

Stages of murine articular cartilage development.

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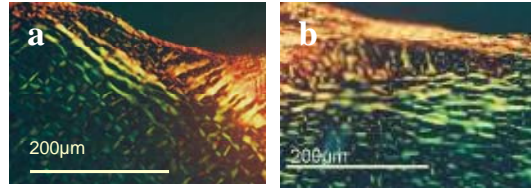
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INTRODUCTION: The structure of adult articular cartilage (AC) is intimately related to its function as a load bearing tissue¹. Determining the sequential development of this complex tissue will enhance the understanding of factors that may influence AC formation and expose possible mechanisms that may be used to aid tissue repair. Studying mouse tissue enabled a comprehensive examination of each stage of development due to the relatively condensed period of maturation.

METHODS: Mouse tibiae were dissected from pups aged 1-25 days old, raised either in natural litters or litters culled to 3 or 10 pups. The tibial plateaux were kept intact and fixed in formaldehyde in 0.1M Piperazine_1, 4- bis-2-ethanesulfopnic acid (PIPES) buffer (pH 8.5) for 24 hours, dehydrated in an ascending ethanol series, transferred to xylene, embedded in paraffin wax, sectioned at a thickness of 7.5µm and stained in picosirius red². Sections were imaged using light and polarised light microscopy. Additional mouse tibiae were plunge frozen in propane cooled by liquid nitrogen, freeze substituted for 5 days in 58% acetone, 30% methanol, 10% acrolein and 2% tannic acid and a further 5 days in 100% acetone. Samples were then critical point dried, fractured and coated in platinum/palladium (80/20) for examination by scanning electron microscopy.

RESULTS: Several developmental stages were determined. At birth there was no birefringent collagen matrix or clear chondrocyte orientation. Subsequent development was characterised by an alignment of chondrocytes and collagen matrix parallel to the articular surface. This was apparent in tissue from mice up to 6 days old. An area lacking birefringence, containing chondrocytes obliquely orientated with respect to the articular surface, was apparent in the load-bearing regions of cartilage from mice 7 days and older. Progressive development of this region of reorganisation (ROR) was observed in the increasingly determinate appearance of collagen



Figures 1a and b show polarised light microscope images of 7 and 8 day old mouse articular cartilage respectively. The ROR can clearly be seen in figure 1a and perpendicular alignment of the collagen fibres can be observed within the ROR in figure 1b.

fibres and columns of chondrocytes aligned perpendicular to the articular surface. Extension of the ROR from the load-bearing region to cover the condyles was observed, finally resembling adult AC. Development of AC correlated with the initiation of weight bearing on the hindlimbs and anatomical maturation of the pups. Additionally, the rate of AC development was retarded in pups raised in large litters, which also affected the appearance of the tissue in skeletally mature adults.

DISCUSSION & CONCLUSIONS: The development of mouse AC appeared to be related to that of other species. Similar stages of development are observed in bovine tissue, and clearly affect the biomechanical properties of the cartilage³. The formation of the ROR and realignment of the tissue to a structure that resembled adult AC occurred at the same time as the formation of the secondary centre of ossification. The subsequent expansion of the tissue to adult depths, which is observed in some species⁴, appears to occur by a mechanism of appositional growth⁵ but was not observed in mouse tissue.

REFERENCES: ¹Hughes *et al.*, (2005) *Eur. Cell Mater.* 9: 68-84; ²Sweat *et al.*, (1964) *Arch. Pathol.* 78:69-72; ³Camosso and Maroti (1962) *J. Bone Joint Surg.* 44A (4): 699-709; ⁴Wei *et al.*, (1998) *Osteoarthritis Cartilage* 6: 400-409; ⁵Hayes *et al.*, (2001) *Anat. Embryol.* 203: 469-479.

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