Zebrafish tools for the study of ectopic calcification disorders

Date: July 12-14, 2023

Location: University of Algarve, Campus of Gambelas, Faro, Portugal

Applications: Limited to 15 participants. Applicants should be enrolled in an institution from the INTEC network and have basic knowledge on biological/biomedical sciences. Participants must bring their personal laptops for this training school.

Register at https://www.ccmar.ualg.pt/en/webform/intec-zebrafish-workshop

Contact of the organizing committee: bioskel.lab@gmail.com

Application schedule: from 1st of May to 15th of June, 2023

Description of the TS:

1. Overview

Bone and ectopic calcification disorders affect millions of people worldwide and current treatments have limited efficacy often accompanied by side effects. The zebrafish have emerged as a model for the study of human bone and calcification-related diseases, but also as a system for the screening of compounds with the potential to treat or prevent these diseases. Beyond the technical advantages over traditional models (e.g. rodents), the high similarity of targets, physiology, drug metabolism and pharmacology in comparison to humans further highlight the potential of zebrafish as a robust model for drug screenings. The BIOSKEL lab (CCMAR, University of Algarve) has developed several zebrafish assays to evaluate the osteogenic potential of natural or synthetic compounds and molecules that will be focused in the scope of a workshop, providing the theoretical and practical knowledge required to go through all the steps of those procedures.

2. Objectives

This workshop aims at introducing the zebrafish system for research in skeletal formation and calcification disorders and providing advanced training to both academic and industrial staff on the use of specific zebrafish systems toward mineralization analysis and drug screening. It will also foster knowledge exchange between attendees and contribute to the establishment of scientific collaborations on bioprospection using zebrafish. Specifically, this workshop will transfer conceptual and practical known-how on the following topics:

- Basis of zebrafish biology and husbandry;
- Current knowledge on models for calcification disorders;
- Routes of compounds exposure in zebrafish;
- Imaging of calcified structures and morphometric analysis;
- Assessment of compounds with pro and anti-osteogenic as well as regeneration potential.

3. Planning

A 3-day program divided into a series of introductory lectures on selected topics and practical sessions involving manipulation of adults, larvae and embryos of zebrafish.

Day 1 – Background and hands-on on zebrafish biology	
Welcome reception **10:00**	Welcome reception and overview of the INTEC objectives *(M. Leonor Cancela, CCMAR-UALG; Olivier Vanakker, UGhent)*
Lecture 1 **10:15 - 11:00**	Basis of zebrafish biology and husbandry. *(Gil Martins, CCMAR)*
Coffee break (30 min)	
Lecture 2 **11:30 - 12:30**	Bone as a Model for the Uncoupling of Mineralisation and Matrix Formation *(P. Eckhard Witten, UGhent))*
Lunch break (1h30)	
Lecture 3 **14:00 - 15:00**	Fish skeletal biology and metabolism: cell types and mechanisms underlying bone formation and remodeling. *(Paulo Gavaia, CCMAR)*
Hands-on session 1 **15:00 – 17:30**	**Hands-on 1.1:** Visit to the zebrafish facilities **Hands-on 1.2:** Manipulation of zebrafish embryos and identification of embryonic stages using a bright-field stereomicroscope **Hands-on 1.3**: Manipulation of zebrafish larvae and adults: accommodation in different settings/plates and exposure to experimental compounds ***Coffee-break at 16:00 (30 min)***

Day 2 – Drug screening for osteogenic molecule using zebrafish based-systems	
Lecture 4 **09:30 – 10:15**	Fundamentals, principles and importance of drug screenings: Introduction on zebrafish screening systems *(Vincent Laizé, CCMAR)*
Lecture 5 **10:15 – 11:00**	Zebrafish as model for ectopic calcification disorders: the use of mutants and transgenic fish to unveil molecular and cellular mechanisms involved in pathologies with ectopic calcification. *(M. Leonor Cancela, CCMAR-UALG)*
Coffee break (30 min)	
Lecture 6 **11:30 – 12:30**	Using medaka as model for abnormal calcification pathologies (*Christoph Winkler, National University of Singapore*)
Lunch break (1h30)	

Hands-on session 2 **14:00 – 18:00**	Introduction to operculum screening system; ImageJ and macros **Hands-on 2.1:** Alizarin red staining of zebrafish larvae and visualization of calcified structures and warfarin-induced vascular calcification **Hands-on 2.2:** Imaging of the opercular bone using a fluorescence stereomicroscope. Visualise transgenic fish expressing fluorescent markers of different bone proteins **Hands-on 2.3:** Image analysis and morphometric assessment of zebrafish structures. ***Coffee break at 16:00 (30 min)***

Day 3 – Drug screening and regeneration: the caudal fin based-system	
Lecture 6 **10:00 – 11:00**	Basis of regeneration and regenerative medicine. Zebrafish caudal fin as biomedical tools for the screening of pro-osteogenic, pro-resorptive and regenerative molecules. *(João Cardeira, MPI-HLR)*
Coffee-break (30 min)	
Lecture 7 **11:30 – 12:30**	Basis of caudal fin imaging acquisition, image analysis and data processing regarding the osteogenic/regenerative potential of compounds. Pipeline of procedures and visualization of an explanatory video on caudal fin amputation. *(Marco Tarasco, MPI-HLR)*
Lunch break (1h30)	
Hands-on session 3 **14:00 – 17:30**	**Hands-on 3.1:** Exposure of regenerating caudal fin to experimental compounds and alizarin red staining **Hands-on 3.2:** Visualization of new bone structures and imaging of the newly formed lepidotrichia using a fluorescence stereomicroscope **Hands-on 3.3:** Image analysis and morphometric assessment of new bone mineralization and patterning ***Coffee break at 16:00 (30 min)***

4. Expected outcomes for trainees

Zebrafish tools presented in this workshop provide state-of-the-art information on how to use the zebrafish to study calcification disorders or for drug screening. Attendees will have the opportunity to experience the potential of the operculum and the caudal fin systems as screening tools for pro and anti-osteogenic effects and for regenerative capacity. Zebrafish first-line screening systems are at the first steps of drug discovery but represent a critical filter as a whole animal screening allowing to reduce the amount of compounds to be further tested and are therefore time and money saver. The workshop will provide both conceptual and experimental know-how on zebrafish screening systems for bone disorders. Attendees will have the opportunity to practice all the procedures from compound exposure to the assessment of the osteogenic effect. This workshop is expected to capacitate each attendee to reproduce these procedures at their home institutions.

5. Eligibility and selection criteria

Trainees will be selected from institutions from the INTEC network