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Finite element modelling predicts changes in joint shape and cell behaviour due to loss of muscle strain in jaw development

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ABSTRACT

Abnormal joint morphogenesis is linked to clinical conditions such as Developmental Dysplasia of the Hip (DDH) and to osteoarthritis (OA). Muscle activity is known to be important during the developmental process of joint morphogenesis. However, less is known about how this mechanical stimulus affects the behaviour of joint cells to generate altered morphology. Using zebrafish, in which we can image all joint musculoskeletal tissues at high resolution, we show that removal of muscle activity through anaesthetisation or genetic manipulation causes a change to the shape of the joint between the Meckel's cartilage and Palatoquadrate (the jaw joint), such that the joint develops asymmetrically leading to an overlap of the cartilage elements on the medial side which inhibits normal joint function. We identify the time during which muscle activity is critical to produce a normal joint. Using Finite Element Analysis (FEA), to model the strains exerted by muscle on the skeletal elements, we identify that minimum principal strains are located at the medial region of the joint and interzone during mouth opening. Then, by studying the cells immediately proximal to the joint, we demonstrate that biomechanical strain regulates cell orientation within the developing joint, such that when muscle-induced strain is removed, cells on the medial side of the joint notably change their orientation. Together, these data show that biomechanical forces are required to establish symmetry in the joint during development.

1. Introduction

The development of reciprocal interlocking joints at cartilage elements is central to ensuring normal skeletal function (Nowlan et al., 2010). Processes that disrupt joint shape formation can cause abnormal loading and joint function (Michaeli et al., 1997), e.g. hip shape correlates strongly with risk of osteoarthritis (Jacobsen and Sonne-Holm, 2005). The initial formation of the cartilage template from mesenchymal cell condensations, mostly replaced by bone, is well understood (DeLise et al., 2000; Thorogood and Hinchliffe, 1975). However, how the early joint structures undergo morphogenesis to form their mature shape remains less clear (Pacifici et al., 2005).

Zebrafish, with relevant fluorescent transgenic lines (Apschner et al., 2014; Hammond and Moro, 2012), allow dynamic imaging of the musculoskeletal system at cellular resolution. Zebrafish are, therefore, a useful model to examine how mechanical loading from muscle impacts on cartilage behaviour. By 48 h post-fertilisation (hpf), mesenchymal cells have condensed to form the mandibular arches (Eames et al., 2013; Kimmel et al., 1995). At 53–55 hpf, the cartilaginous elements of the Meckel's cartilage (MC), palatoquadrate (PQ) and ceratohyal appear, as does the adductor mandibulae jaw musculature, with the intermandibularis anterior and protractor hyoideus, identifiable by 62 hpf (Schilling and Kimmel, 1997). Larval zebrafish use the protractor hyoideus to constrict the buccal chamber of the jaw and adductor mandibulae to close the mouth (Diogo et al., 2008; Hernandez et al., 2002). The joint between the MC and PQ (Figs. 1A and 2A) is described as the jaw joint in (Talbot et al., 2010) and referred to as such hereafter. In the joint structure, the retroarticular process (RAP) of the MC protrudes ventrally to interlock with the PQ (Miller et al., 2003), typically cavitation of this joint occurs at around 7 dpf (http://zfatlas.psu.edu/).

Many studies have linked absence of muscle activity with abnormal joint shaping and fusions of articular surfaces in long bones. Early studies used Decamethonium Bromide and botulinum toxin to generate paralysis in chick models; leading to a flattening of articular surfaces and a failure of joint cavitation (Drachman and Sokoloff, 1966; Murray and Drachman, 1969). More recently, (Roddy et al., 2011b) found that rigid paralysis of chicks during early development caused the knee joint to appear flattened.

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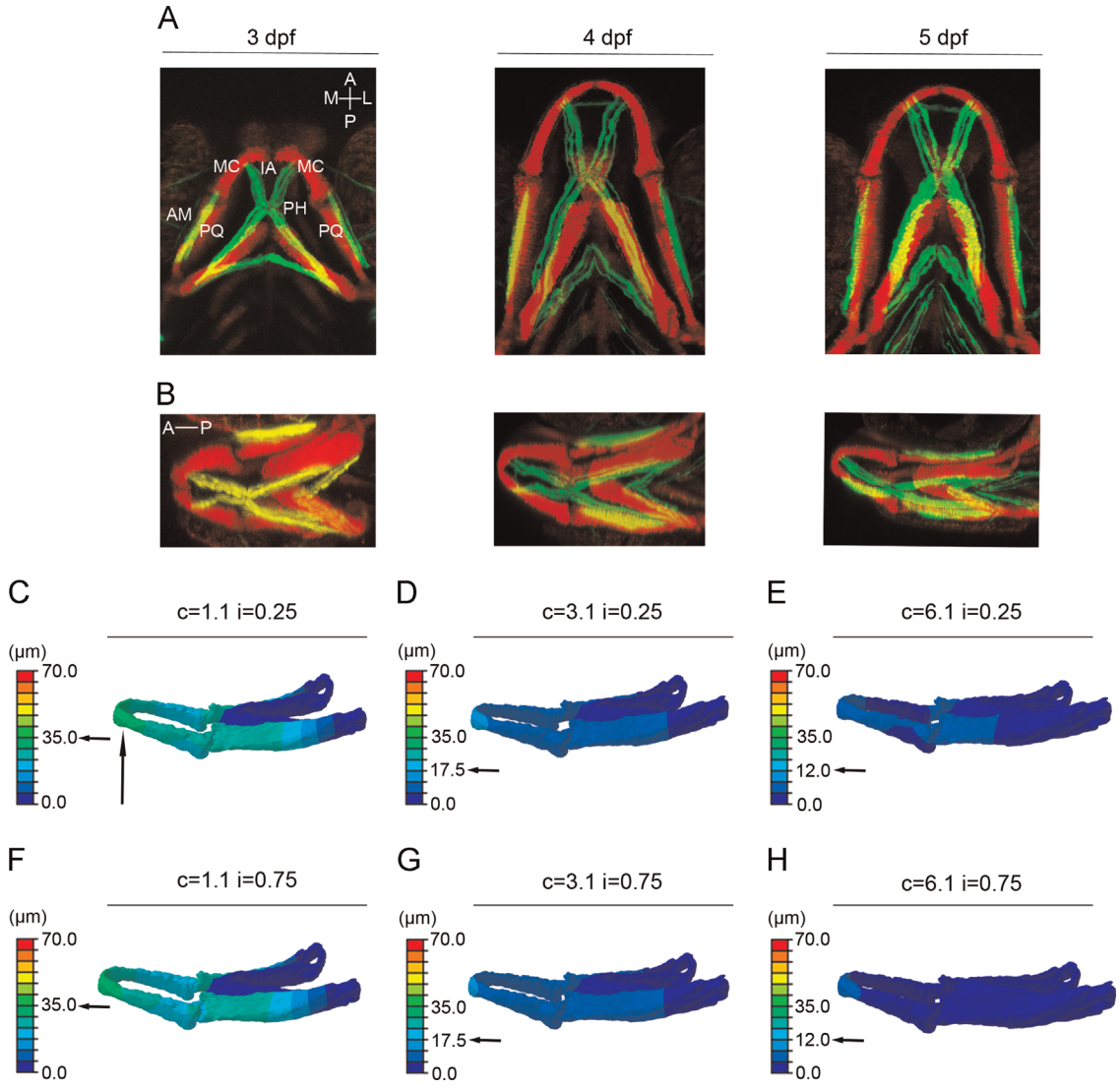

Fig. 1. FE-model simulating jaw displacement in a 5 day old fish (5 dpf) for different cartilage and interzone Young's moduli. (A, B): Confocal image stacks of the ventral (A) and lateral (B) zebrafish jaw at 3, 4, and 5 dpf with cartilage labelled by *Tg(Col2a1aBAC:mcherry)* and muscle labelled by *Smyhc:GFP*. AM=adductor mandibulae, IA=intermandibularis anterior, PH=protractor hyoideus, MC=Meckel's cartilage, PQ=palatoquadrate, M=medial, L=lateral, A=anterior, P=posterior. (C–H): Jaw displacement (open to closed in μm) is marked on the jaw; recorded using the colour key. Each model (C–H) has a different combination of cartilage (c=1.1, 3.1, or 6.1 MPa) or interzone (i=0.25 or 0.75 MPa) properties. Horizontal black arrow highlights the value of jaw displacement at the tip of the Meckel's cartilage (represented by the vertical black arrow).

Additionally, genetic models in mice, such as Splotch mutants that lack limb muscle, exhibit abnormal limb joint shaping (Kahn et al., 2009; Nowlan et al., 2010). While muscle force has been shown to be necessary for normal joint morphogenesis and chondrocyte intercalation (Shwartz et al., 2012), it remains largely unclear how cells within the joint interpret such forces to bring about changes in behaviour. Little is known concerning the effects of paralysis on jaw joint morphology, though the temporomandibular joint region shows signs of adaptation when the mechanical environment is altered (Enomoto et al., 2014; McNamara and Carlson, 1979).

Finite element (FE) models simulating the biophysical response to muscle-induced loading have been used to investigate joint development, endochondral ossification, and joint development, including developmental dysplasia of the hip (DDH) (Carter and Wong, 1988; Heegaard et al., 1999; Nowlan et al., 2008; Roddy et al., 2011a; Shefelbine and Carter, 2004). Thus far, developmental FE-models have focused primarily on the femur, particularly in humans and chick. Whilst there are a handful of studies using FE to deduce the mechanical performance of extant shark jaws (Ferrara et al., 2011) and other jawed vertebrates (Rayfield, 2007), FE-modelling has not been used to explore the mechanobiology of the developing zebrafish jaw pre-cavitation.

Here, we document the process of joint morphogenesis in wild type zebrafish jaws from the time of first muscle activity to generation of the refined interlocking joint shape. Then, by removing muscle activity pharmacologically and genetically, we quantify the timing and extent of the response to lack of muscle activity on joint morphogenesis. Using FE analysis (FEA) we identify the locations of muscle-induced strain acting on the zebrafish jaw cartilage throughout normal joint morphogenesis. To understand the mechanobiological changes that underpin joint shape we quantify differences in cellular orientation between wild type, anaesthetised and mutant models.

2. Methods

2.1. Zebrafish husbandry/zebrafish lines

Zebrafish were maintained as described (Westerfield, 2000). All zebrafish experiments were approved by the local ethics committee and the Home Office (Project license number 30/2863). The *Tg(Col2a1aBAC:mcherry)* zebrafish line has been previously described (Hammond and Moro, 2012; Hammond and Schulte-Merker, 2009) and labels all chondrocytes (Fig. 2A). The line *Tg(smhyc:EGFP)i104* labels all slow twitch muscle fibres (Elworthy et al., 2008). *myod*^{fh261} mutants have

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