High-field MR microscopy as a tool for comparative morphological studies: soft tissue discrimination in sea urchins

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Introduction

Palaeontological, morphological, and molecular characters are used to unravel the evolutionary development (also termed phylogeny) of animal groups. Sea urchins (Echinoidea), like sea stars or sea cucumbers marine spiny-skinned animals (Echinodermata), represent one of the few animal groups where extensive character sets are available for phylogenetic inferences, including a recently published whole genome sequence for one species (Sodergren et al. 2006). However, the morphological data is characterized by an almost entire absence of soft tissue characters. This is due to problems that are connected with the application of classical histological techniques: sectioning irretrievably destroys and alters the specimen, dissolving of calcite produces large amounts of carbon dioxide that further alter the object, and mechanical stress exerted by the microtome blade causes tissue compression and deformation. A recent study (Ziegler & Angenstein 2007) has shown that *in vitro* analyses of sea urchins are well feasible using MRI. However, the resolution achieved at 4.7 T was only partly satisfactory. Therefore, the purpose of our study was to generate high-resolution MRI data suitable for 3D visualization and a direct comparison with classical overview histology. Another aim was to show that MRI can give rapid access to morphological data from several species, some of them available only as valuable museum specimens, for comparative studies.

Material & Methods

The sea urchin specimens were fixed in a 37% formaldehyde solution and subsequently transferred to a 7% formaldehyde solution for imaging. Spines were dressed for tight fit inside the tubes. MRI measurements at 7 T were performed on a Bruker PharmaScan 70/16 equipped with a 38 mm inner diameter volume resonator and a 300 mT/m gradient system. 3D MR data sets were acquired with a resolution of (117 µm)^3 (TR/TE = 30/6 ms). For MRI at 17.6 T, Magnevist (Schering) was added at a concentration of 2 mM. MRI was performed on a Bruker 750 MHz wide bore spectrometer equipped with a 1 T/m gradient system. 3D gradient echo data sets were recorded with resolutions of (44 µm)^3 (TR/TE = 20/3.0 ms) and 20x18^2 µm^3 (TR/TE = 20/3.3 ms) using bird cage resonators with 20 mm and 5 mm inner diameter, respectively. 3D reconstruction and 3D visualization were performed by converting the 3D data sets into TIFF files using ImageJ 1.36b. The TIFF picture series were employed for segmentation using the brush tool, the blow tool, and the 4-viewer mode in the Amira 3.0.2 (Mercury Computer Systems) Segmentation Editor. Organ designation and detection of borderlines were carried out based on Strenger (1973) and our own histological and previously acquired MRI data.

Results & Discussion

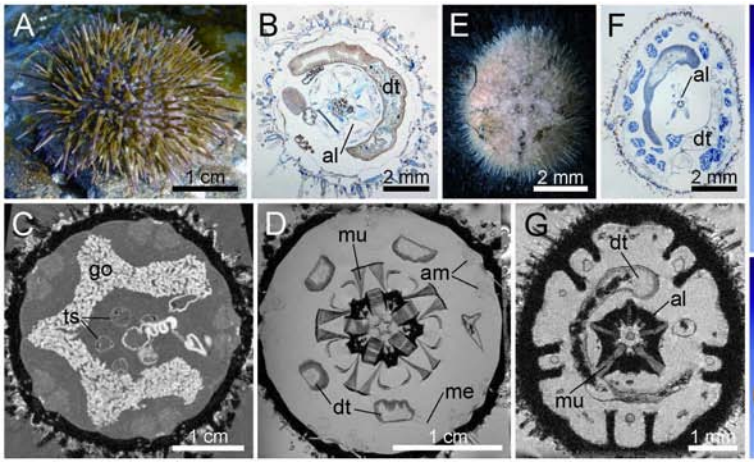
The localization of internal and external organs of the two exemplary species is shown in the figure below. The major internal structures that can be recognized using high-resolution MRI include: muscles, digestive tract, gonads, calcified structures, mesenteries, Aristotle´s lantern, ambulacral system, coelomic compartments, and the axial complex. The mesenteric suspensions of all digestive tract constituents and the outlines of the ampullae are visible, as well as all hard tissue, including the calcite endoskeleton and the calcified components of the Aristotle´s lantern. Several structures that cannot be seen with preparative techniques and that are being destroyed during histological preparation, such as the coelomic sacs of the lantern teeth, can easily be discerned using MRI. The 3D reconstructions of the digestive tracts show that substantial anatomical differences can be observed between the two different species.

Conclusion & Outlook

Instead of applying laborious conventional histological methods, we generated morphological data resembling overview histology in quality and so permitting comparative studies in sea urchins. High-resolution MRI allows for a rapid, non-invasive, and unbiased approach, since the subjective element of image alignment, an essential component of 3D reconstruction from histological sections, can be omitted. In addition, the data is present in digital form from the beginning. MRI can thus substantially complement classical histological data and should be applied in cases where large numbers of specimens need to be screened, e.g. in the course of analyses of sea urchin development or comparative morphological studies. Furthermore, the possibility to rapidly display the data in 3D reconstructions facilitates an understanding of the extremely complex echinoid anatomy and serves as a powerful tool in our effort to add new information to the soft tissue character list for sea urchin phylogeny.

References

Sodergren E et al. (Sea Urchin Genome Sequencing Consortium), Science 314, 941 (2006); Strenger A, *S. granularis*, Gr Zool Pr (1973); Ziegler A & Angenstein F, Mikrokosmos 96 (6), 1 (2007).



A: Adult specimen of *Psammechinus miliaris* (Müller, 1771). **B**: Image of an overview histological section of **A** at the height of digestive tract and Aristotle´s lantern. **C**: 7 T MRI image of **A** at the height of gonads and tooth sacs, (117 µm)^2 resolution. **D**: 17.6 T MRI image of **A** taken at the height of Aristotle´s lantern, (44 µm)^2 resolution. **E**: Adult specimen of *Echinocyamus pusillus* (Müller, 1776). **F**: Image of an overview histological section of **E** at the height of digestive tract and Aristotle´s lantern. **G**: 17.6 T MRI image of **E** taken at the height of Aristotle´s lantern and digestive tract, (18 µm)^2 resolution. **H**: 3D visualization of the digestive tract of **A** - a slightly angled aboral view. **J**: 3D visualization of the digestive tract of **E** - a slightly angled aboral view. **al** = Aristotle´s lantern, **am** = ampullae, **dt** = digestive tract, **go** = gonads, **me** = mesentery, **mu** = muscle, **ts** = tooth sacs.