3-Dimensional modelling of chick embryo eye development and growth using high resolution magnetic resonance imaging

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Magnetic resonance imaging (MRI) is a powerful tool for generating 3-dimensional structural and functional image data. MRI has already proven valuable in creating atlases of mouse and quail development. Here, we have exploited high resolution MRI to determine the parameters necessary to acquire images of the chick embryo eye. Using a 9.4 Tesla (400 MHz) high field ultra-shielded and refrigerated magnet (Bruker), MRI was carried out on paraformaldehyde-fixed chick embryos or heads at E4, E6, E8, and E10. Image data were processed using established and custom packages (MRICro, ImageJ, ParaVision, Bruker and mri3Dx). Voxel dimensions ranged from 62.5 μm to 117.2 μm. We subsequently used the images obtained from the MRI data in order to make precise measurements of chick embryo eye surface area, volume and axial length from E4 to E10. MRI was validated for accurate sizing of ocular tissue features by direct comparison with previously published literature. Furthermore, we demonstrate the utility of high resolution MRI for making accurate measurements of morphological changes due to experimental manipulation of chick eye development, thereby facilitating a better understanding of the effects on chick embryo eye development and growth of such manipulations. Chondroitin sulphate or heparin were microinjected into the vitreous cavity of the right eyes of each of 3 embryos at E5. At E10, embryos were fixed and various eye parameters (volume, surface area, axial length and equatorial diameter) were determined using MRI and normalised with respect to the un-injected left eyes. Statistically significant alterations in eye volume (p < 0.05; increases with chondroitin sulphate and decreases with heparin) and changes in vitreous homogeneity were observed in embryos following microinjection of glycosaminoglycans. Furthermore, in the heparin-injected eyes, significant disturbances at the vitreo-retinal boundary were observed as well as retinal folding and detachment confirming histological observations. These data reveal the utility and superiority of MRI for producing images enabling quantification of experimentally induced changes in eye volume and structure. The results indicate that MRI is an important tool that could become a routine approach for rapid and sensitive phenotypic analysis of normal chick ocular development and morphology as well as potentially the effects of surgical or genetic manipulations of chick embryo eyes in live embryos in ovo.

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1. Introduction

The chicken (Gallus gallus) embryo is a classic developmental system, which, owing to the large size of the embryonic eye, has been employed extensively for studying the development and growth of the eye (Coulombre, 1965, 1969). In the course of these studies, the lens was shown to be pivotal for the development of the eye (Coulombre and Coulombre, 1964; Coulombre and Herrmann, 1965; Piatigorsky, 1981; Wride, 1996; Beebe and Coats,
studies using high resolution MRI to study morphogenesis. The consequent combination of classical embryological manipulation and modern genomics means that the chick embryo is emerging as an extremely useful model for characterising the gene networks that influence development and growth of the eye as well pathologies such as myopia (Schaafel, 2006; Schippert et al., 2006; McGlenn et al., 2007). In particular, the chicken allows for the mapping and identification of quantitative trait loci (QTLs) controlling normal ocular component dimensions in inbred strains or, for example, during form deprivation myopia (Guggenheim et al., 2002).

Therefore, there is a pressing need to develop methodologies that will provide for rapid and sensitive analyses of ocular structural morphology during development, growth and disease. The traditional approach involves histological analysis, but although this provides high spatial resolution, 3-dimensional (3D) visualisation of structures cannot be easily achieved and the unavoidable artefacts of tissue processing complicate the interpretation of data. In contrast, magnetic resonance imaging (MRI) represents a powerful tool for generating high resolution structural and functional image data in three dimensions, allowing the intact specimen to be viewed interactively and analyzed in any plane, either in vivo or postmortem.

MRI is a mature and diverse science that has been widely reviewed (e.g. Potter et al., 1995; Tyszka et al., 2005; Li et al., 2007). Applied to imaging of the eye, MRI offers a useful variety of imaging contrasts (Potter et al., 1995). In particular, the contrast of T1-weighted and T2-weighted images is dramatically different.

MRI has been used for visualisation of human embryo development (Smith, 1999, 2000; Shiota et al., 2007), including cerebellar development (Triulzi et al., 2006) and to analyse developmental anatomy in a number of model systems, including the mouse, rat and non-human primates (Schneider and Bhattacharya, 2004; Ahrens et al., 2006; Drieuhsy et al., 2008). Furthermore, MRI has been used to create an atlas of mouse embryonic development (Dhenain et al., 2001), to investigate cardiac development (Smith, 2001), to characterise cardiac malformations in connexin43 mutant mouse embryos (Wadhiri et al., 2007) and in phosphatidylyserine receptor (Ptdsr) deficient embryos (Schneider et al., 2004), and to analyse pre-gastrulation movements leading to formation of the dorsal marginal zone during Xenopus development (Papan et al., 2007).

High resolution MRI has been utilised to image avian embryos (Effmann et al., 1988; Hogers et al., 2001; Kulesa, 2004), the chick embryo vasculature (Smith et al., 1992) and phenotypic changes following chick limb manipulation (Li et al., 2007). Imaging of chick development is also possible non-invasively from E12 through to hatching (Bain et al., 2007). Recently, an MRI atlas of quail embryonic development has been produced (Ruffins et al., 2007) (http://atlasserv.caltech.edu/Quail/Start_Quail.html).

MRI is also being employed for investigating ocular anatomy in foetal human eyes and during human eye growth, ageing and disease. For example, age-related changes in human ciliary muscle and lens have been determined using MRI (Strenk et al., 1999) as have the effects of age and accommodation on human lens cross sectional area (Strenk et al., 2004). Recently, MRI has been a useful diagnostic tool to determine imaging features of congenital optic nerve head coloboma (Righini et al., 2008) and for analysis of the 3D shape of the human eye (Singh et al., 2006). Finally, MRI has been used recently for the detection of ocular abnormalities in human foetal eyes (Robinson et al., 2008).

Thus, the work described here is a logical addition to other studies using high resolution MRI to study morphogenesis. However, to the best of our knowledge, this is the first study to use MRI to examine chick embryonic eye development. Although, the parameters used in the study by Li et al. (2007) allowed for generation of images of the chick embryo eye, the focus of their report was primarily on the limb. Here, using fixed samples, we have determined optimal parameters for producing MRI-generated images of the embryonic chick eye. Furthermore, we have adapted and developed various software packages for measuring several parameters of eye growth during chick embryo development: internal eye volume, surface area, axial length, and lens height and have used these techniques to examine changes in eye structure and volume following experimental manipulation of embryos in ovo.

2. Materials and methods

2.1. Preparation of chick embryos and eyes for MRI

Fertile white leghorn hen eggs (Hen Stewart and Co. Ltd., Lincolnshire, UK) were incubated at 37.8 °C for 4, 6, 8 or 10 days in a digital tabletop incubator with humidity control (Brinsea Products Ltd., Standford, UK). At the appropriate stage of development, eggs were cooled at 4 °C. Embryos (or heads) were then carefully removed from their eggs and, once the hearts had stopped beating, placed in 4% paraformaldehyde in phosphate buffered saline (PBS) at pH 7.4 for 24 h. The fixed embryos were then washed in PBS and distilled water (three times each for 5 min each at room temperature) and dried with filter paper. Embryos (or heads) were placed in 15 ml plastic tubes (Corning) and immersed in liquid 1% molecular biology grade agarose (Sigma–Aldrich Co. Ltd., Gillingham, Dorset, UK; diluted in distilled water). The embryos were aligned consistently with the head down. Using the appropriate software to rotate the images later compensated for minor deviations from this alignment. Once the agarose had solidified, the tubes were trimmed to within 5 mm of the specimen and the ends sealed with Parafilm. Embedded specimens were stored at 4 °C in a sealed, humid Tupperware container until scanned by MRI.

2.2. MRI

MRI was carried out at the Experimental MRI Centre (EMRIC) in the School of Biosciences Cardiff University (http://www.cardiff.ac.uk/biosi/researchsites/emric/facilities.html) using a Bruker BioSpec 94/20 small-animal scanner operating at 9.4 Tesla (400 MHz for 1H). The system is built around an Ultra-Shielded and Refrigerated (USR) magnet with an inner bore of 20 cm and uses Avance II electronics controlled from a Linux console equipped with Paravision 4.0. The specimen was placed within a 72 mm coil. A transmit-receive quadrature coil was used for the E10 and E8 specimens with foam packing added to fit them centrally within the coil. The E4 and E6 specimens involved the use of a linear coil for RF transmission as well as an additional surface coil for receiving the RF signal. The coil was then tuned, via the RF signal, in order to homogenise the magnetic field once again, as the addition of the sample within the coil creates an inhomogeneous environment in which the signal is poorer and more susceptible to artefacts. A tri-pilot scan was then performed, which set up the sample at the centre of the magnet using visual markers to gain maximum magnetic field energy and to maintain homogeneity. The field of view was then set in all three directions (x, y and z). The parameters for each scan of each embryo/head are provided in Table 1. Images were obtained with a mixture of T1 and T2 weighting, which had been optimised to reveal the fluid filled chambers of the developing eye as well as the lens.
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Table 1
Parameters employed for each MRI scan carried out.

<table>
<thead>
<tr>
<th>Embryo</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>TEeff (ms)</th>
<th>Matrix Size (pixels)</th>
<th>Field of View (cm)</th>
<th>Number of averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st scan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4 (whole)</td>
<td>2400</td>
<td>37.5</td>
<td>75</td>
<td>128 × 256 × 256</td>
<td>0.8 × 1.6 × 1.6</td>
<td>1</td>
</tr>
<tr>
<td>E6 (whole)</td>
<td>2400</td>
<td>37.5</td>
<td>75</td>
<td>160 × 256 × 256</td>
<td>1.0 × 1.6 × 1.6</td>
<td>1</td>
</tr>
<tr>
<td>E8 (head)</td>
<td>2400</td>
<td>37.5</td>
<td>75</td>
<td>256 × 256 × 256</td>
<td>1.6 × 1.6 × 1.6</td>
<td>2</td>
</tr>
<tr>
<td>E10 (head)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>256 × 256 × 256</td>
<td>3.0 × 3.0 × 3.0</td>
<td>2</td>
</tr>
<tr>
<td>E10 (whole)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>256 × 256 × 256</td>
<td>3.0 × 3.0 × 3.0</td>
<td>5</td>
</tr>
<tr>
<td>2nd scan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4 (whole)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>128 × 256 × 256</td>
<td>0.8 × 1.6 × 1.6</td>
<td>6</td>
</tr>
<tr>
<td>E6 (whole)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>160 × 256 × 256</td>
<td>1.0 × 1.6 × 1.6</td>
<td>18</td>
</tr>
<tr>
<td>E8 (head)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>192 × 240 × 240</td>
<td>1.28 × 1.6 × 1.6</td>
<td>6</td>
</tr>
<tr>
<td>E10 (head)</td>
<td>750</td>
<td>37.5</td>
<td>75</td>
<td>256 × 256 × 256</td>
<td>2.4 × 2.4 × 2.4</td>
<td>6</td>
</tr>
</tbody>
</table>

2.3. MRI image analysis

Image data obtained from MRI scans were processed using both established (mri3dX, ImageJ, ParaVision; Bruker) and custom packages (mri3dX). Voxel dimension was 62.5–117.2 μm (for the complete calculations, see Table 2). MRIcro allowed for slicing the generated 3D image in x, y and z planes to produce transverse, sagittal and coronal slices. Oblique slices were also generated using the ‘free rotate’ function. The threshold was altered automatically using the ‘contrast autobalance’ function. Image generated slices of the image and AVI movies (i.e. successive slices through the embryo or of 3D image reconstructions). ParaVision also enabled generation of 3D movies of eyes or lenses by segmentation (removal of regions of the 3D image) to isolate E10 eyes and lenses as 3D images that could be rotated and viewed as movies or saved at any point as an image. Firstly, the thresholds for voxel intensity were set as 0–50, 50–100, 100–150, and 150–200, in order to identify layers of similar voxel intensity that could later be removed by segmentation. The thresholds were sufficient to remove individual layers of similar voxel intensity by segmentation by clicking on the different coloured surfaces (representing different intensities, as governed when setting the threshold intensities). Segmentation data generated in Paravision were subsequently exported to ImageJ.

mri3dX software (http://www.jiscmail.ac.uk/lists/mri3dx.html) was adapted by one of us (KDS) for the purposes described here. This software employs automated segmentation and meshing algorithms and facilitated 3D modelling of the adult human eye (Singh et al., 2006). mri3dX was employed here to determine chick embryo eye internal volume, surface area and axial length, analysing eyes individually. For calculation of chick embryo eye internal volume, the region of the MRI image to be analysed was first labelled using a 3D flood-filling algorithm. The voxel intensity differences between the interior of the developing eye and the retina were sufficient to clearly distinguish the internal eye volume, i.e. flood filling excluded the retina (see Fig. 4). The model of the eye was shown as a mesh of triangular polygons, which were then ‘shrink-wrapped’ to give an approximation to the shape of the eye. After the surfaces of the model were smoothed by local averaging of the voxels, 3D models of each eye were created, allowing the calculation of the total surface area and internal volume and axial length of each eye. Axial length was defined as the distance between the anterior and posterior poles of the eye on an axis passing through the centre of the cornea and perpendicular to the corneal surface to the internal surface of the retina.

2.4. Intravitreal microinjection of glycosaminoglycans and processing for MRI

The methods for intravitreal microinjection of glycosaminoglycans into chick embryo eyes in ovo have been described previously (Halfter, 2008). Briefly, 1 μl of chondroitin sulphate (CS; 25 mg/ml) or heparin (H; 2.5 mg/ml) were microinjected into the right eyes of chick embryos in ovo at E5, while the left eye in each case served as a control. Three different embryos were intravitreally microinjected with each of CS or H at E5. Embryos were killed 5 days following microinjection at E10 and the heads were fixed in 4% paraformaldehyde with 0.1% glutaraldehyde. Heads were then embedded in 1% agarose in plastic tubes as described above. Embryo heads were also processed histologically as described previously (Halfter, 2008). MRI scanning was then carried out using the parameters outlined above. MRI data were imported into mri3dx software and various eye parameters were determined in each case (volume, surface area, axial length and equatorial diameter). To control for variation in the size of each of the three individual embryos, the volumes determined for each embryo right eye were normalised with respect to the non-injected left eye in each case. The degree of statistical significance between injected (right) and un-injected (left) eyes in each case was determined using the Wilcoxon Signed Ranks Test.

3. Results

3.1. 3D reconstruction of chick embryo eyes through development and measurement of various parameters

3D reconstruction of chicken embryo eyes was carried out using mri3dX software. Manual ‘flood-filling’ was required to fill the

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space or area of interest of the E4 eyes (Fig. 1A), whereas the software accomplished this automatically for the other stages (E6, E8, and E10; Figs. B–D). From the ‘flood-filled’ models, morphological parameters were determined for each eye from each embryo (E4, E6, E8 and E10) including: internal eye volume, surface area and axial length (Fig. 2A–C). Mean internal eye volume increased from 2.1 mm$^3$ at E4 to 139.3 mm$^3$ at E10, eye surface area increased from 8.5 mm$^2$ at E4 to 137.7 mm$^2$ at E10 and axial length increased from 1.1 mm at E4 to 6 mm at E10 (Table 3). The values for axial length were comparable to those calculated using the formulae for surface area and volume of a sphere (Table 3).

3.2. Generation of movies of space-filled MRI images of embryos, eyes and lenses

Movies of embryos were generated using ImageJ (see Fig. 3A–C for screen shots) and segmented to reveal eyes and lenses using Paravision (see Fig. 3D–F for screen shots). The movies can be found in the Supplementary information online (Supp_movies 1–6). Segmentation reveals the eye on the right without its lens, revealing the iris (visible in the Supp_movie_5), and the eye on the left with its lens and cornea intact (see Fig. 3E for screen shot; Supp_movie_5). The choroidal fissure (white arrow; Fig. 3E) is also clearly visible. Lenses were subsequently segmented from the E10 eyes (Supp_movie_6; see Fig. 3F for screen shot).

Fig. 1. 3D reconstruction of chick embryo eyes through development. Carried out using mri3DX software, manual “flooding” was required for space filling of the E4 eyes (A), but the other stages (E6 (B), E8 (C) and E10, D) were flooded automatically using the software. Axial lengths in mm are shown on the diagram. Two views of the eye at each stage are shown, each from a different perspective. The eyes at the different stages are not shown to scale. A, anterior; P, posterior.

Fig. 2. Increases over time in internal eye volume, surface area and axial length through embryonic development (E4, E6, E8 and E10). A. Internal eye volume. B. Surface area. C. Axial length (Determined using 3D models built from MRI data; the trendline of best fit in each case is with respect to the left eye). D. Relationship between surface area, volume and radius (half of the axial length) if the eyes were perfectly spherical calculated using the appropriate formulae. The reader is referred to Table 3 for the exact values for these parameters.

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Table 3
Change in mean volume, surface area and axial length of chick embryo eye as determined by MRI in combination with image analysis using mi3Dx software (% values in brackets compare each measurement with the value for that parameter at embryonic day 10). The theoretical axial length measurements calculated using the appropriate formulae for surface area and volume of a sphere at each of the embryonic stages are shown in brackets in the axial length column: (axial length value calculated from MRI measured surface area; axial length value calculated from MRI measured volume).

<table>
<thead>
<tr>
<th>Embryonic Day</th>
<th>Mean Value</th>
<th>Axial Length (mm)</th>
<th>Surface Area (mm²)</th>
<th>Volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.1 (18.3%) (1.59; 1.64)</td>
<td>8.5 (6.2%)</td>
<td>2.1 (1.5%)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.9 (48.3%) (3.70; 3.77)</td>
<td>44.6 (32.4%)</td>
<td>26.5 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.4 (73.3%) (5.62; 5.93)</td>
<td>110.4 (80.2%)</td>
<td>103 (73.9%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6 (100%) (6.43; 6.62)</td>
<td>137.7 (100%)</td>
<td>139.3 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Generation of serial sections through the eye of the E10 embryo using MRI

A panel of serial sections of the E10 embryo from the back to the front of the eye in the coronal plane was generated using MRI (Fig. 4A–N). The images are of sufficient resolution to visualise the pecten (Fig. 4C and D). The lenses of both eyes, which are also clearly delineated in these sections, are virtually black at their centres (the lens nucleus), indicating dehydration, while the spaces occupied by the vitreous and aqueous are white, indicating high levels of hydration (Fig. 4F and F’). The retina is also delineated clearly in these images.

3.4. Increasing number of averages per scan significantly improve image quality of young embryo eyes by increasing the signal to noise ratio of images

The different embryos were scanned several times in order to produce the optimum images in terms of contrast and resolution (Tables 1 and 2). There are two main factors to be considered: production of optimum intensity of scans and comparable scans between embryos. The project began with the optimum parameters, but initially for the E4–E8 embryos, the scan times were too short and produced noisy images, due to the trade off between scanning time, resolution and noise. The three younger stage embryos were initially scanned sequentially. However, as the scanning time was reduced, the split between the three embryos resulted in a decreased signal-to-noise ratio, due to a reduction in the number of averages (acquisitions) obtained. For the second set of scans, the parameters were set to be comparable to the first scan of the E10 embryo. When the scans were repeated, there was an increase in the number of averages, which produced clearer images.

3.5. Detection of changes in eye parameters at E10 determined using MRI following intravitreal microinjection of glycosaminoglycans into E5 chick embryos

Intravitreal microinjection of two different glycosaminoglycans (CS or H) was carried out on individual embryos at E5. The embryos were then killed and fixed at E10 and scanned using MRI. Measurements of various eye parameters (eye volume, surface area, axial length and equatorial diameter) were determined. The individual MRI measurements made for each embryo are presented in Table 4. Morphological changes in chick eye embryos were determined using conventional histology (Fig. 5A, B, D, C) in addition to MRI (Fig. 5C, E, F) on fixed tissue at E10 following in ovo intravitreal microinjection of chondroitin sulphate (CS) at 25 mg/ml (B, C) and heparin (hep; D–F) at 1 mg/ml into the right eye in each case at E5. A control embryo microinjected with PBS is shown for comparison (Fig. 5A). Cryostat-sectioned embryos were either photographed (Fig. 5A, B, D) or a screen shot of MRI images was generated using ImageJ (Fig. 5C, E, F). The eyes injected with CS at E5 showed an obvious increase in eye size in the right, experimental (Ex) eyes at E10 compared to the left eyes using both histological and MRI approaches (Fig. 5B, C). The vitreous in the injected right eye (Fig. 5C) exhibited a ‘stippled’ texture using MRI, indicating that it is less homogenous than in the un-injected left eye. Eyes injected with heparin at E5 showed a decrease in eye size in the experimental eyes at E10 (Fig. 5D–F). Infoldings of the retina were clearly visible (Fig. 5E, F; black arrows) extending in to the vitreoretinal boundary region. One of these folds is shown in a conventionally stained plastic section of the retina (Fig. 5F; R; retina). Using MRI, a band of reduced pixel intensity was clearly visible at the vitreoretinal boundary in the heparin-injected right eye compared to the un-injected left eye (highlighted by red bars in Fig. 5E), indicating withdrawal of the vitreous from the retina. Bar charts (Fig. 5H, I) demonstrated a statistically significant increase in eye size after CS injection or a decrease in eye size after heparin injection. The graph in Fig. 5H is based on eye weight measurements of control (C, red bars) or experimental (Ex, green bars) eyes injected with CS from bovine trachea (CS1; n = 8), shark fin (CS2; n = 9) or heparin (hep; n = 6). The weight differences were statistically significant (p < 0.01). MRI-based volume measurements showed a similar increase (×1.8; p < 0.05) or decrease (∗×0.68; p < 0.05) in eye size following intravitreal microinjection of CS or heparin. Error bars represent SEM; “statistically significant difference at p < 0.05.

4. Discussion

Here, we generated images and movies (see Supplementary data) from early chick embryos derived using high resolution MRI. MRI scan parameters were determined that provided optimal images of the developing chicken eye through a range of developmental stages. MRI, in combination with appropriate software packages, generated space-filled 3D models of chick embryo eyes allowing for accurate measurement of axial length, volume and surface area.

Furthermore, we have provided data demonstrating the potential utility of MRI for generating a high quality atlas of chick eye development (Figs. 3 and 4; Supp_Figs. 1 and 2), which would complement existing histological atlases of chick development (Bellairs and Osmond, 2005) and the recently created quail development atlas (Ruffins et al., 2007). Indeed, a 3D digital mouse MRI atlas has been developed (Dhenain et al., 2001), but no such comprehensive atlas of chick embryo development has been created and there is no atlas of eye development in any species using MRI.

4.1. MRI as a tool for analysing chick embryo eye development: 3D model construction

We have generated 3D models of chick eye development. Such models are advantageous compared to classical histology as a much more complete picture of spatial relationships within the eye can be captured than is possible when attempting to reconstruct a 3D image from many serial sections. Conversely, classical histology can provide significantly higher resolution (i.e. of 5 μm compared to the 62.5 μm for MRI obtained here or the 30 μm resolution obtained in some other studies; e.g. Hogers et al., 2001), but histological processing becomes more difficult when using larger late-stage embryos, due to the increased number of sections required to reconstruct the tissue of interest (Dhenain et al., 2001). Furthermore, histological methods do not preserve the 3D geometry of the tissue (Fraser et al., 2002). MRI is a powerful imaging technique that preserves the true 3D geometry of the specimen, making it
straightforward to produce 3D models from any perspective (see Figs. 3 and 4 and the Supplementary movies). While the studies described here utilised fixed tissue, they are nevertheless important as proof of principle towards examination of ocular development and/or growth in fresh un-fixed tissue and/or in live embryos in ovo. For example, it has previously been demonstrated that live chick embryos can be imaged using MRI from day 12 of incubation through to hatching (Bain et al., 2007). Clearly, given the
Fig. 4. Panel of serial sections of the E10 embryo from the back to the front of the eye (A–N) in the coronal plane obtained using MRI. The images are of sufficient resolution to visualise the pecten in the eye (arrow in C). The nucleus of the lens in both eyes is significantly darker than the lens cortex (F). (F’) A higher magnification of the region in the white square in F with enhanced contrast highlighting the dark regions. The lenses are indicated by arrows.

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data presented here, eye development could be imaged using MRI in embryos as young as day 4 of incubation.

An MRI-based atlas would be particularly suitable for measuring volumes and areas as well as analysing morphological features within a specimen and the relationship of these features to each other. MRI allows creation of images that are “stereotactic,” as they preserve distances, areas, volumes and angular orientations, which can be manipulated easily (Fraser et al., 1994).

We have also determined the optimum conditions for contrast and resolution of MRI images of chick embryo eyes. We carried out consecutive scans with different RF pulse sequences in order to alter the weight of the T1 relaxation signals and/or T2 decay-signals to account for the specific nature of chick embryo eye tissue (e.g. degree of hydration). During this investigation, in order to obtain optimum contrast of the eyes, we utilised mixed T1/T2 weighted images. In future studies, we plan to explore further the benefits of different methodologies for achieving optimal contrast when using MRI to examine eye development and growth in the chick; e.g. the use of novel contrast agents such as gadolinium, which has successfully improved the preparation of chick embryos for modelling heart development using MRI (Zhang et al., 2003) and for obtaining images to build models of avian limb development (Li et al., 2007).

4.2. Growth of the chick embryo eye determined by MRI: axial length, surface area and volume

The space-filled models of embryonic chicken eyes at different stages of development (Fig. 1) allowed us to determine values for internal eye volume, surface area and axial length (Fig. 2A–C). It is clearly apparent that the eyes have a linear rate of growth between E4 and E10 and these values are comparable to the theoretical calculations for eye surface area and volume for a perfect sphere for radii in this range (Fig. 2D). Furthermore, calculations for radii, assuming perfectly spherical eyes (clearly not the case as seen in our space-filled models; see Fig. 1), which we determined from the actual volume and surface area values obtained using MRI (discussed further below), indicate that the axial length values we obtained using MRI are similar to the theoretical values calculated using the appropriate formulae for surface area and volume of a sphere at each of the embryonic stages examined (Table 3), supporting the contention that the MRI measurements are accurate.

Growth rates of chick embryo eyes of ages between E4 and 1-day post hatch have been determined previously (Neath et al., 1991). That study divided the linear growth into two distinct phases, the first of which is a rapid growth phase (occurring between E4 and E10), while the second (from E10 onwards) is a slower phase. During the rapid phase, the average diameter of the eye increased at a constant rate of 1.193 mm/day. This is comparable with the growth rate for the early phase we observed here using space-filled models generated from MRI; specifically for axial length, an average of 0.817 mm/day (6 mm at day 10 minus 1.1 mm at day 4 = 4.9 mm; 4.9 mm/6 days = 0.817 mm/day), as shown in Fig. 2C and Table 3. Furthermore, the measurement of eye diameter at E10 (6 mm) that we obtained using MRI is identical to that determined from Hamburger and Hamilton (Hamburger and Hamilton, 1951) and is comparable to the value recently obtained for the quail embryo at E10 (Ruffins et al., 2007).

We also observed that the pixel intensities of the cortical (outer) regions of the E10 lens were higher than those in the central (nuclear) region (Fig. 4). This suggests that, even at E10, there is a significant amount of dehydration occurring in the nucleus of the chick embryo lens. Indeed, age-related changes in water and crystallin content of the foetal and adult human lens have previously been demonstrated using a microsectioning technique (Bours et al., 1987). These authors showed that the lens nucleus contains the lowest amounts of water and water-soluble crystallins, there is a relative dehydration of the lens during development and ageing and that this is most pronounced in the lens nucleus. Our MRI data on the chick embryo lens agree with this study and further suggest that these processes are occurring at least as early as E10 in the chick embryo lens. Furthermore, our results could be relevant to attempts to understand the mechanisms of glucocorticoid-induced cataract formation in chick embryos. Administration of hydrocortisone hemisuccinate sodium to E15 chick embryos resulted in elevation of glucose, dehydration and cataract formation (Nishigori et al., 1987). Thus, high resolution MRI could enable quantification of the extent of dehydration in chick embryo lenses and the relationships between glucose levels, dehydration and cataract formation. It may also be possible to follow lens-specific metabolic changes resulting in cataract formation using non-invasive MRI on live chick embryos in ovo (Bain et al., 2007).

Eye growth is dependent upon increasing intraocular pressure (IOP) (Neath et al., 1991), and the vitreous provides the tensile pressure, which causes rapid growth of the eye (Coulombre et al., 1963). Interestingly, images generated using our MRI data showed that there is an increase in pixel intensity of the vitreous between E4 and E10. This suggests an increase in hydration, potentially resulting in the increases in IOP that drives the accompanying linear eye growth that we observed here. This observation that MRI can detect changes in the extent of vitreous hydration/homogeneity during embryonic eye development was born out in our
additional studies in which glycosaminoglycans were injected into the vitreous of E5 embryos in order to analyse their effects on eye growth at E10 (discussed further below).

3D models were generated using mri3dX to investigate various spatial relationships of eye growth during normal embryonic development. Fig. 1 shows the relative sizes and shapes of the left and right eyes at each stage of development between E4 and E10. Once again, linear increases were observed as development proceeds (Figs. 1 and 2; Table 3).

The 3D images underwent ‘segmentation’ (or isolation) of individual regions: the developing eyes from the E10 embryo and the lenses from the eyes of the E10 embryo (Fig. 3E, F and Supp_movies 5 and 6). These data illustrate the point that the resolution we achieved here using MRI is sufficient to allow for recognition of small voxel intensity differences in embryonic tissue in order to generate 3D images/movies, which can be analyzed further in order to make precise measurements or to carry out shape analysis.

4.3. Detection of changes in eye parameters at E10 determined using MRI following intravitreal microinjection of glycosaminoglycans into E5 chick embryos

We went on to examine the nature of the vitreous and to make precise measurements of various eye parameters with MRI compared to histological approaches to determine morphological changes following microinjection of glycosaminoglycans into chick embryo eyes in ovo and by weighing the eyes. We detected alterations in the normally uniform consistency of the vitreous,
particularly in the CS injected eyes (Fig. 5), but also to a lesser extent in the H injected eyes as well as a brighter area adjacent to the retina at the vitreoretinal border in the H injected eyes, revealing a region of the vitreous that had withdrawn from the retina (Fig. 5E, F). MRI may also be valuable for comparing morphological differences in ovo.

Conclusion

The results described here complement numerous other studies using high resolution MRI in studying morphogenesis and are the first to use MRI to carry out a quantitative analysis of eye development and growth in the chick embryo. The data indicate that MRI facilitates the phenotypic analysis of chick ocular development and growth in fixed samples and strongly suggest that it could also be useful for the non-invasive monitoring of chick eye development in ovo in live embryos. In addition, using intravitreal microinjection of glycosaminoglycans into the E5 chick eye, we have demonstrated that MRI can be used for analyses of changes in ovoid structural morphology in response to surgical or genetic manipulations of chick embryos in ovo (particularly of overall eye growth), for determining changes in the extent of ocular tissue hydration status (particularly of the vitreous and lens) and for revealing the integrity of the vitreoretinal boundary. The results further indicate that MRI may also be valuable for comparing morphological differences during eye development in chicken mutants with eye defects, something that we are currently investigating.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.exer.2009.05.014

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