Sewing wounds up: the epithelial morphogenesis as a central mechanism of *Leucosolenia complicata* (Porifera, Calcarea) regeneration

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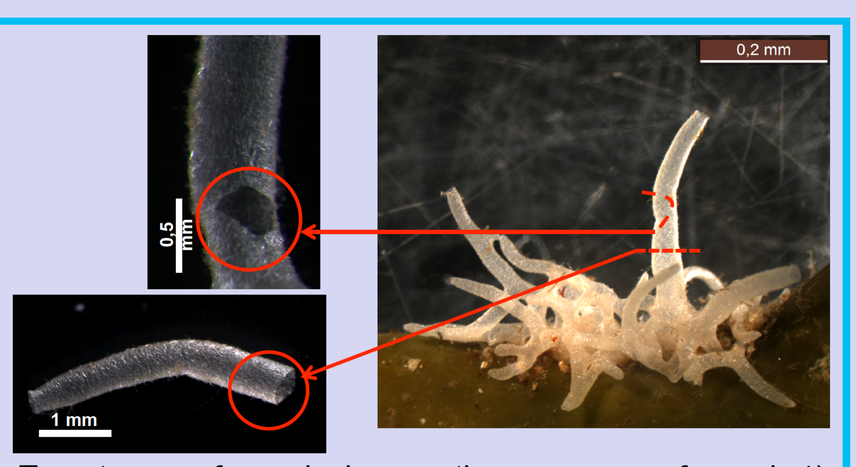
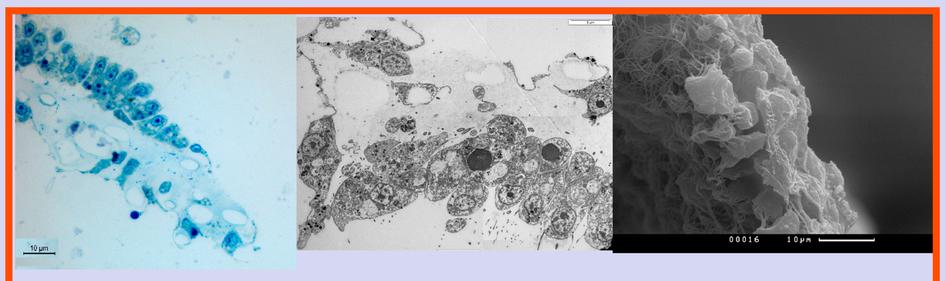
The ability to regenerate is widespread in the animal kingdom, but the regenerative capacities and mechanisms vary widely. To understand evolutionary history of the diverse regeneration mechanisms, regeneration processes must be studied in early evolved metazoans along with the traditional cnidarian and bilaterian models. For this purpose, we have combined several microscopy techniques to study cellular mechanisms of the regeneration in the calcareous sponge *Leucosolenia complicata*. The current study is aimed at revealing the cells and morphogenetic processes involved in this process.

Materials & Methods

Leucosolenia complicata is a common species in littoral habitats along the North European coasts, and is accessible throughout the year. The body of *L. complicata* composed of a basal reticulation of ascon tubes, from which erect oscular tubes. The cormus of *L. complicata* is always simple, without subdivisions or differentiation into regions with distinct functions. The body wall (thickness is 18-30 μm) has three layers: an outer layer is the exopinacoderm, a central region – the loose mesohyl, and an inner layer – the choanoderm. The cutting exposes choanocytes, the inner epithelial cells responsible for generating of the water movement and capturing of food particles.

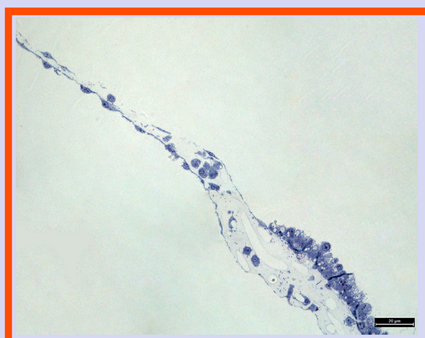


Two types of surgical operations were performed 1) excision of a small part of a body wall at the base of an oscular tube, and 2) amputation of oscular tubes. The observations and fixations were done at 3, 6, 12, 24, 48, 72, 96, 120 and 144 hours post operation (hpo).



Stage I – internal milieu isolation (wound healing)

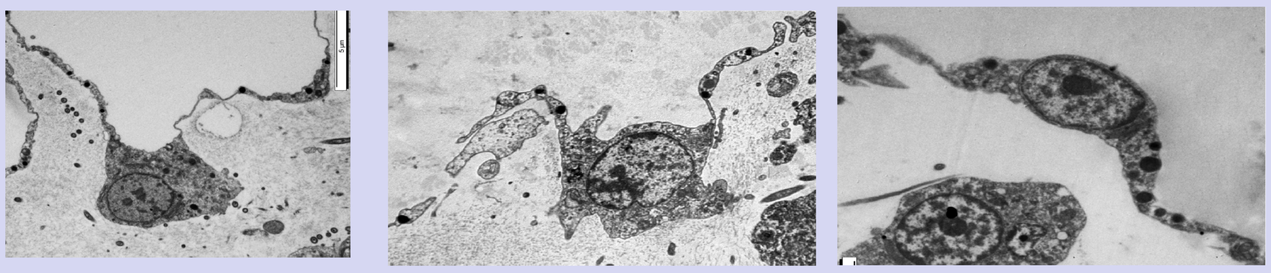
After 6 hpo the wound edges become almost smooth. The exopinacoderm, adjacent to the wound, spreads and bends over the open mesohyl, covering the edges and isolating the internal milieu of the sponge. Distal parts of the exopinacocytes produce very flat cytoplasm extensions, forming leading edges with pseudopodi that come into contact with the transdifferentiating choanocytes.



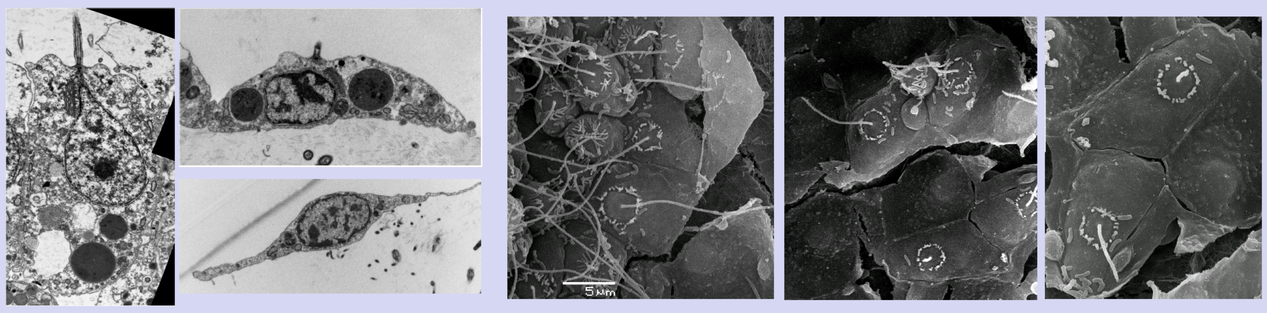
Regenerative membrane

Stage II – Formation of the regenerative membrane 12-24h

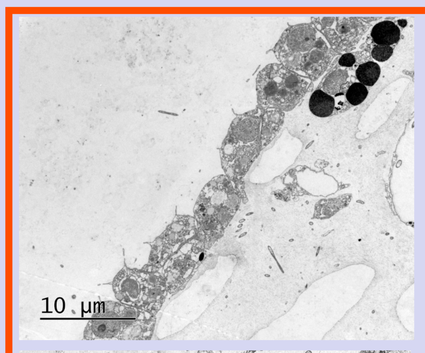
This membrane growths in the centripetal direction and gradually covers the wound orifice. The regenerative membrane consists of two cell layers: the external layer of the exopinacocytes and the internal layer of the endopinacocytes. The thin layer of the mesohyl is located in-between these pinacoderm layers. The regenerative membrane growths due to the spreading of the intact exopinacoderm and arising of the new endopinacoderm through the choanocyte transdifferentiation.

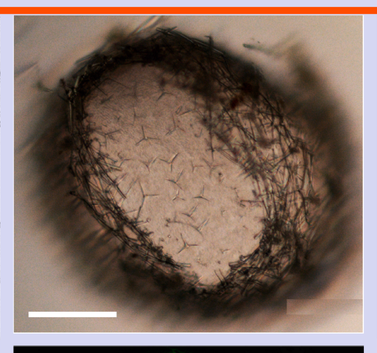


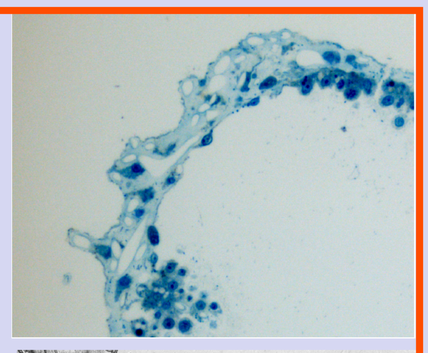
The exopinacoderm increases its surface and grows due to the transformation of the U-shaped into flat ones.



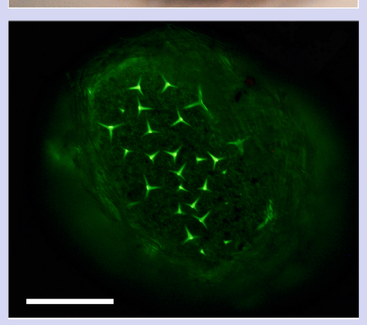
The choanocytes, adjacent to the wound, transdifferentiate into the endopinacocytes through the flattening and resorption of the flagellum and microvilli.



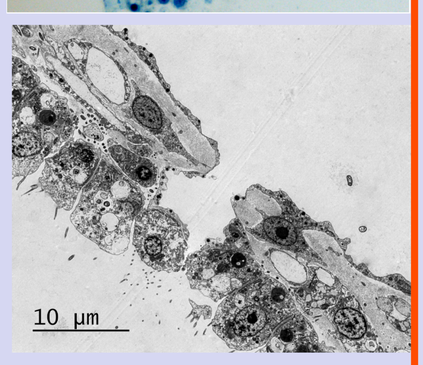




Mesohyl cells repopulate the regenrative mebrane



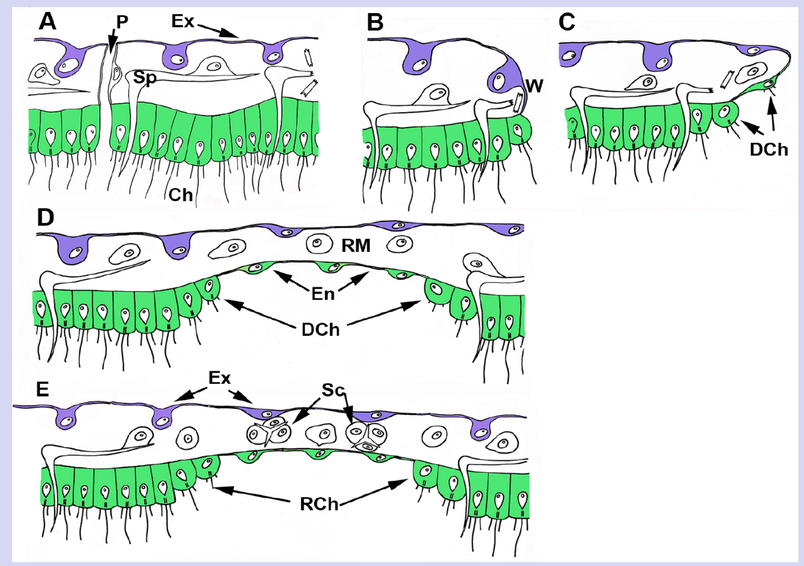
New spicules are synthezed in the regenerative membrane



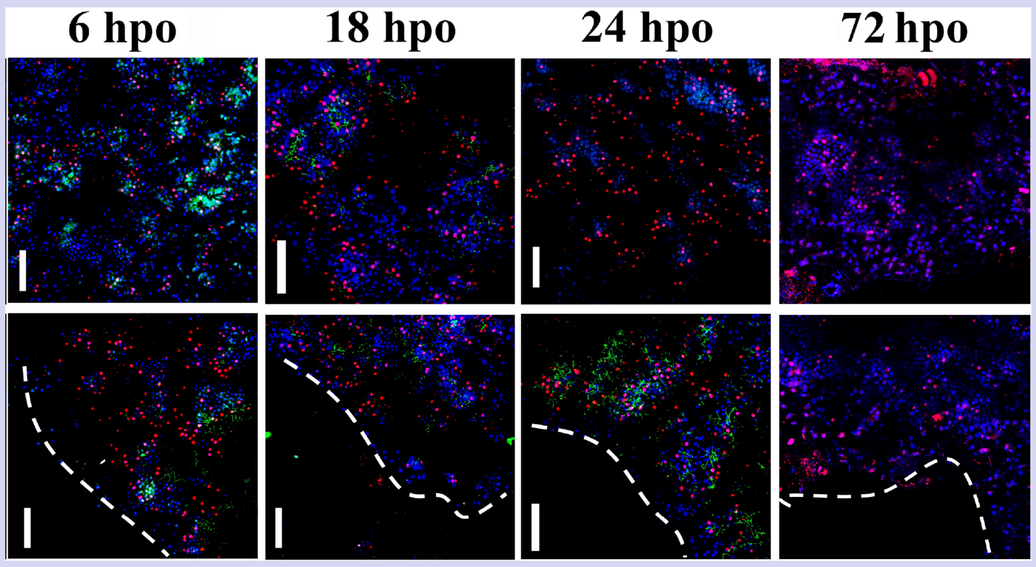
New porocytes develops by transdifferentiation of the exopinacocytes

Stage III – transformation of the regenerative membrane into normal body wall (48-144h)

After the complete formation, the regenerative membrane gradually transforms into the normal body wall. This transformation includes the restoration of the intact mesohyl cell composition, skeleton formation, porocyte development and choanoderm redifferentiation.



Diagrams of *Leucosolenia* regeneration. A – the intact sponge body wall. B – the internal milieu isolation. C – the beginning of regenerative membrane development. D – the wound orifice healing. E – the transformation of the regenerative membrane into the normal body wall.



Cell proliferation dynamics

The cells retain their proliferation activity during the body wall regeneration. The proliferation activity in the tissues distant from the wound, which should not be affected by the surgical procedures and subsequent regeneration processes, shows the same pattern with the tissues of intact sponge: they contain many EdU-labelled cells in the choanoderm. The tissues adjacent to the wound also demonstrate no changes in their proliferation activity.

Conclusion

The regeneration in *Leucosolenia* represents a rare example of the «true» morphallaxis. All processes occur solely through spreading and fusion of the epithelia surrounding the wound, accompanied by the transdifferentiation of the choanocytes and exopinacocytes. There are no changes in activity and distribution pattern in the proliferating cells, in both tissues, adjacent and distant from the wound. There are also no blastema nor any elements of mesenchymal-epithelial transitions. The transdifferentiation capacity of choanocytes and their involvement in regeneration supports the hypothesis that these cells combine features of somatic and stem cells.

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