Analysis of the development and regeneration of the killifish heart

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Key Words:
killifish
heart regeneration
heart developemnt

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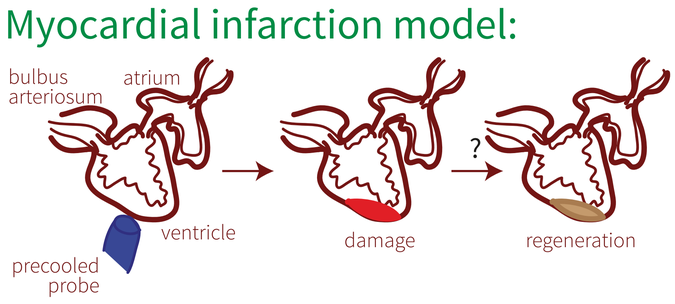
Introduction

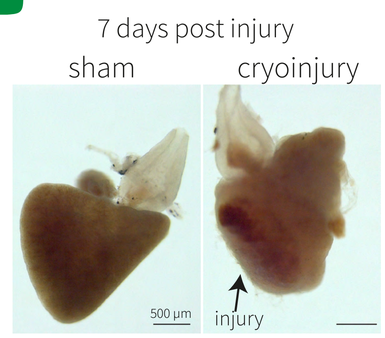
Humans suffer from age-related diseases when tissue homeostasis gradually declines. It is therefore of great interest to understand molecular mechanisms underlying regeneration processes to potentially improve regenerative strategies in humans. Regeneration of the killifish heart post resection has been reported (Wang *et al*., Science, 2020).

The **aim of** this project is therefore to analyze how age impacts the regenerative capacity of the fish heart, using *Nothobranchius furzeri* as a model. To this end, we established the cryoinjury method for adult *N. furzeri* to study heart regeneration after myocardial infarction and we generated a genetic cell ablation model to study the heart *in vivo*.

1 Myocardial infarction

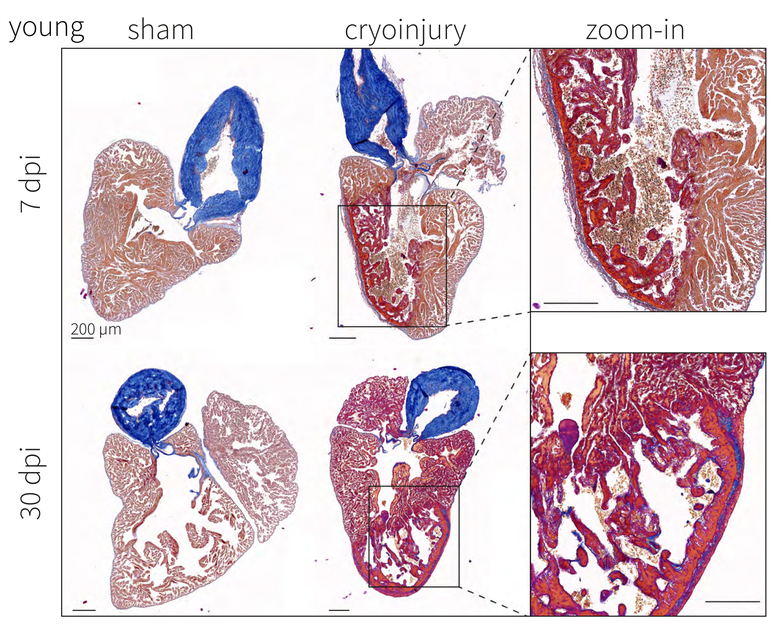
Myocardial infarction model:



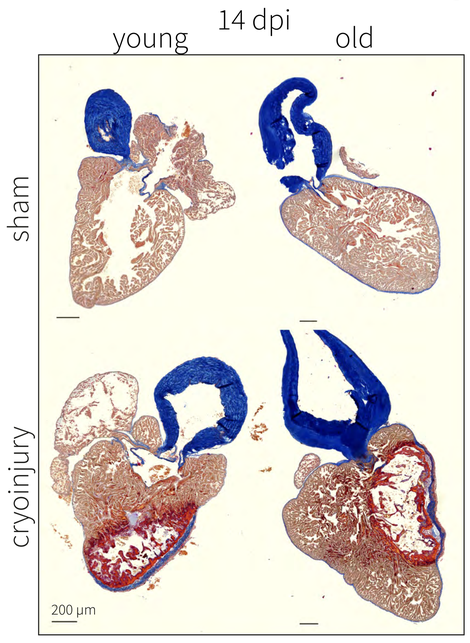


2 Response to cryoinjury in *N. furzeri*

Acid Fuchsin Orange G staining
Blue: collagen Red: fibrin Orange: muscle



young: 11-12 weeks post hatching
old: 46 weeks post hatching

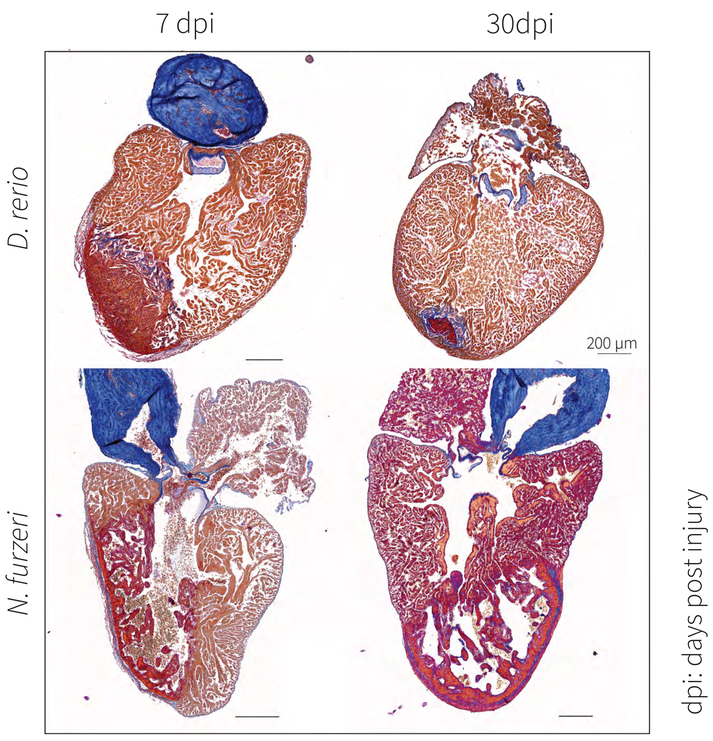


Cryoinjury method was established for *N. furzeri*. Injuries were still present at 30 dpi. Injury size was similar in young and old hearts at 14 dpi

dpi: days post injury

3 Comparison to zebrafish

Acid Fuchsin Orange G staining
Blue: collagen Red: fibrin Orange: muscle

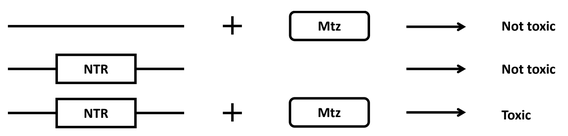


dpi: days post injury

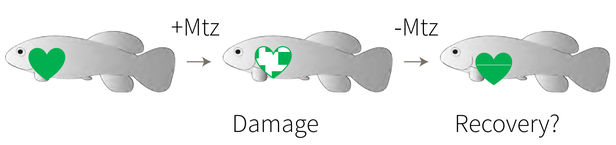
While the wound decreases in size by 30 dpi in *Danio rerio*, it is still prominent in *Nothobranchius furzeri*.

4 NTR-System

Directed cell ablation
The Metronidazol-Nitroreductase-System:



Generation of a transgenic line:
- in the transparent *klara* background
- heart specific ***myl7*** promoter
- expressing NTR
- GFP reporter



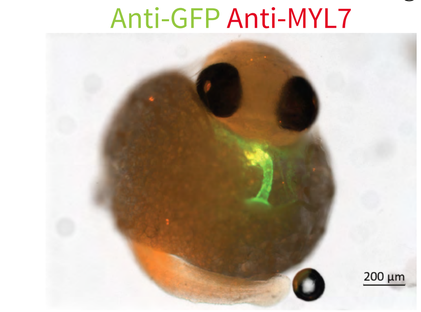
In combination with a heart specific promoter, the Mtz-NTR-system can be used as a non invasive injury method.

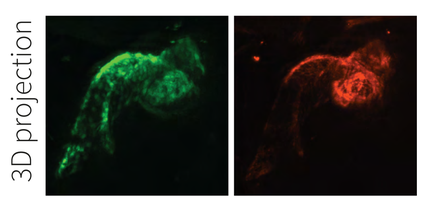
5 Heart development in *N. furzeri*

dpc: days post egg collection

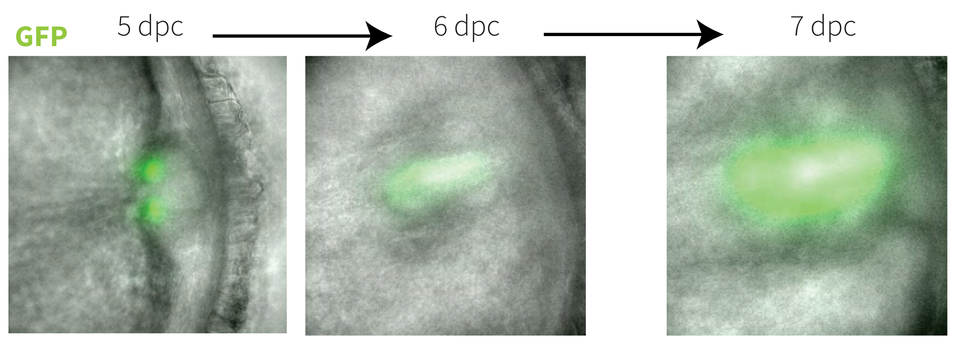
klara-Tg(myl7:NTR-mCherry;myl7:GFP)

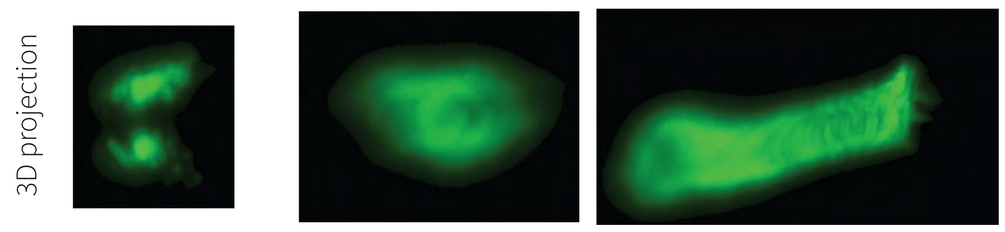
Whole mount immunostaining
Anti-GFP Anti-MYL7





live imaging - lightsheet microscopy





The newly generated transgenic line cannot only be used for regeneration studies, but also for analysing the development of the killifish heart *in vivo* by light sheet microscopy.

Summary

Establishement of a myocardial infarction model in *N. furzeri*

Reduced or delayed heart regeneration in comparison to zebrafish

Accumulation of collagen with age

Generation of a transgenic line for non-invasive *in vivo* regeneration analyses

Embryonic heart development: two heart fields that merge, elongate to form a tube, that finally bends and balloons to form the chambered heart.

Outlook

- Comparative analysis of the response to myocardial infarction in *D. rerio* and *N. furzeri.*
- Ablation studies with Mtz using different age groups to study heart regeneration *in vivo.*

Acknowledgements:

This project was started in a collaboration with M. Dalvoy and G. Weidinger (Ulm University).

Englert Lab

Leibniz Association

Fish facility | Imaging facility | Histology service