then observe how the cluster reacts. However, this method is too “gross motoric” to achieve replicable and controllable test conditions. The findings obtained in this way are correspondingly imprecise.

The Stuttgart researchers have now developed a method, with which the cells are exposed to gentle light pulses instead of a sharp tip. Moreover, they do not produce relatively random scratched-out areas but can produce precisely defined and replicable areas and shapes. Thus the scientists can observe in detail how the cells behave on these geometries.

The key to this improvement is a completely newly developed substrate on which the cells grow. The properties of this material can be altered using light. Body cells need contact with each other and with the “fibrous” part of the tissue, the extracellular matrix. The substrate imitates important characteristics of the matrix so that the cluster of epithelial cells can attach to it and thrive on it. However, there is nothing new about this. What is new is the application of a layer of polyethyleneglycol (PEG) molecules, which cover the substrate and initially prevent contact with the cells. This layer can then be dissolved through brief irradiation with ultraviolet (UV) light which does not damage the living cells. Everything is transparent and can be viewed

under an optical microscope.

All cells move rapidly in very small clusters

The researchers begin by irradiating the slide with UV light through a photomask. In this way, they can generate circles, rings or areas shaped as required, on which the PEG molecules release the substrate. The cells can then seed in these areas – the scientists even produced a living version of the Max Planck Society's logo to demonstrate the potential offered by their method. The researchers then wait a few hours, an incubation period in which the cell clusters can organize themselves. Finally, they release the remaining PEG-molecule cover using a second, wide beam of UV light. The cells can then spread around their initial spot.

The Stuttgart scientists studied this behaviour using kidney epithelial cells, the "lab rat" of this kind of research for decades. They succeeded in observing for the first time, for example, that the size of a circular spot influences the migration behaviour of the cells. If it has a radius of up to 75 micrometers (millionths of a metre), all of the cells in the cluster move very fast. If, however, it is 104 micrometres or more in size, the inner cells tend to remain at a standstill. "All of the cells on the smaller island appear to sense the outer environment," concludes Kemkemer. The process resembles the behaviour of people in crowds: in a smaller group, the people standing on the inside still experience something of the surroundings outside the group and may react to them, however in bigger groups they are cut off from the outside.

The incubation period also influences the movement process. After only nine hours waiting time between the two light pulses, the cells move faster to the outside than they do after around 25 hours. "In the first case, they still tend to behave like individual cells," explains the biophysicist. "After longer periods they have formed a collective." The

geometry of the photomask used is also important. With ring-shaped masks, it emerged that the cells in the inner circle remain relatively immobile unlike those in the outer circle. The outer concave form clearly places the cells under greater mechanical tension and tends to incite the cells there to migrate more than those inside. Moreover, during migration, the cells organise themselves into “leader cells”, which, as pioneers, take to their pseudo-feet (lamellipodia) first, and into “follower” cells, the group that follows the leaders. It is not yet known what triggers this division of labor and the scientists would also like to investigate this phenomenon using the new method.

Ralf Kemkemer stresses that this research could only have been carried out through cooperation in an international team. The fact that Claudio Rolli had spent several months in Japan and was able to establish the invaluable contact with Jun Nakanishi’s team was crucial to the success of the project. The Japanese chemists were responsible for the important contribution of the trick with the PEG molecule.

More information: Claudio G. Rolli, et al., Switchable adhesive substrates: Revealing geometry dependence in collective cell behavior, *Biomaterials* March 2012; doi:10.1016/j.biomaterials.2011.12.012

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