Mesenchymal condensation sets the stage for intramembranous bone development

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Abstract

Mesenchymal condensation is one of the earliest, and most critical, stages in the formation of intramembranous (directly ossifying) bone. It is during the condensation phase that key osteogenic genes are first upregulated and during which collagen I, the scaffold on which the inorganic portion of bone will be deposited, is first established. Thus, morphogenesis and tissue specific gene expression occur concurrently. Previously, we used microarray analysis and *in situ* hybridisation to determine which genes are important and at what stage of development these genes act during intramembranous ossification in chick and zebrafish. In the current study, we utilize a combination of light and electron microscopy to better understand the patterning and polarization of cells in early osteogenic condensations. We compare neural crest-derived intramembranous bones, specifically the scleral ossicles of the chick (*Gallus gallus*) and the opercula of the zebrafish (*Danio rerio)*. While our preliminary results in the chick suggest osteoblasts are polarized toward the center of the early condensations, more data is required to make a solid conclusion. Data collection in zebrafish is also ongoing. Combined, these studies provide a means to correlate gene expression and morphogenesis during intramembranous bone formation. This work was primarily funded by the Nova Scotia Health Research Foundation (Canada) and our lab is funded by Natural Sciences and Engineering Research Council of Canada.

Introduction

During the development of intramembranous (direct developing) bone, neural crest-derived mesenchymal cells aggregate to form areas of high cell density, known as osteogenic condensations, where they differentiate into osteoblasts and begin to lay down a collagen I-rich extracellular matrix (ECM)[1,3]. This process determines the location, size, and shape of the future bone[3]. Here, I use light and electron microscopy to study cell patterning and ultrastructure in osteogenic condensations.

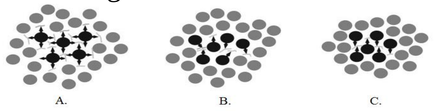


Figure 1 – Hypothesized scenarios of osteoblast arrangement and polarization in early osteogenic condensations[1].
A. Cells are not polarized; collagen secretion occurs in all directions.
B. Cells are polarized but not arranged.
C. Cells are polarized and arranged.

Objectives

To describe the arrangement of osteoblasts and the direction of collagen secretion in osteogenic condensations. This will be undertaken in a comparative manner using two intramembranous neural crest-derived bones, chick scleral ossicles and zebrafish opercles.

Results

The Zebrafish Opercle

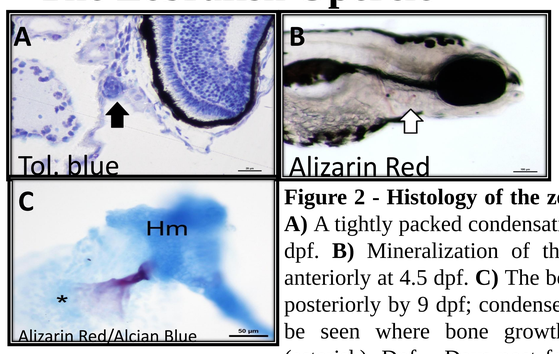


Figure 2 - Histology of the zebrafish opercle. **A)** A tightly packed condensation is present at 4 dpf. **B)** Mineralization of the spicule begins anteriorly at 4.5 dpf. **C)** The bone (red) fans out posteriorly by 9 dpf; condensed osteoblasts can be seen where bone growth is occurring[4,5] (asterisk). Dpf – Days post fertilization. Hm – hyomandibular cartilage.

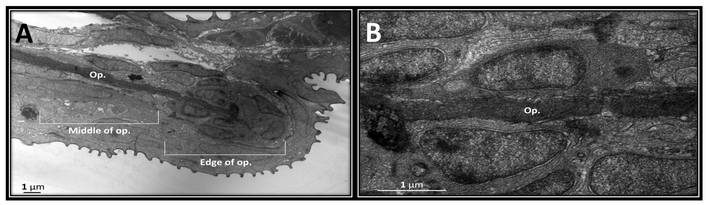


Figure 3 – Ultrastructure of the zebrafish opercle. 23 dpf. **A)** Cells at the edge of the opercle are rounded and contain dark cytoplasm, indicating bone deposition. Cells adjacent to the middle of the bone are flattened, indicating lower activity. These cells likely contribute to appositional growth **B)** High magnification of cells at the edge of the opercle. These cells contain large nuclei, numerous Golgi, and stacks of RER. Op – opercle.

Chick Scleral Ossicles

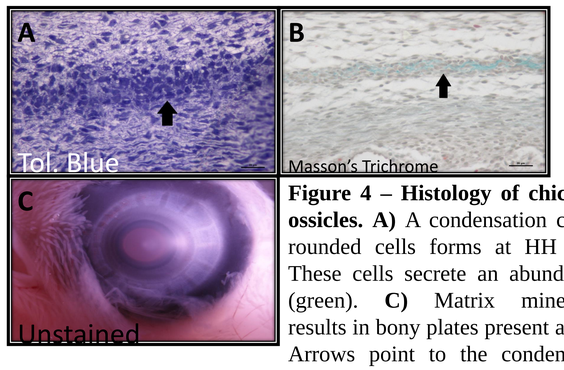


Figure 4 – Histology of chick scleral ossicles. A) A condensation containing rounded cells forms at HH 36.5. **B)** These cells secrete an abundant ECM (green). **C)** Matrix mineralization results in bony plates present at HH 38[2]. Arrows point to the condensation in each. HH – Hamburger and Hamilton stages.

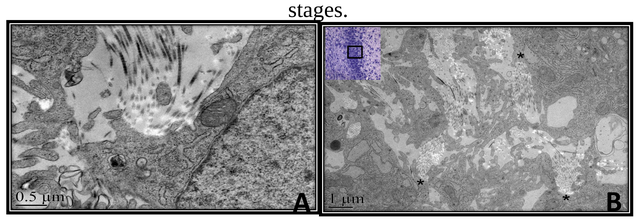


Figure 5 - Collagen secretion in chick osteogenic condensations. HH 36.5. **A**) Large clusters of collagen accumulate just around the edge of this actively secreting osteoblast. **B**) Inset shows a condensation in a vertical orientation; boxed area is enlarged. Three cells are shown secreting collagen (asterisks) parallel to the long-axis of the condensation.

Materials and Methods

Zebrafish

4-23 dpf

Chick

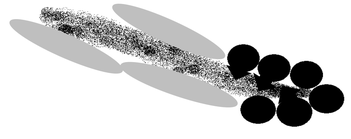
HH 36.5
10.5 days

Fix → Embed → Section → Stain → View

Fix: PFA/ glutraldehyde in sodium cacodylate
Embed: LR White (fish) Epon (chick)
Stain: Uranyl acetate/ lead citrate (TEM) Toluidine blue Masson's Trichrome

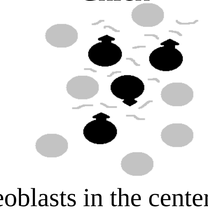
Conclusions

Zebrafish



Osteoblasts at the edge of the opercle actively lay down bone matrix, thereby contributing to directional bone growth.

Chick



Osteoblasts in the center of the chick scleral ossicle condensations are polarized and arranged such that the direction of collagen secretion is parallel to the long axis of the condensation.

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