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TESTICULAR ORGANOIDS IN TELEOST FISH: CURRENT PROGRESS AND PERSPECTIVES

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During the last decades, several strategies have been developed to study spermatogenesis *in vitro*. However, most of the existing methods were not able to reproduce the cellular interactions as similar as in the native testis. Recently, the establishment of a culture system based on scaffolds would provide a 3D microenvironment to support testis cells growth and development, facilitating the *de novo* testis organogenesis and functioning. This artificial system, named as organoid, would permit a more detailed investigation of physiological testicular functions, including the interactions between germline stem cells (GSCs) and somatic cells, as well as mechanisms involved in male infertility (disease, ecotoxicology). Moreover, for species or individual with high genetic value, organoids would also allow the amplification of GSCs for the conservation of genetic resources and the regeneration of the cohorts of interest. Considering this background and the lack of testicular organoids in teleost fish, the current study aimed to develop a scaffold-based testis culture system in fish. The first approach consisted in developing an endogenous hydrogelscaffold from decellularized extracellular matrix (dECM) of rainbow trout immature testes. The second approach aimed to test different commercially available hydrogels (natural and synthetic) for establishing fish testicular organoids. In the first part, we successfully obtained dECM from immature trout testes without affecting its natural composition and structure, as shown by histochemistry, immunofluorescence, and scanning or transmission electron microscopy. These data open future possibilities to develop testicular organoids using a natural/endogenous scaffold. With regards to the second strategy, we showed that zebrafish testicular cells were able to form cell clusters when encapsulated into commercial hydrogels (Vitrogel or Matrigel). The use of testicular cells from different reporter lines demonstrated that these clusters were not derived from partial cell dissociation but were rather formed from spontaneous reaggregation of previously dissociated cells. The cell clusters encapsulated into Matrigel presented a more regular and round shaped morphology as compared to Vitrogel. We also observed that cell clusters changedin morphology, cell density and some exhibited bud formation during the culture period (19 days). Future studies will be required to further characterize the structure of these cell clusters and improve the culture medium to promote cell growth and *de novo* testis organogenesis.

Keywords: teleost fish; testicular organoids; stem cell; germinal niche; biotechnology

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