REPARATIVE REGENERATION OF WHITE SEA SPONGE *LEUCOSOLENIA COMPLICATA* (PORIFERA, CALCAREA): MORPHOGENESIS AND CELLULAR SOURCES

A. Ereskovsky[1,2], A. Lavrov[3], F. Bolshakov[3]
[1]*Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille Université, CNRS, IRD, Marseille, France*
[2]*Dept. Embryology, Faculty of Biology, Saint-Petersburg State University, Russia*
[3]*Pertsov White Sea Biological Station, Biological Faculty, Lomonosov Moscow State University, Russia*
e-mail: aereskovsky@mail.ru

Introduction

The morphological and cellular plasticity of sponges (phylum Porifera) allows them to adapt to the variations in the environmental conditions. Therefore, they often dominate the benthic communities in the diverse marine and freshwater ecosystems from tropical to polar regions. The ecological success of the sponges is partially a result of their rapid regeneration capacity enabling them to recover from damages (Ayling, 1983; Luter et al., 2012; Wulff, 2013). Sponges are known to possess remarkable regenerative and reconstitutive abilities ranging from the wound healing or body part regeneration to re-building of a functional body from dissociated cells (Korotkova, 1997). Moreover, sponges show big diversity of the regeneration mechanisms (Ereskovsky et al., 2015, 2017; Borisenko et al., 2015; Lavrov, Kosevich, 2016).

The present study is aimed at revealing morphogenetic mechanisms and determining the cell types involved in the reparative regeneration in White Sea *Leucosolenia complicata* (Montagu, 1818) (class Calcarea) using *in vivo* light microscopy and histological studies.

Leucosolenia complicata is a common species in the littoral habitats along the North European coasts from the English Channel to the White Sea and is accessible throughout the year. It is an asconoid sponge with the body formed by the anastomosing hollow tubes with the internal cavities completely lined with choanocytes, and external surface covered with the layer of the flat and T-shaped exopinacocytes.

Materials and methods

The specimens of *L. complicata* were collected in the environs of Pertsov White Sea Biological Station of Moscow State University (Kandalakshsky Bay, White Sea) (66°34′ N, 33°08′ E) from the upper subtidal zone (0-2 m). The sponges were maintained in the 100 l aquarium with biological filters and natural sea water at temperature 6-10°C.

Two types of experiments on *L. complicata* regeneration were performed: 1) the regeneration of the body wall, and 2) the regeneration of the amputated oscular tube. Twenty-seven individuals were used in the body wall regeneration experiments. Twenty-seven oscular tubes obtained from nine individuals were used in the oscular tube regeneration experiments.

The staining with 100 mg/l calcein disodium salt solution was used for visualization of spicule synthesis in the regenerative membrane.

The fixation of the specimens for the histological studies by 2.5% glutaraldehyde on 0.2M Millonig's phosphate buffer (pH 7.4) were performed at 3, 6, 12, 18, 24, 36, 48, 72 and 96 hours post operation (hpo).

Twenty-four individuals with the excised body wall and three intact individuals were used in cell proliferation studies. The 5-Ethynyl-2′-deoxyuridine (EdU) was used as label for the proliferating cells.

Results

Regardless of the experiment type the wound healing can be subdivided into three stages according to the *in vivo* observations: 1) the alignment of the wound edges (0 – 6 hpo), 2) the regenerative membrane formation (6 – 24 hpo), and 3) the restoration of the body wall intact structure (4 – 5 days).

The complete wound healing occurs within 4 – 5 days post operation (dpo). The regeneration begins with the cleaning of the wound surface ending at 3 hpo. After 6 hpo the continuous epithelium appears on the wound edges. It is formed by joining of the intact exopinacocytes and endopinacocyte arising from intact choanocytes near the wound edges through their transdifferentiation.

After the alignment of wound edges, the development of regenerative membrane begins. The regenerative membrane grows from the periphery of the wound to its center, closing the hole in the body wall or opening in the basal part of the amputated oscular tube. This membrane completely closes wound at 24 hpo.

The epithelial morphogenesis plays the main role during the development of the regenerative membrane. It forms due to the convergent spreading and fusion of epithelial layers: the exopinacoderm on the external side of the membrane and the endopinacoderm – on the internal. The formation of the endopinacoderm occurs due to the transdifferentiation of the choanocytes near the wound edges through their flattening and losing of flagellum and microvilli collar.

Such a rapid formation of the regenerative membrane is associated with the necessity to restore the normal water flow through the aquiferous system of the sponge as soon as possible. The formation of the regenerative membrane quickly returns the process of water pumping to the normal mode.

After the full development of the regenerative membrane, the restoration of the intact structure of the body wall proceeds. By 36-48 hpo, the wound size decreases. By 72 hpo, the wound surface further decreases in size. The mesohyl of the regenerative membrane obtains intact structure – the mesohyl cells become more abundant and spaces between them occupy by ECM. At this stage the new spicules appear in the regenerative membrane. The calcein staining shows that these spicules are synthesized in the regenerative membrane.

Cells retain their proliferation activity during the whole time of the body wall regeneration. The proliferation activity of the cells in the tissues adjacent to the wound and distant from it does not change after the operation and shows the same pattern with the intact sponge tissues. Thus, the cell proliferation does not play significant role in the regeneration processes in *L. complicata*.

Conclusion

The epithelial morphogenesis, mainly spreading (flattening) and fusion of epithelial sheets is involved in the reparative regeneration in *L. complicata*. These processes are accompanied by transdifferentiation of choanocytes to endopinacocytes. Epithelial-mesenchymal transitions are absent during *L. complicata* regeneration. Therefore, regeneration in *Leucosolenia* has a mode in which lost body parts are replaced by the remodeling of the remaining tissue.

The epithelial nature of the regeneration in *Leucosolenia* makes it similar to the regeneration in homoscleromorphs sponges, which was described in detail in *Oscarella* (Ereskovsky et al. 2015), and Eumetazoans, like *Hydra*, and to the last phase of the regeneration in the triploblastic animals (Salvenmoser et al. 2001; Galliot, Ghila 2010).

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