In vivo magnetic resonance microimaging in adult zebrafish

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Introduction

The zebrafish (*Danio rerio*) is an important model organism for the study of vertebrate biology [1]. However, optical *in vivo* studies in zebrafish are restricted to very early developmental stages due to opaqueness of the juvenile and the adult stages [2]. Application of high resolution *in vivo* Magnetic resonance microimaging (µMRI) has not yet been explored in adult zebrafish. Being a small aquatic animals zebrafish requires special setup and several precautions for supporting *in vivo* imaging. In the present study we optimized the MR setup and µMRI sequences to visualized high resolution structural details in adult zebrafish. In addition to ex vivo studies, a flow through setup has been designed for *in vivo* µMRI studies of adult zebrafish and first results of *in vivo* µMRI are presented.

Methods

For *in vivo* µMRI measurements, fish was anesthetised by adding 0.001% MS222 (ethyl meta aminobenzoate metanesulfonic acid salt) to pH controlled water. Subsequently fish was transferred to a closed flow-through chamber, which was specially designed to support living zebrafish inside the magnet. The flow-through setup was then inserted in the centre of the volume coil (1 cm diameter, 4 cm length) inside the microimaging probe, which was then inserted in the bore of the vertical MR magnet. Aerated water with anaesthetic was pumped from a temperature controlled aquarium to a tube fixed on lower end of the flow-through cell, which was close to the mouth of the fish. After passing the chamber the water was transported back to the aquarium. After the MRI measurements, zebrafish recovered uneventfully from the experimental treatment. MR images were acquired using a 9.4-T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. A series of coronal and sagittal T_2-weighted images were acquired using the rapid acquisition with relaxation enhancement (RARE) sequence. The settings used were , TE = 10.5 (22.5 ms effective), TR =1000 ms, RARE factor (echo train length) = 4 and averages = 2. An in-plane resolution of 78 x 78 µm was achieved with slice thickness of 500 µm in an acquisition time as low as 4 minutes.

Results and Discussion

Although *in vivo* MRI has become an approved tool in medicine and pharmacological research, very few studies use this method to uncover physiological issues in aquatic organisms [3]. In this study we applied and optimized high resolution µMRI methods to examine anatomical structures non-invasively in adult zebrafish. Fig. 1 depicts image slices of fixed adult zebrafish in sagittal (Fig. 1A) and coronal planes (Fig. 1B) obtained using the RARE sequence at 9.4T. A small flow-through chamber, designed to support imaging of living zebrafish, is shown in figure 2A. The chamber could be fitted into a cylindrical resonator for a homogeneous excitation profile (Fig. 2B). Clear morphological proton images were obtained using RARE sequences revealing many anatomical details in living zebrafish in a short time (4 minutes) (Fig. 2C).

Conclusion

Our results show that high field µMRI provides sufficient resolution to get rapid anatomical details in adult zebrafish *ex vivo* as well as *in vivo*. This study pave the way for applying high resolution µMRI for *in vivo* studies in adult zebrafish for analyzing disease development, biological pathways, toxicological mechanisms and possible drug screening during various developmental stages in same living zebrafish non-invasively.

References:

Van der Sar and Bitter et al. Trends Microbiol 12 (2004); (2) Freidlin and Basse et al. Proc Intl Soc Magn Med 11 (2004).
(3) Van der linden and Bock et al. Magn Reson Mat Physics Biol Med 17 (2004).

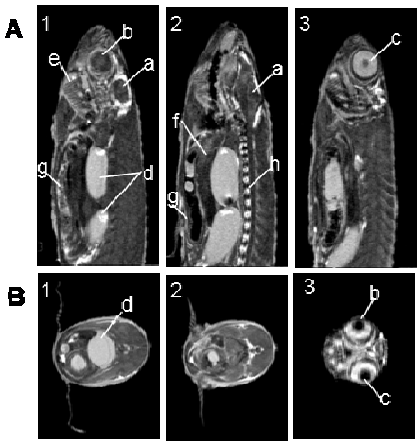

Fig. 1: High resolution images of adult zebrafish at 9.4T. Successive Slices (1-3) in sagittal (A), and coronal (B) planes. The image resolution is 78 µm. Slice thickness 0.2 mm. (a) brain; (b) left eye; (c) right eye; (d) swim bladder; (e) gills; (f) ovary; (g) intestine; (h) horizontal myoseptum; (i) heart.

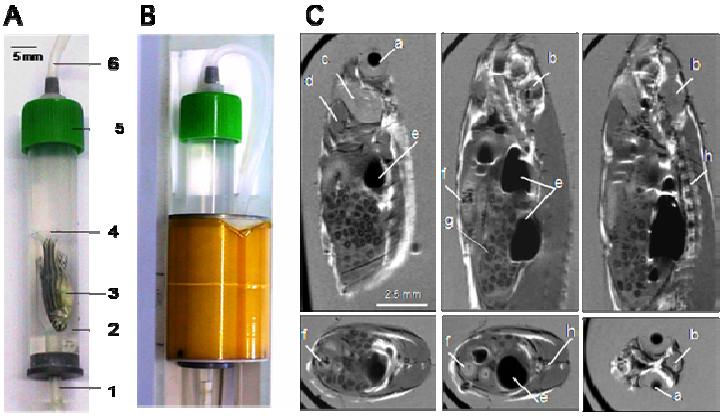

Fig 2: (A) Design of flow-through chamber for in vivo MRM measurements of living adult zebrafish. 1, water inlet; 2, U-shaped PVC chamber; 3, a specimen of adult zebrafish; 4, a variable slide barrier to fit the size of the fish with a hole at the bottom; 5, chamber closet; 6, water outlet. (B) Flow-through chamber fitted into the volume coil of microimaging probe. (C) MRI images of anaesthetized living adult zebrafish obtained at 9.4T. Slices in sagittal (upper row) and coronal (lower row) planes. The image resolution is 78 µm. Slice thickness 0.5 mm. (a) eye; (b) brain; (c) gills; (d) heart; (e) swim bladder; (f) intestine; (g) eggs; (h) horizontal myoseptum.