Review article

Effects of immobilization on chondrocytes and pericellular matrix in articular cartilage of patella in rats

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Abstract

The articular cartilage can be divided into four distinct zones: a superficial (or tangential) zone, a transitional (or intermediate) zone, a deep (or radial) zone and a calcified cartilage zone. Each zone contains a distinct subpopulation of chondrocytes that differs in morphology, distribution within the matrix as well as metabolic activity. Articular cartilage of patella in rats consists of a highly organized matrix with a sparse population of highly specialized chondrocytes. The chondrocytes in the articular cartilage are surrounded by a specialized microenvironment, which effectively insulates the chondrocytes and physically separates the cell from direct interaction with the bulk of the load bearing matrix. The present study was conducted to see histological changes in pericellular matrix and also in chondrocytes by varying periods of immobilization. Methods: 30 male rats belonging to Sprague Dawley strai n were procured from National Institute of Health Islamabad and the study was carried out at the animal house College of Physician and Surgeons Islamabad. These animals were divided into three groups. The right hind limbs of rats were immobilized with plaster of Paris cast. Animals in these groups were immobilized, remobilized and sacrificed at different periods a given below. Group 1: Control group of 10 animals who were left un-immobilized. Group 2: Experimental group of 10 animals who were immobilized for two weeks. Group 3: Experimental group of 10 animals who were immobilized for four weeks. The skin over knee joint was dissected and the joint along with patella was exposed. The knee joint was cut in sagittal plane. It was stored in 10% formalin for 48 hours. After processing for making paraffin blocks sections were cut from the same block and stained with Alcian Blue and H&E stain. Result: In the group immobilized for two weeks in only th ree experimental animals the cells were like fibroblasts and no loss of staining was seen in the pericellular matrix in any of the experimental animal as compared to the control group. After four weeks immobilization the diffuse necrosis of chondrocytes, with pit formation on the surface and disruption loss of staining with Alcian Blue stain was seen to some extent in all the animals. Conclusion: The chondrocytes as well as the pericellular matrix of articular cartilage shows changes on immobilization and surface chondrocytes are vulnerable to immobilization injury.

Keywords: chondrocytes, immobilization, pericellular matrix.

1 Introduction

The articular cartilage can be divided into four distinct zones: a superficial (or tangential) zone, a transitional (or intermediate) zone, a deep (or radial) zone and a calcified cartilage zone. Each zone contains a distinct subpopulation of chondrocytes that differs in morphology, distribution within the matrix as well as metabolic activity. Articular cartilage of patella in rats consists of a highly organized matrix with a sparse population of highly specialized chondrocytes (WATRIN, RUAUD, OLIVIER et al., 2001). Chondrocytes provide 10% or less of the total volume of cartilage; consequently, the functional properties of cartilage, including stiffness, durability, and distribution of load, rely on the extra cellular matrix (JADIN, WONG, BAEG et al., 2005). The chondrocytes and matrix depend on each other. The chondrocytes in the articular cartilage are surrounded by a specialized microenvironment, which effectively insulates the chondrocytes and physically separates the cell from direct interaction with the bulk of the load bearing matrix (FARNUM and WILSMAN 1983). The pericellular microenvironment must therefore play an important role in mediating the interaction between the chondrocytes and its extra cellular matrix. Collectively, the chondrocytes and its pericellular microenvironment are thought to represent the chondron, arguably the primary functional and metabolic unit of hyaline cartilages (ALEXOPOULOS, WILLIAMS, UPTON et al., 2005). With the identification of the chondron as a true microstructure of articular cartilage, current studies have focused on defining the composition and organization of the chondron, and its role in chondrocyte - matrix interactions (POOLE, AYAD and GILBERT, 1992). Researchers have conducted researches in order to know composition of pericellular matrix (JULKUNEN, WILSON and JURVELIN, 2009). In one research proteoglycans were found to be component of chondron while others believe that this region is rich in proteoglycans and non-collagenous proteins, like cell membrane-associated molecule anchorin. The presence of proteoglycan in the matrix raises the point of interest that whether this matrix is as responsive to mechanical effects of loading and unloading like the extra cellular matrix so the present study was conducted to see histological changes in pericellular matrix and also in chondrocytes by varying periods of immobilization. The objective of the study was to determine the histological changes in shape of chondrocytes and proteoglycan content

in the pericellular matrix of articular cartilage of patella by varying the periods of immobilization.

2 Material and methods

30 male rats belonging to Sprague Dawley strain were procured from National Institute of Health Islamabad and the study was carried out at the animal house College of Physician and Surgeons Islamabad. These animals were divided into three groups. The right hind limbs of rats were immobilized with plaster of Paris cast. Care was taken to cover the knee joint completely. Animals in these groups were immobilized, remobilized and sacrificed at different periods a given below.

Group 1: Control group of 10 animals who were left un-immobilized.

Group 2: Experimental group of 10 animals who were immobilized for two weeks.

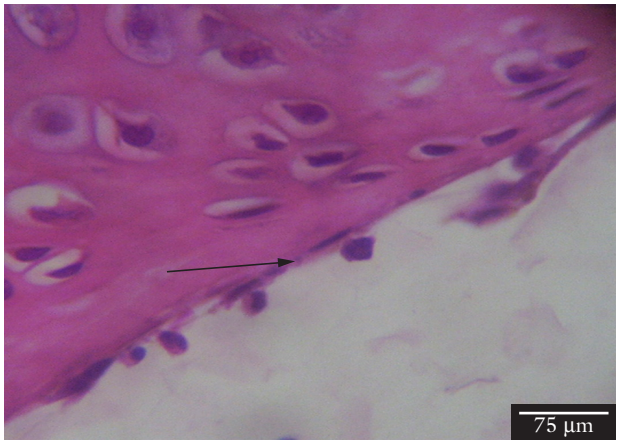

Figure 1. Photomicrographs of articular cartilage of patella showing necrotic chondrocytes shedding from surface of articular cartilage. Arrow showing fibroblast like cells in the superficial zone. H&E stain. Bar 75 µm.

Group 3: Experimental group of 10 animals who were immobilized for four weeks.

At the end of experimental period the rats were anaesthetized with chloroform. The skin over knee joint was dissected and the joint along with patella was exposed. The knee joint was cut in sagittal plane. It was stored in 10% formalin for 48 hours. Specimen was decalcified using Ethylene diamine tetra acid (EDTA).

After processing for making paraffin blocks 10 and 7 µm sections were cut from the same block and stained as given below.

- Alcian Blue stain was used for 10 µm thick sections to demonstrate proteoglycan content;
- H & E stain was used for 7 µm thick sections to study routine histology of patellar cartilage.

3 Results

In the group immobilized for two weeks in only three experimental animals the cells were like fibroblasts and no loss of staining was seen in the pericellular matrix in any of the experimental animal as compared to the control group. After four week's immobilization in the experimental group the superficial chondrocytes were necrotic. The chondrocytes were in form of elliptical cells and were comparable to fibroblasts. There was shedding of cells from superficial zone (Figure 1). There was loss of staining from the pericellular matrix as seen in Alcian Blue stained sections After four weeks immobilization the diffuse necrosis of chondrocytes, with pit formation on the surface and disruption loss of staining with Alcian Blue stain was seen to some extent in all the animals (Figure 2). There were small and thin cells adhering to the surface in the hollows. The nuclei of these cells were flattened. The chondrocytes showed elongated or flattened morphology with many branched cytopalsmic processes. Many cells were seen shedding from the superficial surface of cartilage (Figure 3).

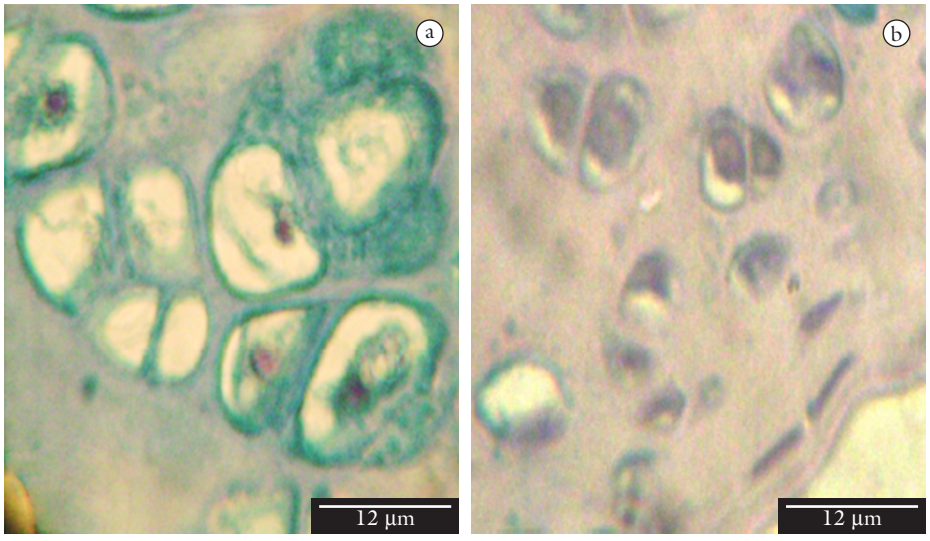

Figure 2. Photomicrographs of articular cartilage of patella. a) shows intense staining in the pericellular matrix in control group. b) shows the loss of staining from the pericellular matrix in the experimental group. Alcian Blue stain. Bar 12 µm.

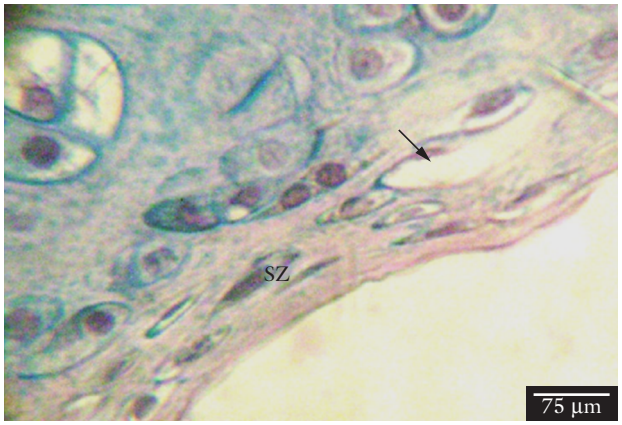

Figure 3. Photomicrograph of articular cartilage of patella in group immobilized four weeks. Black arrow shows the empty spaces in superficial zone. Alcian Blue stain. Bar 75 µm.

4 Discussion

In this study it was seen that on two weeks immobilization no significant change was observed in chondrocytes. After four week's immobilization in the experimental group there was loss of staining from the pericellular matrix and the surface layer of the cartilage showed disruption (Figure 2). The superficial chondrocytes were necrotic. The chondrocytes were in form of elliptical cells and were comparable to fibroblasts. In past it has been proved that the chondrocytes respond to specific loading conditions through anabolic or catabolic reactions induced by the stress and strain imparted to the cells by physical stimulation. Loss of staining from the pericellular matrix has been observed by previous researches (KAAB, RICHARDS, GWYNN et al., 2003). It has been speculated that the chondron functions to absorb mechanical loads and provide hydrodynamic protection for the chondrocytes. The composition of cartilage reflects the net response of the chondrocytes to the prevailing loading pattern, with cartilage proteoglycan content highest in heavily loaded regions and removal of load leading to cartilage thinning and proteoglycan loss (SOOD, 1971). During immobilization, especially in the extended position, articular hypoxia occurs due to a decreased amount of synovial fluid, the increased compression of the cartilage surfaces and the increased intra-articular pressure. This causes degeneration and necrosis of the superficial chondrocytes and the superficial cartilage layer (FINSTERBUSH and FRIEDMAN 1973).It was seen that the superficial chondrocytes showed necrotic changes and were seen shedding from the superficial surface. This is consistent with the finding of past researchers (POOLE, KOJIMA, YASUDA et al., 2001; CANDOLIN and VIDEMAN, 1980). Numerous studies have demonstrated that joint immobilization induces degenerative changes in articular cartilage. The duration of immobilization to produce degenerative changes is disputed. Some authors postulate that a much longer duration of immobilization is necessary to cause necrosis but some think that even one week immobilization can profoundly affect the cartilage (COLE JUNIOR, NARINE, ELLINGER et al., 1983; GUILAK, 1995). Presence of empty lacunae show that on four weeks immobilization apoptosis might be going on as has been proved in past studies (LANGENSKIOD, MICHELSSON and VIDEMAN, 1979). It is clear from the present study that the pericellular matrix is sensitive to the changes on immobilization but to study in depth the regeneration and repair of cartilage damaged by injury or disease, a major goal of orthopedic science, depends on understanding the structure and function of both the extra cellular matrix and the chondrocytes.

5 Conclusion

The chondrocytes as well as the pericellular matrix of articular cartilage shows changes on immobilization and surface chondrocytes are vulnerable to immobilization injury.

References

ALEXOPOULOS, LG., WILLIAMS, GM., UPTON, ML., SETTON, LA. and GUILAK, F. Osteoarthritic changes in the biphasic mechanical properties of the chondrocyte pericellular matrix in articular cartilage. *Journal of Biomechanics*, 2005, vol. 38, p. 509-517. PMid:15652549.

CANDOLIN, T. and VIDEMAN, T. Surface changes in the articular cartilage of rabbit knee during immobilization. A scanning electron microscopic study of experimental osteoarthritis. *Acta Pathologica et Microbiologica Scandinavica, 19*80, vol. 88, p. 291-97

COLE JUNIOR, MB., NARINE, KR. and ELLINGER, J. Morphological evidence of the shedding of chondrocytes from the articular surface in neonatal rats: relationship to the interlacunar network. *Anatomical Record*, 1983, vol. 206, p. 439-46. PMid:6625202. http://dx.doi.org/10.1002/ar.1092060409

FARNUM, CE. and WILSMAN, NJ. Pericellular matrix of growth plate chondrocytes: a study using postfixation with osmium-ferrocyanide. *Journal of Histochemistry and Cytochemistry*, 1983, vol. 31, p. 765-75. http://dx.doi.org/10.1177/31.6.6841972

FINSTERBUSH, A. and FRIEDMAN, B. Early changes in immobilized rabbits knee joints: a light and electron microscopic study. *Clinical Orthopaedics*, 1973, vol. 92, p. 305-319. PMid:4710841. http://dx.doi.org/10.1097/00003086-197305000-00027

GUILAK, F. Compression-induced changes in the shape and volume of the chondrocyte nucleus. *Journal of Biomechanics*, 1995, vol. 28, p. 1529-42. http://dx.doi.org/10.1016/0021-9290(95)00100-X

JADIN, K., WONG, B., BAEG, W., WILLIAMSON, A. and SCHUMA, B. Depth-varying density and organization of chondrocytes in immature and mature bovine articular cartilage assessed by 3d imaging and analysis. *Journal of Histochemistry and Cytochemistry*, 2005, vol. 53, p. 1109-19. PMid:15879579. http://dx.doi.org/10.1369/jhc.4A6511.2005

JULKUNEN, P., WILSON, W., JURVELIN, JS. and KORHONEN, RK. Composition of the pericellular matrix modulates the deformation behaviour of chondrocytes in articular cartilage under static loading. *Medical & Biological Engineering & Computing*, 2009, vol. 47, p. 1281-90. http://dx.doi.org/10.1007/s11517-009-0547-8

KAAB, MJ., RICHARDS, K., GWYNN, I. and NOTZIL, HP. Deformation of chondrocytes in articular cartilage under compressive loads: morphological study. *Cells Tissues Organs*, 2003, vol. 175, p. 133-9. http://dx.doi.org/10.1159/000074629

LANGENSKIOD, A., MICHELSSON, JE. and VIDEMAN, T. Osteoarthritis of the knee in the rabbit produced by immobilization. Attempts to achieve a reproducible model for studies on pathogenesis and therapy. *Acta Orthopaedica Scandinavica*, 1979, vol. 50, p. 1-14. http://dx.doi.org/10.3109/17453677909024083

POOLE, AR., KOJIMA, T., YASUDA, T., MWALE, F., KOBAYASHI, M. and LAVERTY, S. Composition and structure of articular cartilage: a template for tissue repair. *Clinical Orthopaedics and Related Research*, 2001, vol. 391, p. 26-33. PMid:11603679.

POOLE, CA., AYAD, S. and GILBERT, RT. Chondrons from articular cartilage. Immunohistochemical evaluation of type VI collagen organisation in isolated chondrons by light, confocal and electron microscopy. *Journal of Cell Science*, 1992, vol. 103, p. 1101-10. PMid:1487492.

SOOD, SC. A study of the effects of experimental immobilisation on rabbit articular cartilage. *Journal of Anatomy*, 1971, vol. 108, p. 497-507. PMid:4102518. PMCid:1234185.

WATRIN, A., RUAUD, JPB., OLIVIER, PTA., GUINGAMP, NC., GONORD, PD. and NETTER, PA, etal. T2 mapping of rat patellar cartilage. *Radiology*, 2001, vol. 219, p. 395-2. PMid:11323463.

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