Original Article

Effects of immobilisation and re-mobilisation on superficial zone of articular cartilage of patella in rats

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Abstract

Objective: To determine the staining grades and morphological changes in the cells of articular cartilage of patella on immobilisation and re-mobilisation in rats.

Methods: A total of 120 Sprague Dawley rats were divided into four groups of 30 animals. The study was done between July and December 2009. Group I consisted of control animals that were not immobilised. Group 2 were immobilised for four weeks. Group 3 consisted of animals that were immobilised for four weeks and re-mobilised for four weeks. Group 4 consisted of animals that were immobilised for four weeks and re-mobilised for eight weeks. At the end of the period, the knee joint was dissected in sagittal plane along with patella. Tissue specimens were stored in 10% formalin for 48 hours. After processing for making paraffin blocks, 10µm and 7 µm sections were cut from the same block and stained with Alcian Blue and Haematoxylin and Eosin stain.

Results: Extensive necrotic changes were observed on four weeks immobilisation. On four weeks re-mobilisation after four weeks of immobilization, the superficial zone was sloughed off, but on eight weeks re-mobilisation after four weeks of immobilization, regeneration was seen in the superficial zone. The superficial zone was affected both in immobilisation and re-mobilisation.

Conclusion: After eight weeks re-mobilisation, regeneration was still going on. No conclusion can be drawn regarding the exact time required for complete reversibility of the changes in the cartilage.

Keywords: Immobilisation, Re-mobilisation, Rats, Articular cartilage, Patella (JPMA 62: 531; 2012).

Introduction

Cartilage is a firm and flexible connective tissue capable of rapid turnover and specialised to absorb and resist compression. There are three types of cartilages; hyaline, elastic and fibro.[1] Articular cartilage is a hyaline cartilage which lines the articular margins of long bones and normally functions as a low-friction, shock-absorbing and load-bearing material in joints. Histologically, the rat cartilage is composed of chondrocytes embedded in a matrix consisting of highly anionic, hydrated proteoglycans, and a network of collagen fibrils. The structure of mature articular cartilage is organised in four zones; superficial (5% of cartilage thickness), transitional (35% of cartilage thickness), radial and hypertrophic (60% of cartilage thickness). In immature form, it lacks the radial zone.[2] The most superficial tangential layer consists of collagen fibers and a few ovoid to flattened chondrocytes arranged in a meshwork pattern. The intermediate or transitional layer contains chondrocytes that are larger, randomly spaced with collagen fibers randomly oriented. In the radial layer, chondrocytes are in vertical columns separated by collagenous fibrils. The deepest layer is called the hypertrophic layer that contains very large chondrocytes which are at various stages of degeneration.[3]

Cartilage is avascular as well as aneural.[4] The popular concept is that loading and unloading plays a role in nutrition, and immobilisation causes degenerative changes. Those degenerative changes have been studied by researchers over the years. Until now the focus of research has been the whole cartilage.[5-8] and zonal changes have not been studied in detail individually. Since superficial zone is the first in line to face friction, wear and load-bearing, so, keeping this in mind, the present study was designed to observe the effects of immobilisation and re-mobilisation on the superficial zone of articular cartilage of patella in young rats.

Material and Methods

Simple random sampling technique was used. Each of the N population members (rats) was assigned a unique number. The numbers were placed in a bowl and thoroughly mixed. Then n numbers was selected. Population members having the selected numbers were included in the sample. For group 2, we used sampling with replacement. For other groups we used sampling without replacement. An interventional experimental design was used for the study which was carried out during July and December 2009.

For the study, male rats belonging to the Sprague

Dawley strain were procured from the National Institute of Health, Islamabad, and the study was carried out at the animal house of the College of Physicians and Surgeons (CPSP), Islamabad. The rats 12-weeks-old were included because at this age all zones can be observed microscopically. Rats found limping or with any limb deformity were excluded. The final study population comprised 120 rats.

These animals were divided into four groups. The animals were kept in separate cages and numbers were assigned to each animal with the help of a marker. The number of groups was written on the cages. The right hind limbs of rats were immobilised with Plaster of Paris (PoP) cast. Care was taken to cover the knee joint completely. Animals in these groups were immobilised, re-mobilised and sacrifised at different periods: Group 1 - Control group of 30 animals who were left un-immobilised; Group 2 - Experimental group of 30 animals who were immobilised for four weeks, Group 3 - Experimental group of 30 animals who were immobilised for four weeks and then re-mobilised for four weeks; Group 4 - Experimental group of 30 animals who were immobilised for four weeks and then re-mobilised for eight weeks.

At the end of the experimental period, the rats were anaesthetized with chloroform. Each group was dissected, and the dissected specimens were labelled accordingly so that the specimen could be studied individually. The dissection along with processing and staining was performed by one of the researchers in the laboratory of the Anatomy Department of the CPSP regional centre in Islamabad. The skin over the knee joint was dissected and the joint along with patella was exposed. The knee joint was cut in the sagittal plane and patella with its hyaline cartilage covering was stored in 10% formalin for 48 hours. The specimen was de-calcified using ethylene diamine tetra acetic acid (EDTA).

After processing for making paraffin blocks, 10µm and 7µm sections were cut by using microtome from the same block and stained. The groups were assigned codes and stickers of code were applied on each slide. The particular group was given a code by one researcher, while the others did not know which code was for which group. Alcian Blue stain was used for 10µm-thick sections to demonstrate proteoglycan content.[9] Haematoxylin and Eosin (H&E) stain was used for 7µm-thick sections to study routine histology of patellar articular cartilage.

Parameters studied included the number of cells /unit area in control, immobilised and re-mobilised groups; and the intensity of staining in control, immobilised and re-mobilised groups using Alcian Blue stain for evaluating cartilage matrix.

The data was analyzed using SPSS version 10. Quantitative data was interpreted with the help of unpaired Student's' test. Regarding qualitative data, percentages of staining grades in various groups were calculated. The quantitative data included number of cells with different shapes. A p-value of ≤ 0.05 was taken as significant and p-value of ≤ 0.001 was taken as highly significant. A p value of > 0.05 was taken as insignificant.

Results

When the knee joint was dissected, adhesions were found in 10 animals between the joint spaces in immobilised animals. In control animals, the joint was normal with glistening surface.

The microscopic structure of the articular cartilage of all control rats exhibited the normal histological architecture. In the group immobilised for four weeks it was noted that in 12 of the immobilised animals, in the superficial zone the linear orientation of elliptical cells was disturbed. There was migration of cells from the superficial to the transitional zone. The cells were also spindle shaped in two of the specimen. In some specimen empty lacunae were observed. The number of elliptical cells was decreased highly significantly in the immobilised group ($p < 0.001$) (Table-1).

When the patellofemoral joint was dissected, overlying skin was difficult to remove in 10 immobilised animals. The skin over the joint was ulcerated. In control animals the joint was normal.

In the immobilised animals extensive necrotic changes were observed. Erosion began from the superficial zone and spread towards the transitional and radial zones. In four immobilised animals, the entire articular cartilage was eroded (Figure-1). The number of cells were 04.93±1.48 in group 3 while in the immobilised group, it was14.93 ± 0.89 which showed a highly significant decrease (Table-1). Loss of staining was also observed in 80% of the specimen as compared to the control group (Table-2). In each group, the number of specimen

Table-1: Number of cells/unit area in the control and experimental groups.

Group	Number of Cells/Unit Area
Group 1	32.93 ±1.48
Group 2	14.93 ± 0.89***
Group 3	04.93±1.48***
Group 4	33.43±0.43***

Values are Mean ± S.D. Significant difference by student't test. p<.001***; comparison between group 1 and group 2, group 2 and 3 and group 2 and group 4.

Table-2: Staining grades in superficial zone in control and experimental groups.

Group	% of specimen with Intensely stained superficial zone	% of specimen with unstained superficial zone
Group 1	100	00
Group 2	20%	80
Group 3	15%	85
Group 4	40%	60

which had lost staining was calculated and their percentages were calculated accordingly. Large population of inflammatory cells had invaded the patellofemoral joint space. The necrotic area was flooded with inflammatory cells.

When the knee joint was dissected, patellofemoral joint space was reduced in all experimental groups, while in control animals, the joint was normal.

In the group re-mobilised for four weeks after four weeks of immobilization, all the animals showed there was shedding of superficial zone (Figure-2). In six animals, the entire superficial zone was sloughed off. Split segments were seen separating and lodging in the patellofemoral space. The number of cells were increased in a significant manner as compared to the immobilised group. Loss of staining with Alcian Blue stain was also observed in 85% of the specimen as compared to the control group. As compared to the immobilised group, the staining grades were almost the same at 85, and in 80% full staining intensity was observed.

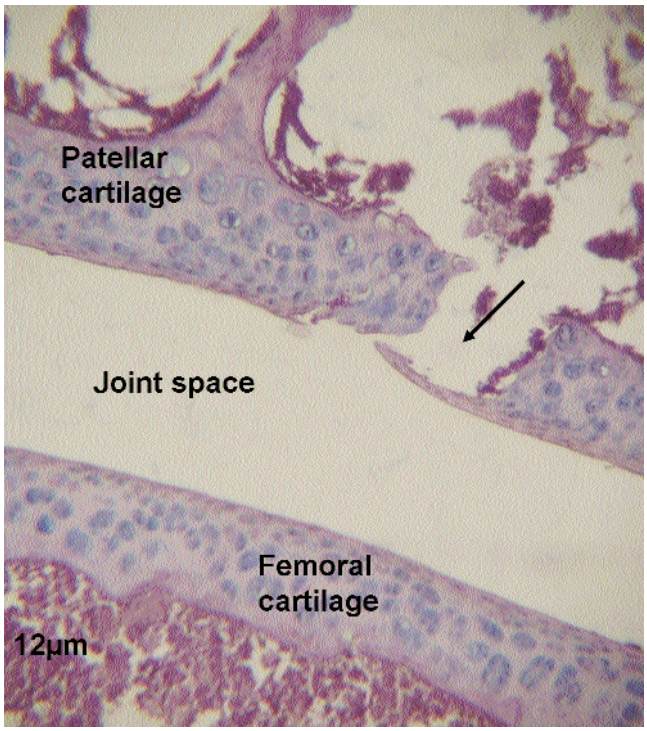

Figure-1: Photomicrograph showing patellar cartilage and joint space. Arrow showing disruption along entire thickness of cartilage. Alcian blue stain. Bar 100 µ m.

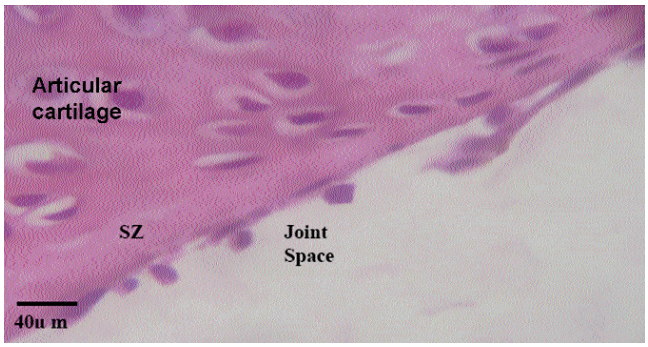

Figure-2: Photomicrograph showing a section of articular cartilage of patella. Cells from the superficial zone (SZ) can be seen projecting from the surface, ready to be shed into the joint space. Bar 40 µ m. H&E stain.

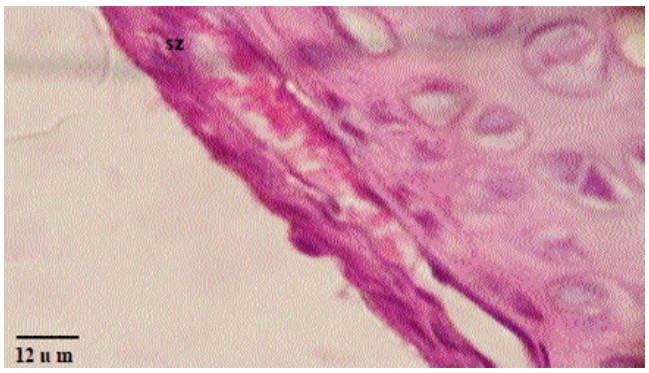

Figure-3: Photomicrograph showing superficial zone of the articular cartilage of patella. Vessel can be seen in the superficial zone. H & E Stain. Bar 12µm.

In the group re-mobilised for eight weeks after four weeks of immobilization, small regenerated areas were observed in 10 re-mobilised animals. Regeneration began in the superficial zone. The re-generated tissue was vascularised; and the vessels could be seen clearly (Figure-3). Population of round cells was seen in addition to the elliptical cells in the re-mobilised group as compared to the immobilised group, but it was not statistically significant. The percentage of intensely stained specimen was 40, and the loss of staining was still 60% as compared to 100% in the control animals. The percentage of intensely stained specimen was 40% as compared to 20% in the immobilised group.

Discussion

On four weeks immobilization, necrotic areas were observed in superficial zone in 12 immobilised animals. Other authors have described proliferation of fibrous connective tissue spreading from the superficial zone in the knee joint.[10] In a study done on rabbits after four weeks immobilisation, there was pressure necrosis and marked decrease in the thickness of superficial zone in the cartilage of knee joint.[8]

It is the prevailing view that once the integrity of the superficial zone of cartilage is lost, the underlying cartilage is subjected to abnormally high strains.[10] Some studies show that the superficial zone contains various factors for the maintenance of normal healthy cartilage.[10] Identification of progenitor/stem cell populations specific to the articular cartilage is an unmet priority for cartilage tissue engineering.[11] It was seen in the present study that the degeneration started from the superficial zone and then involved the other zones. In the past, researchers

have described the incidence of cleft formation, matrix fibrillation and decrease in thickness in articular cartilage of femur in rats whose knees had been immobilized.[12,13]

Fibrillation was seen in the superficial zone which has been described by pervious researchers as the process in which the matrix becomes frayed and splits in the direction of the principal collagen fibrils.[14] The pathogenesis of fibrillation can be that due to immobilisation the cells undergo hypoxia and necrosis follows. Degraded cartilage is seen and cells in immediate vicinity become deformed.[15]

In the present study, the superficial layer of the cartilage was the first to show this change, but when compression was maintained for longer periods, the cells of the deeper part of the cartilage were affected involving the whole thickness layer after layer. Decrease in the number of cells can be related to apoptosis as has been proved by past researchers.[12] Surface fibrillation of articular cartilage is an early sign of degenerative changes in the development of osteoarthritis.[16] The prominent features of necrosis as observed in this study were lesions seen in sections that appeared as clefts and decreased staining of matrix in that area was identified. Clefts and fissures were large enough and also cystic areas were observed in between the clefts, and the fissures serve as source of cells which help in regeneration.[17-19]

Compared with the knowledge on the affects of immobilisation, the effects of re-mobilisation on musculoskeletal tissues have not been well established.[20,21] It would not be surprising if the serious structural changes induced by immobilisation were unrestorable.[22] In the present study, it was identified that on four weeks re-mobilisation, the superficial zone was sloughed off. This finding can be correlated to the fact that sometimes the process of degeneration goes on even on re-mobilisation because researches have shown that shielding or preventing the newly formed cartilage from stress also leads to better regeneration of the cartilage.[21,22] Premature and intensive mobilization after immobilisation at this time leads to breakdown of the newly-formed tissue that was produced during the immobilisation period.[23] When the duration of re-mobilisation was lengthened, recovery was seen and regeneration also started in the superficial zone. The regenerated tissue was vascularised and vessels could be seen clearly. On re-mobilisation, the appearance of round cells can be related to the migration of cells from other zones.

The study showed that the superficial zone of the articular cartilage was affected both on immobilisation and re-mobilisation. With further studies on the superficial zone, more effective treatment plans can be initiated and modified.

The study had its limitations because complete blinding of the person collecting data and the one doing the analysis was not possible because research was part of a thesis. But allocation of codes was done to eliminate the chance of bias.

Conclusion

Superficial zone is the first zone to sustain changes on immobilisation and re-mobilisation. After eight weeks re-mobilisation regeneration was still going on in the superficial zone. Hence, it can be said that a much longer duration of re-mobilisation as compared to immobilisation was required for healing in the articular cartilage.

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