A new approach for immunostaining nervous systems in isolated organs and whole animals

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Abstract.

The problem to which the claimed study is to improve the methods of immunostaining of large biological objects, including mammalian embryos, isolated whole organs, and marine invertebrates through the use of penetrating agents and detergents for the delivery of antibodies and their subsequent visualization after chemical clearance. The advantages of the proposed method are adaptability for different biological objects.

The aim of the present study was to improve the method of immunostaining biological objects up to 10 cm3 (up to 5x2x1 cm in length, width and depth, respectively) through the use of penetrating agents and chemical enlightenment in order to achieve optical transparency for confocal scanning microscopy.

Results

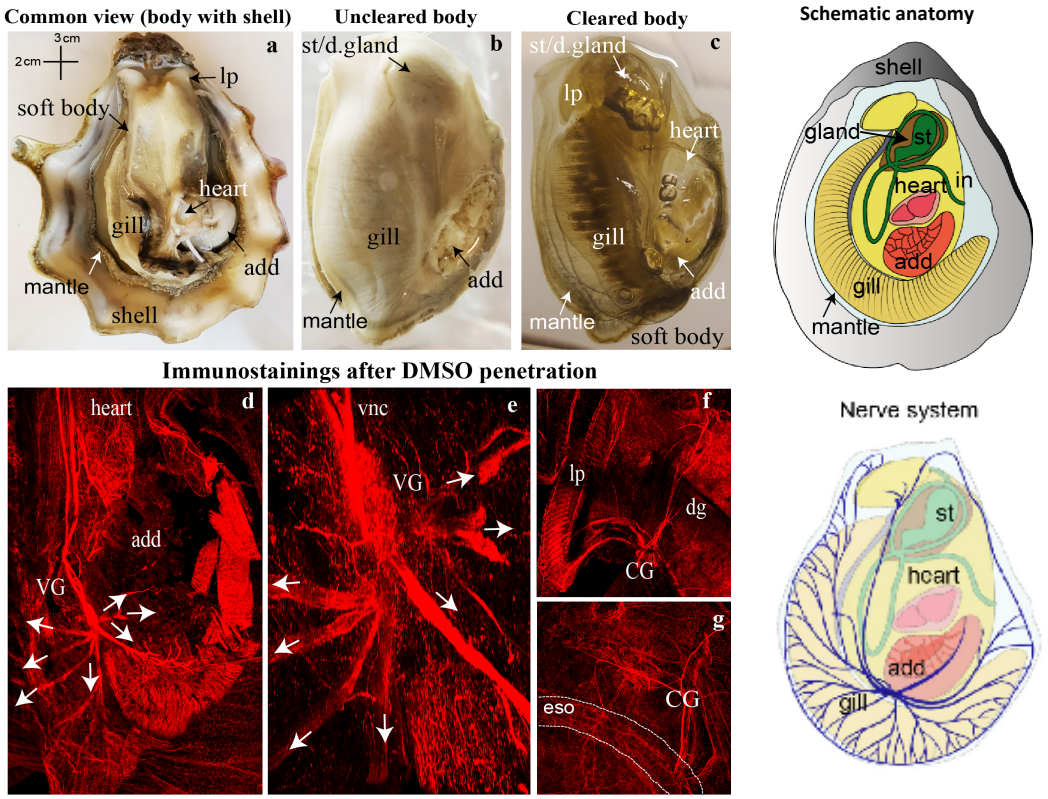

Figure.1. New method of identification inner morphological structures by DMSO penetration and BABB clearance. (a) common view of adult oyster *Crassostrea gigas* before experiments. (b) Unshelled oyster and external morphology. (c) cleared oyster soft body after BABB. (d-f) immunostaining by serotonin (5-HT) soft body of oyster after penetration by DMSO, confocal microscopy. Schematic anatomy and nervous system presented on right side. Abbreviations, lp, labial palps; add, adductor; st/d.gland, stomach/digestive gland; VG, visceral ganglion; vnc, ventral nerve cord; dg, digestive gland; CG, cerebral ganglion.

Novelty

This method allows to easily visualizing the deeper tissue structures of organs, embryos of vertebrates, and also, for the first time, the method is adapted for invertebrate animals. The method involves the use of DMSO penetrating agents, solutions of blocking and incubation with antibodies and chemical enlightenment using a mixture of benzyl alcohol and benzyl benzoate.

Conclusion: Developed protocol is a new tool for morphological studies and has cell resolution. This protocol have several variations depending on density, size and lipid composition of tissues, suitable for adult invertebrates, vertebrates.

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