Development of an innovative two-chamber skin explant model for marine fish

Vercauteren Maaike[1], De Swaef Evelien[2], Devriese Lisa[3-4], Polet Hans[4], Decostere Annemie[4] and Chiers Koen[4]

[1] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
E-mail: maaike.vercauteren@ugent.be
[2] Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[3] Flanders Marine Institute (VLIZ), InnovOcean site, Wandelaarkaai 7, 8400 Ostend, Belgium
[4] Institute for Agricultural and Fisheries Research (ILVO), Ankerstraat 1, 8400 Ostend, Belgium

Since 2011 a remarkable increase in the prevalence of skin ulcerations in common dab (*Limanda limanda*) was noted in the Belgian part of the North Sea. Although the etiology remains largely unknown, we have identified two bacterial species, *Vibrio tapetis* and *Aeromonas salmonicida*, that are involved in the development of these lesions. Both bacteria are able to invade damaged skin and cause skin ulcerations. It is however likely that other factors, such as temperature, salinity, and even human activities such as fisheries can play a direct and/or indirect role in the development of skin ulcerations.

Until now, the involvement of both bacteria in the pathogenesis of skin ulcerations was confirmed via *in vivo* experiments using wild caught dab. However, alternative *in vitro* or *ex vivo* techniques, whereby cells, tissues or explants of animals are used for testing under controlled laboratory environments, could be considered to be ethically superior to an *in vivo* technique since they can remarkably reduce the number of experimental animals used and reduce the pain of the used animals. Moreover, the use of *in vitro* techniques often offer more precision in experimental assays whereby individual variability and unpredictability can be minimalized or eliminated.

In marine fish, studies with skin explants are challenging due to differences in salinity between their inner (body fluids, ± 0.9 PSU) and outer environment (seawater, ± 31 PSU). To mimic this *in vivo* situation, we have designed a two-chamber skin explant model. By mounting the skin explant in a 3D-printed apparatus made of bioplastic (Poly lactic acid; PLA), the epidermis can be exposed to a seawater environment in the upper chamber, and the underlying tissue can be exposed to the physiological fluids, provided in the lower chamber.

The model will be further developed and different steps will be undertaken starting with decontamination of the skin explants. The development will continue with testing the performance of the designed 3D-printed apparatus, with a focus on leakage of the apparatus and subsequent changes in the inner and/or outer medium. The performance of the skin explant will be studied with main focus on the viability of the tissue and plausible changes in the structure of the skin. This, hopefully, will lead to a maximal performance of both the apparatus and the skin explant itself.

To validate the model, we will use the pathogenic *Vibrio tapetis*. Results of an infection of the skin explants with this bacteria can be compared to results of the *in vivo* experiments performed previously by our research department. Importantly, the skin explants might behave differently outside their natural environment, therefore results will be interpreted with imperative caution.

The developed two-chamber skin explant model offers an opportunity to treat the skin locally in a small treatment spot, mimicking a certain environmental impact such as pollution, changes in environmental salinity and/or the presence of pathogens, and simultaneously keep the inner environment separate and stable. It therefore could be a useful alternative *in vitro* technique to study various factors compromising the health of the skin of marine fish.

Keywords: common dab (Limanda limanda); skin explant; in vitro; two chamber skin explant model