Analysing the effect of Hedgehog on zebrafish tail regeneration

Harrison Vasudeva
Department of Biomedical Sciences
Supervisor: Dr. Henry H. Roehl

Introduction

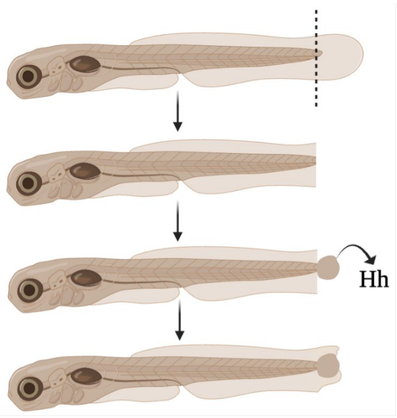


Figure 1: The formation of the notochord bead in zebrafish tail regeneration.

Regeneration is the process by which an organism regenerates damaged or lost tissue. Zebrafish serve as a suitable model for vertebrate regeneration as their regenerative capacity is far less limited compared to humans. In larval zebrafish, tail regeneration is characterised by the protrusion of the notochord, forming a ball of cells known as the **notochord bead** (Roehl, 2018) [Figure 1]. **Hedgehog** (Hh) is a protein that is expressed in the notochord bead, and has been shown to be **required** for zebrafish regeneration after the notochord bead has formed (Romero *et al*., 2018). It is yet to be determined whether Hh is **sufficient** to induce regeneration, which is the purpose of this project. Zebrafish kept in isotonic solution have been shown to have reduced regenerative capacity compared to hypotonic solution (Cheung, 2019) [Figure 2], and this provided a suitable condition to study whether Hh has an inductive role in larval zebrafish tail regeneration. By studying model organisms such as zebrafish, it may be possible to identify the molecular machinery that underpins their regeneration and then use this knowledge to enhance the regenerative capacity of humans.

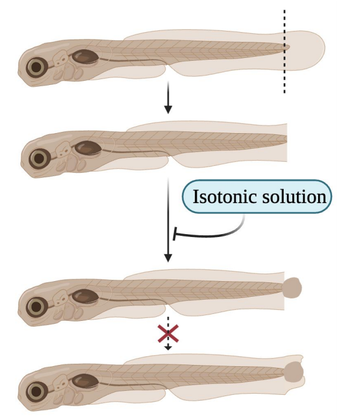


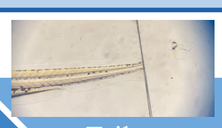
Figure 2: Isotonic solution prevents the formation of the notochord bead after tail amputation.

Methods

Isotonic experiment:

Larval fish collected at 72hpf.

Fish were placed in hypotonic or isotonic media with or without Hh [0.4mg/ml]. Hypotonic/Hh- provided a negative control, isotonic/Hh- provided a positive control.



Tails were cut at the ventral melanophore gap, and fixed at 8 or 24 hours post amputation (hpa).

A hybridisation chain reaction was carried out for *ptch1*, a target gene of Hh. Fish were then imaged for their morphology and expression of *ptch1*.

Bead experiment:

Larval fish collected at 24hpf.

Fish were then implanted with Hh- beads or Hh+ beads (beads submerged in 9.0mg/ml Hh solution).

Fish were left at RT for 19 hours.

Fish were imaged for their morphology only.

Results

Isotonic experiment

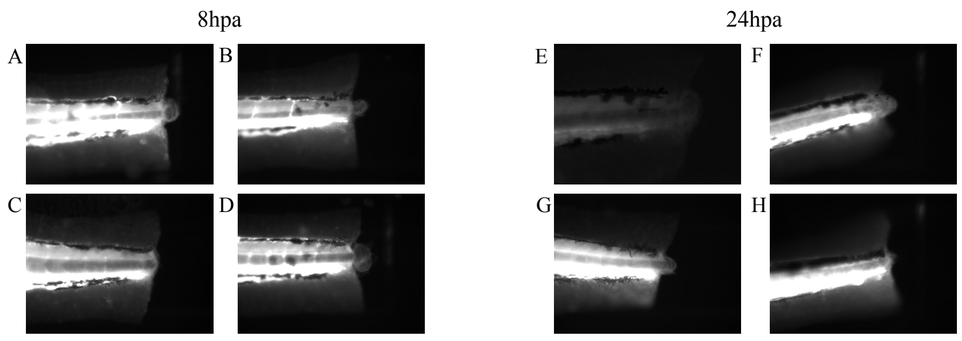


Figure 3: ***Ptch1* expression in zebrafish tails 8hpa (A-D) and 24hpa (E-H)**. Fish were cut in either hypotonic/Hh- (A/E), hypotonic/Hh+ (B/F), isotonic/Hh- (C/G) or isotonic/Hh+ (D/H) conditions (n≥ 7 for each condition 8hpa, n≥ 2 for each condition 24hpa. Shown 1 fish per condition). Magnification: 90x.

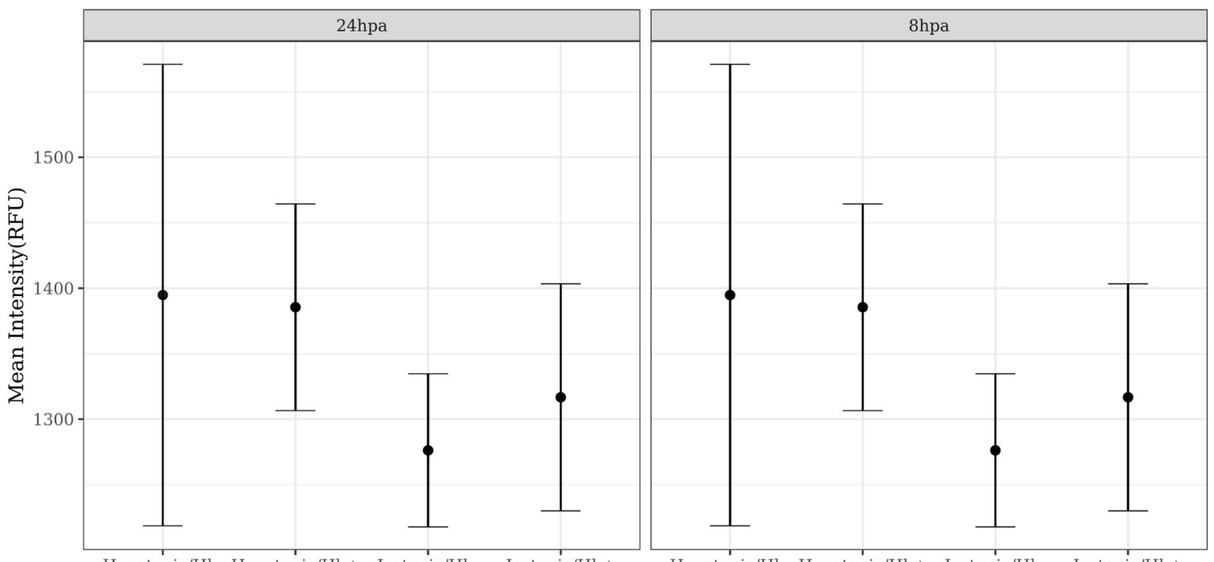


Figure 4: **Mean fluorescence between each condition at 8 and 24hpa.** Error bars represent the standard error of mean. At both time points, a **decrease in fluorescence can be seen** from both hypotonic conditions to the isotonic/Hh- condition, followed by a **partial restoration** in fluorescence in the isotonic/Hh+ condition. A two-way ANOVA test was used to analyse the effect of Hh and media (hypotonic/isotonic) on mean fluorescence at 8 and 24hpa. At both 8/24hpa, **there was not a significance difference** from: (A) The interaction between the effects of Hh and isotonic solution ($F=0.064$, $p=0.801$ and $F=0.839$, $p = 0.384$ respectively), (B) The effect of isotonic compared to hypotonic solution alone ($F = 0.053$, $p = 0.066$ and $F = 0.898$, $p = 0.368$ respectively) and (C) The effect of Hh alone ($F = 0.009$, $p = 0.925$ and $F = 0.790$, $p = 0.397$ respectively).

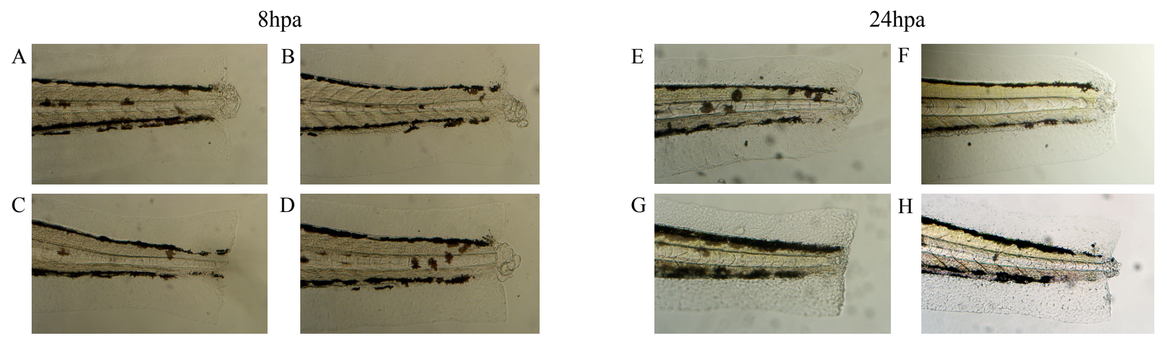


Figure 5: **The morphology of zebrafish tails 8hpa (A-D) and 24hpa (E-H)**. Fish were cut in either hypotonic/Hh- (A/E), hypotonic/Hh+ (B/F), isotonic/Hh- (C/G) or isotonic/Hh+ (D/H) conditions (n≥ 7 for each conditions 8hpa, n≥ 2 for each condition 24hpa. Shown 1 fish per condition). Magnification: 100x. 100% of fish in conditions A and B, 30% of fish in condition C and 100% of fish in condition D formed a notochord bead. 100% of fish in conditions E and F, 20% of fish in condition G and 60% of fish in condition H formed a notochord bead. The mean notochord bead size did not vary significantly between treatment A and B (Welch's $t = 0.922$, d.f. = 14.2, $p = 0.372$), despite being 116.3% larger in condition F than E. Epithelial migration in the fin fold increased 35.2% in condition F compared to condition E. Notochord beads appear to have altered morphology in conditions B and D compared to condition A, which may reflect differing cell adhesion upon Hh treatment.

Bead experiment

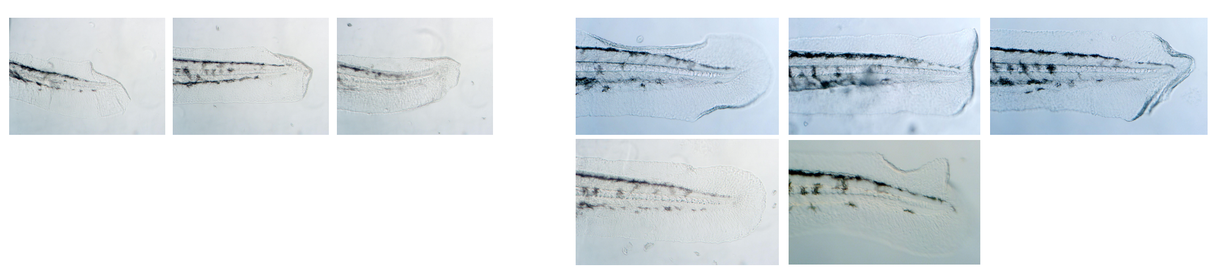


Figure 6: **Images of zebrafish 19 hours post bead implantation**. Two of five fish implanted with Hh+ beads showed a clear loss of material in the fin fold. The significance of this is unclear, as, although no material was lost, all 3 control fish that were implanted with Hh- beads had fin fold defects. Magnification: 100x.

Conclusions and future work

- The restoration of the notochord bead upon Hh treatment at 8/24hpa in isotonic solution supports **the hypothesis that Hh has an inductive role** in zebrafish tail regeneration. This may be indicative of an early role for Hh in tail regeneration, in addition to the role after notochord bead formation seen in the literature. Future studies are needed to explore this possibility further.
- This hypothesis is also supported by increased epithelial migration in the fin fold at 24hpa, indicating **accelerated regeneration**.
- **No significant difference** was found in *ptch1* expression upon Hh treatment. More research at the molecular level, such as single cell RNA-seq, is needed to explain the **notochord bead recovery** in isotonic solution, the **increased fin fold epithelial migration** in hypotonic solution and the **abnormal notochord bead structure** upon Hh treatment.
- The results of the bead experiment were **inconclusive**, due to low sample size and defects in control fish. However, a phenotype as extreme as large losses of material should be researched further. Repeating this experiment using a time-lapse and/or staining for *ptch1* may provide a greater insight into how this material is lost.

References

1. Roehl, H.H 2018 'Linking wound response and inflammation to regeneration in the zebrafish larval fin', *International Journal of Developmental Biology*, 62 (6-8), 473-477. doi: https://doi.org/10.1387/ijdb.170331hr.
2. Romero, MMG. *et al*. 2018 'Damage-induced reactive oxygen species enable zebrafish tail regeneration by repositioning of Hedgehog expressing cells', *Nat Commun*, 9(1), 4010. doi: 10.1038/s41467-018-06460-2.
3. Cheung K.Y. 2019 'Understanding the Role of Wound-Induced Calcium and Reactive Oxygen Species During Zebrafish Larval Tail Regeneration', Master's thesis, University of Sheffield, Sheffield.