

October, 2016

Isolated proliferating cells with stem cell properties from adult tissue of poikilothermic animals, stable cell cultures thereof and method for their production

Key words: poikilothermic animals, cells, cell culture, head kidney, pylorus, gonads

Stable, long-term cell cultures from poikilothermic animals, especially from fishes, are important tools for research into animal diseases, virus research, developmental biology, physiology and phylogeny or as model systems for (eco)toxicology studies, pharmaceuticals or chemicals, just to name the most important topics. The cells may be cultured 2- or 3-dimensional and model systems may contain differentiated cells or stem/progenitor cells for different applications. Furthermore, cell cultures can also be used to produce biomass and used directly for in vitro meat, as surrogate for fish meal, to isolate fish oil or proteins with an amino acid composition typical for fish. However, in-vitro production systems can also be established from cell cultures of poikilothermic animals in order to produce, for example, cold-adapted enzymes or other substances that are naturally occurring in the source animal.

The Invention

The invention relates to a method for isolating adult, proliferating cells with stem cell properties, which can differentiate at least into cells from ectodermal and mesodermal germ layers. The cells shall be isolated from the head kidney, pyloric appendices or gonads (male or female) from poikilothermic animals, which can be fishes, amphibians or reptiles. The isolation includes the mechanical and enzymatic digestion of the aforementioned tissues in such a way that tissue aggregates smaller than 200 μm persist. The minced tissue is cultivated in a culture vessel under such conditions that differentiated cells will undergo apoptosis within few days, while the stem cells will adhere to the culture vessel. The non-adherent cells and left over tissue will be removed from the culture dish by medium exchange. The cells are cultured at temperatures beneath 37°C, preferably at room temperature, to save energy costs. The cells show high proliferation potential, also after cryoconservation. The invention further comprises cell cultures from the aforementioned cells, which do not contain a feeder layer and can be cultivated ideally for more than 100 passages. Cells may also be differentiated using a differentiation medium which does not contain additional growth or differentiation factors. All of the aforementioned cell cultures can be used for developing tissue-like or organ-like multicellular systems in vitro that can be used as model systems e.g. for chemicals, pharmaceuticals or as production systems for proteins, omega-3 fatty acids and other substances.

Market potential

- Model system for (eco)toxicology studies, pharmaceuticals or chemicals
- Substitute for animal feed, in vitro meat, as surrogate for fish meal, to isolate fish oil or proteins with an amino acid composition typical for fish
- Production system for cold-adapted enzymes

State of Development

The Fraunhofer EMB has established more than 80 long-term cell cultures from 17 different marine and fresh water fish species. The cultures are derived from different tissues or from whole larvae and stored under liquid nitrogen. Some of the cell cultures were passaged more than 100 times and were shown to differentiate into multiple cell types. First concepts of bioreactors for producing high amounts of biomass from adherently growing cells are already being tested and partially patented as well.

Branch

Basic research, veterinary medicine, toxicology studies, food industry, feed industry, pet food industry,

Patent situation

Patent granted (JP, DE)

Offer

Co-operation, Contract research, License, Sale

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