Structural and mechanical properties of the proliferative zone of the developing murine growth plate cartilage assessed by atomic force microscopy

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Abstract

The growth plate (GP) is a dynamic tissue driving bone elongation through chondrocyte proliferation, hypertrophy and matrix production. The extracellular matrix (ECM) is the major determinant of GP biomechanical properties and assumed to play a pivotal role for chondrocyte geometry and arrangement, thereby guiding proper growth plate morphogenesis and bone elongation. To elucidate the relationship between morphology and biomechanics during cartilage morphogenesis, we have investigated age-dependent structural and elastic properties of the proliferative zone of the murine GP by atomic force microscopy (AFM) from the embryonic stage to adulthood. We observed a progressive cell flattening and arrangement into columns from embryonic day 13.5 until postnatal week 2, correlating with an increasing collagen density and ECM stiffness, followed by a nearly constant cell shape, collagen density and ECM stiffness from week 2 to 4 months. At all ages, we found marked differences in the density and organization of the collagen network between the intracolumnar matrix, and the intercolumnar matrix, associated with a roughly two-fold higher stiffness of the intracolumnar matrix compared to the intercolumnar matrix. This difference in local ECM stiffness may force the cells to arrange in a columnar structure upon cell division and drive bone elongation during embryonic and juvenile development.

Introduction

The growth plate (GP), situated between the epiphysis and diaphysis at both ends of long bones [1,2] is a unique and essential cartilaginous structure which is responsible for the elongation of bones formed by endochondral ossification. In this process, first skeletogenic mesenchymal stem cells condense and differentiate into chondrocytes prefiguring the shape of the future bone. In subsequent steps, the chondrocytes of the cartilaginous template undergo a differentiation cascade [3] within the GP which drives the longitudinal growth of the skeletal elements through chondrocyte proliferation, cellular enlargements via hypertrophy, extracellular matrix (ECM) synthesis and controlled matrix degradation [4–7]. The fully matured GP is organized into horizontal zones of resting, proliferative and hypertrophic chondrocytes (Fig. 1), which vary in cellular arrangement, function and matrix composition [8–10]. In the resting or germ layer of the GP the chondrocytes are roundish and rarely divide. In the proliferative zone, the cells undergo rapid division, are flattened along the mediolateral axis, and form columns along the proximodistal axis of the long bones. Column elongation occurs through spatially coordinated cell

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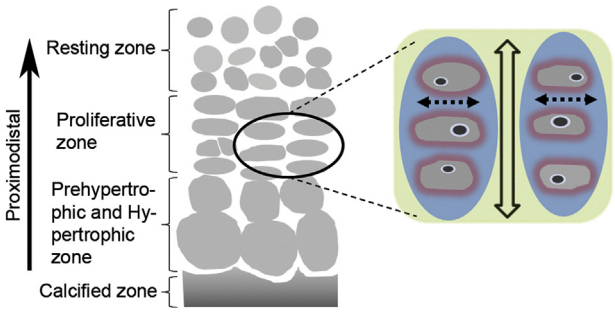

Fig. 1. Zonal arrangement of growth plate cartilage and its ECM. Cartilage growth plate is divided into horizontal zones called resting, proliferative, hypertrophic and calcified. Within the proliferative zone, chondrocytes are flattened and arranged into characteristic columns caused by clonal expansion. Chondrocytes are surrounded by a thin pericellular matrix (PCM, red), which is enveloped by the territorial matrix (TM, blue). The PCM and the TM between the cells of a column define the so called transverse septum (dashed arrow). The ECM around the columns is called the interterritorial matrix (ITM green), which fills up the longitudinal septum between the chondrocyte clusters (solid arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

division and rotational movements [11]. The division axis of the flattened chondrocytes is parallel to the proximodistal direction resulting in semi-circular daughter cells which initially lie along the mediolateral axis. Subsequently, the cells gradually flatten and glide around each other to arrange into the longitudinal column. Chondrocytes at the proximal end of the column then exit from the cell cycle, and increase their volume to become hypertrophic before the zone is calcified and replaced by trabecular bone.

One column in the proliferative zone usually consists of four to eight flattened chondrocytes surrounded by different matrix compartments. The pericellular matrix (PCM), which is rich in proteoglycans (PGs), collagen VI and very fine fibrils [12–14], immediately surrounds the chondrocytes. The adjacent territorial matrix (TM) compartment has a fine network of cross-banded, heterotypic fibrils composed of collagen types II, IX, and XI. The PCM and TM together with the clustered chondrocytes define the columnar chondron, the functional unit of the cartilage [15], which is believed to play an important role in regulating the interactions between chondrocytes and their surrounding matrix [16]. The interterritorial matrix (ITM), which is located between the columns contains thick collagen fibrils that largely run parallel to each other in the fully matured growth plate [17]. At a higher structural level the TM–PCM compartments account for the transverse septum (TS) matrix, separating the stacked chondrocytes within a column, while the ITM primarily accumulates in the intercolumnar longitudinal septum (LS) (Fig. 1).

In each matrix compartment, the interfibrillar space is filled with various types of proteoglycans like aggrecan, decorin, and fibromodulin as well as non-collagenous glycoproteins such as cartilage oligomeric matrix protein and matrilins [18]. The tension of the collagen fibrils and the osmotic pressure of the highly charged proteoglycan aggrecan are responsible for the specific mechanical properties of cartilage that withstands tensile, compressive and shear stress.

Although the columnar organization of the chondrocytes is essential for bone elongation, and despite the fact that the structure and molecular composition of the different matrix compartments of the GP has been intensively studied, the mechanism guiding the chondrocytes to rotate around each other and arrange in a columnar structure upon cell division still remains unclear. There is an increasing body of evidence that the fate of cells, and therefore the morphology and function of tissue is tightly controlled by the mechanical properties of their micro-environment [19,20]. In the case of cartilage, this mechanical micro-environment is mainly determined by the composition and structure of the cartilage ECM. Numerous studies have revealed that the mechanical properties of articular cartilage correlate with and depend on the collagen/proteoglycan composition, not only during development [21,22] but also in osteoarthritis [23,24]. However, the literature is sparse regarding the mechanobiology of the growth plate, even though there is an emerging perception that limb and growth plate morphogenesis is strongly influenced by both extrinsic and intrinsic mechanical cues, which may play an instructive role for chondrocyte differentiation and organization. In fact, local ECM biomechanics might even guide chondrocytes to arrange into a columnar stack and thus drive the linear elongation of endochondral bones [25–29].

Some recent studies investigating the intrinsic mechanical properties of the GP, using unconfined compression tests, have demonstrated zone- and developmental stage-dependent variations in elasticity [30–32]. Nevertheless, to elucidate how GP

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