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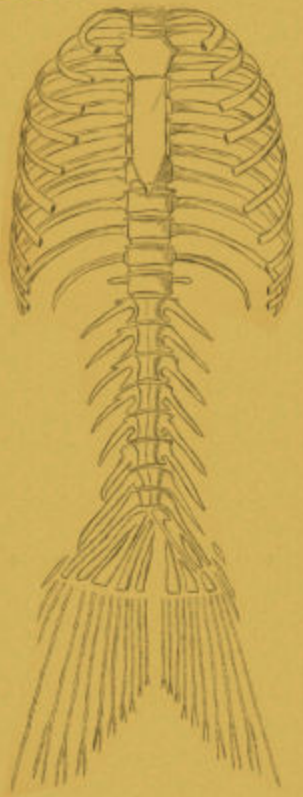

Performed at:

GHENT UNIVERSITY

Zebrafish Facility Ghent

FACULTY OF MEDICINE AND HEALTH SCIENCES

The zebrafish in biomedical research

A vertebrate model to study skeletal disorders

Public PhD defense to obtain the degree of 'Doctor in Health Sciences'

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23rd June 2021

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Summary

Due to the complex nature of skeletal development and related disorders, *in vitro* assays are fundamentally limited in clinical translational value. Animal models on the other hand have contributed significantly to the field of skeletal biology and are indispensable in the search for novel therapeutics.

In recent years, ethical and practical concerns with traditional mammalian model organisms have stimulated research into alternative models such as the zebrafish. In this thesis we first provide a detailed overview of zebrafish in skeletal research. This includes a description of the zebrafish skeletal system and the different techniques involved in laboratory experiments.

Next, we demonstrate the application of photoconvertible fluorescent proteins in skeletal imaging and advocate the benefits compared to the standard fluorescent proteins.

Subsequently, we demonstrate an application of photoconvertible fluorescent proteins in the description of a novel *lrp5* loss-of-function zebrafish osteoporosis mutant. We show that this mutant has the phenotypic and molecular hallmarks of human osteoporosis and can potentially be used in the discovery of new therapeutics.

In parallel we show that F0 somatic zebrafish mutants, called crispants copy both the skeletal and molecular phenotype of the *lrp5* loss-of-function mutant. This demonstrates the application of crispants in reverse genetic screening of osteoporosis (and potential other) candidate genes.

Finally we provide a detailed construction guide for the *Zebrafish exercise-tunnel*, a system we specifically designed for forced swimming experiments. In the future, this system will be used to study the impact of exercise on skeletal development and health.

Samenvatting

De complexiteit van botontwikkeling en gerelateerde aandoeningen zorgt ervoor dat *in vitro* onderzoek fundamenteel gelimiteerd is in klinisch translationeel onderzoek. Significante vorderingen of het gebied van bot biologie en gerelateerde therapieën zijn juist gemaakt door het gebruik van proefdieren.

Recent hebben ethische en praktische overwegingen in het gebruik van traditionele proefdieren ervoor gezorgd dat alternatieve diermodellen zoals zebravis populair zijn geworden. In deze thesis geven we een gedetaileerd overzicht van zebravis skeletaal onderzoek. Initieel geven we een overzicht van het zebravis skeletale systeem en de technieken die in het laboratorium gebruikt worden.

Daarna demonstreren we het gebruik van foto-converteerbare fluorescente eiwitten in skelet microscopie en laten we de voordelen zien ten opzichte van standaard fluorescente eiwitten.

Vervolgens passen we dit toe in de beschrijving van een nieuw *lrp5* loss-of-function zebravis osteoporose model. Deze mutant heeft de zelfde fenotypische en moleculaire kenmerken als patiënten en kan gebruikt worden om nieuwe geneesmiddelen te vinden.

In parallel laten we zien dat somatische F0 zebravis mutanten, genaam crispanten, dezelfde skeletale en moleculaire fenotypes hebben als de *lrp5* loss-of-functie mutanten. Dit demonstreert dat crispanten gebruikt kunnen worden om osteoporose candidaat genen relatief snel te testen.

Als laatste geven we een uitgebreide handleiding voor de constructie van de *Zebrafish exercise-tunnel*. Dit systeem is speciaal ontworpen voor zwem-experimenten die in de toekomst gebruikt zullen worden om de invloed van beweging op het skelet te onderzoeken.

Publications included in this thesis:

Photoconvertible fluorescent proteins: a versatile tool in zebrafish skeletal imaging. (2020)
Jan Willem Bek, Adelbert De Clercq, Hanna De Saffel, Mieke Soenens, Ann Huysseune, Paul E. Witten, Paul J. Coucke, Andy Willaert.
Journal of Fish Biology
doi: 10.1111/jfb.14335

The ZE-Tunnel: An Affordable, Easy-to-Assemble, and User-Friendly Benchtop Zebrafish Swim Tunnel. (2021)
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Zebrafish
doi: 10.1089/zeb.2020.1948

Zebrafish: A Resourceful Vertebrate Model to Investigate Skeletal Disorders. (2020)
Francesca Tonelli†, **Jan Willem Bek†**, Roberta Besio†, Adelbert De Clercq†, Laura Leoni, Phil Salmon, Paul J. Coucke, Andy Willaert‡ and Antonella Forlino‡.
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Frontiers in Endocrinology
doi: 10.3389/fendo.2020.00489

Lrp5 mutant and crispant zebrafish faithfully model human osteoporosis, establishing the zebrafish as a platform for CRISPR-based functional screening of osteoporosis candidate genes. (2021)
Jan Willem Bek, Chen Shochat, Adelbert De Clercq, Hanna De Saffel, Annekatrien Boel, Juriaan Metz, Frans Rodenburg, David Karasik, Andy Willaert, Paul J. Coucke.
Journal of Bone and Mineral Research
doi: 10.1002/jbmr.4327