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Commentary

Embryonic Cell Culture in Zebra Fish

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DESCRIPTION

The zebrafish is a model vertebrate becoming progressively important for several fields of biomedical research. The foremost advantage of zebrafish is the nearly perfect transparency of early zebrafish embryos which develop promptly outside the mother allowing for excellent *in vivo* time lapse imaging. In addition, manipulations of the zebrafish genome are somewhat easy to perform. In consequence, a wide range collection of transgenic reporter lines presents with cell type-specific expression of various fluorescent proteins. Thus, cell populations and even entire organs can be observed in an intact developing organism and specific cell types can be recognized and molecularly targeted by transgene expression. For example, fluorescent reporter lines have been effectively used to identify diverse scientific questions such as the identification of different neuronal subclasses in the embryonic zebrafish brain to recognize their development and function, microglial phagocytosis *in vivo* in complete brains of zebrafish larvae or the identification of radial glia cells as ancestors of distinct neuronal and glial cell types in the zebrafish spinal cord.

Although the initial zebrafish embryo is almost transparent, detectable light has a limited penetration depth and fluorescent emission is extremely dispersed when passing through tissue. This stops effective photon recovery from cells deep inside zebrafish larvae especially when small, sporadically labeled cellular structures are imaged. Moreover, due to a rapid embryogenesis, the arrangement of the different tissues is incessantly changing thus making observation of cellular structures stimulating. Even with highly progressive imaging systems, important details such as clear cellular morphologies, organelle dynamics or the subcellular localization of a zebrafish protein of interest in a particular cell type can sometimes be difficult to resolve. Further, the effect of defined biochemical causes on individual cell function, differentiation or behavior cannot be analyzed easily in whole bases.

Some of these questions can be studied either by using one of the few available zebrafish cell lines which are considered as deeply selected and adapted or by employing immortalized mammalian cell lines. Yet, these two methods may not reflect well the specific properties of a certain cell type of interest. Thus, it is preferable to use primary cells, which are cultivated directly from the embryo and preserve cell type-specific key features. Primary cell cultures resulting from zebrafish embryos are easy and cost-efficient to formulate and have been used in the past to analyze the growth of neurons or to screen for bioactive substances promoting cell differentiation. However, zebrafish primary cell cultures are still far from broadly used and their promising potential for live cell imaging on the cellular and subcellular level has still to be developed. One explanation for that could be the absence of reliable transfection protocols for primary zebrafish cells.

Here presented a fast and simple protocol for obtaining embryonic zebrafish primary cells. By exploiting the rich resource of cell type-specific fluorescent zebrafish reporter lines, different types of distinguished cells are cultured and checked in detail, representing that they maintain their original morphology in culture for several days, and the exact cell types can be enriched by flow cytometry prior to culturing.

CONCLUSION

In order to ease subcellular imaging, several fluorescent vital dyes are positively tested. Moreover, a robust electroporation protocol permitting is established for single, double and triple plasmid transfections of distinguished primary cells for the visualization of organelles by the expression of fluorescent marker proteins. By providing an in-depth protocol, the use of zebrafish primary cell culture as essential research resource for cell biological questions adding *in vivo* studies, thereby closing the gap between imaging of the whole zebrafish embryo and live cell imaging.

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