TRAUMATIZED ARTICULAR CARTILAGE OF KNEE JOINT TREATED WITH BONE MARROW MESENCHYMAL STEM CELLS IN WISTAR ALBINO RATS

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Abstract
The aim of this study is to find out the effect of bone marrow mesenchymal stem cells (BMSCs) in repairing the traumatized articular cartilage of knee joint in Wistar albino rats and to assess its healing potential by gross motor activities and micro anatomy. study. Materials and Methods: 14 male Wistar albino rats were divided into 2 groups (Control and Experimental) Control group - Normal rats (4 rats) and Experimental groups. Experimental group is further divided into 2 groups, Sub Group A - Injured articular cartilage without BMSCs (5 rats) and Sub Group B - Injured articular cartilage with BMSCs (5 rats). For experimental group rats left knee joint was selected randomly and it was opened and articular cartilage was damaged. In Sub Group B alone BMSCs was injected at the site of injury. Experimental groups rats were observed for gross motor activities on 3rd, 10th and 30th day and Histology study was also done at the same days. Results: Previous study revels BMSCs had positive impact in structural reformation of a damaged menisci in knee joint and this study shows rapid functional and structural improvement in rats treated with BMSCs than rats without BMSCs treatment. Conclusion: Bone marrow mesenchymal stem cells can be used as an alternative treatment for osteoarthritis in human beings.

Key words: Stem cells, BMSC, Multipotent cells, Adult stem cells, Wistar Albino rat, Obligatory differentiation cells.

Introduction
Bone Marrow Mesenchymal Stem Cells(BMSCs) has ability to differentiate into either bone, cartilage, muscle, tendon, ligament, nerve, skin, fat cells, so a study was done to find out the effect of BMSCs in repairing the damaged articular cartilage of knee joint in Wistar albino rats. Articular cartilage was selected because age induced (degenerative) damage is more common to it in human beings, example Osteoarthritis.Osteoarthritis (OA) is the most common type of arthritis. Its high prevalence especially in the elderly made them disability and deformity. OA affects certain joints yet spares others. Commonly affected joints include cervical and lumbar spine, hip, knee and first metatarsal phalangeal joint (MTP). It was estimated that over 33 billion dollar are spent annually in the united states for osteoarthritis treatment.

OBJECTIVES
- To assess the repairing potential of BMSCs in damaged articular cartilage.
- To assess the functional status of the repaired articular cartilage by gross motor activities.
- To assess the structural status of the repaired articular cartilage by micro anatomy study.

MATERIALS REQUIRED
- 14 male Wistar albino rats
- Cultured BMSCs from 1 young Wistar albino rat
- Antibody CD 44 and CD 34
- 1 ml syringe (12 nos)
- Ketamine (500 mg, 1 vial)
- Dissection kit
- Hand glove
- Dressing kit
- Small stair case (15 steps)
- Transparent glass cylinder (25 cm height × 10 cm breadth)

METHODOLOGY
15 male Wistar Albino rats (100-200 gm weight) were taken, of these 1 young rat (6 months age ) used for cell culture and rest 14 rats of age group above 1 year were divided into Control group and Experimental group.

1) Control group (4 rats).
2) Experimental groups were divided into
   - Sub group A (5 rats ) - Rats with articular cartilage damage deprived of BMSCs
   - Sub group B (5 rats) - Rats with articular cartilage damage treated with BMSCs

Rats was placed and maintained in same environment in the cage . Each cage was labeled with their group name and body weight. Food and fresh water was provided for rats.
PROCEDURE
Bone marrow was aspirated from 1 young rat under anesthesia. Aspirated bone marrow stem cells were kept in T flask containing CULTURE MEDIUM - Dulbecco's Modified Eagle's Minimal essential medium (DMEM). Cultured bone marrow mesenchymal stem cells were observed on the first, second and up to the sixth passage. The growths of the cells were slow and cell with process were seen. The second passage was done 20 days after the first passage. The time interval between the subsequent passages reduced. This showed that the cells adapted to the in-vitro environment. 6th passage cells were used for the transplant.

Bone marrow mesenchymal stem cells (BMSC) were separated from in vitro culture by adding Antibody CD44 and CD34. BMSCs were counted and sorted by flow Cytometer. Now BMSCs are ready for transplantation. Wistar Albino rats were divided into

1) Control group (4 rats).
2) Experimental groups were divided
   - Sub group A (5 rats ) - Rats with articular cartilage damage deprived of BMSCs
   - Sub group B (5 rats) - Rats with articular cartilage damage treated with BMSCs

With permission from Institutional Animal Ethical Committee (IAEC/SMC/I/03/2013). All 14 rats were given gross motor activities training for a week before experiment. In operation theater with the help of veterinary surgeon following procedures were carried out. To make rats anesthetized, Ketamine 1 ml (conc.100mg/ml) , Xylazine 0.5 ml (conc. 20 mg/ml) and 8.5 ml normal saline 0.9% were mixed. From this mixture 0.08 ml per 10 g body weight was administered intra peritoneally. In Sub group A and B left knee joint of rat was selected uniformly. Hair over left knee joint of rats were shaved using sterilized blade. Surgical incision was made on anterior aspect of knee joint. Patella bone along with ligamentum patellae was retracted. After reaching the articular ends of knee joint , articular cartilage and sub chondral bone (full thickness articular cartilage damage) along lower end of femur condyle was excavated and removed.

In Sub group A rats incision closed , allowed for natural healing. In Sub group B rats 1 ml of BMSCs injected at injured site and incision was closed. 1 ml contains 1-2 millons BMSCs (Rishbud et al 2008). Incision area was sutured with black silk suture and dressed with betadine. Check computerized tomography was taken for left knee joint to confirm whether articular cartilage was damaged or not and this was compared with the normal right side knee joint. Rate of recovery was observed in both groups. Microanatomy study and functional analysis (gross motor activities like walking, running, hind limb standing and stair climbing) were done at 3rd day, 10th day and 30th day for control and experimental groups rats. Using these findings recovery of articular cartilage was analyzed. After completion of this study Wistar albino rats used were given rehabilitation.

EXPECTED OUTCOME
Faster recovery was expected in Sub group B rats than in Sub group A rats in 3 to 4 weeks.

GRADING FOR GROSS MOTOR ACTIVITIES

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<td>—</td>
<td>Absent</td>
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<tr>
<td>+</td>
<td>Mild</td>
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<td>++</td>
<td>Moderate</td>
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<td>+++</td>
<td>Marked</td>
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</table>

RESULTS

GROSS MOTOR ACTIVITIES

Table 1: Control group (Normal rats) results.
Experimental groups:

Table 2: Sub group A (Rats with articular cartilage damage deprived of BMSCs treatment) results

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ACTIVITIES</th>
<th>DAY 3</th>
<th>DAY 10</th>
<th>DAY 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Walking</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2.</td>
<td>Running</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>3.</td>
<td>Hind limb standing</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>4.</td>
<td>Stair climbing</td>
<td>—</td>
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</tbody>
</table>

Table 3: Sub group B (Rats with articular cartilage damage treated with BMSCs)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ACTIVITIES</th>
<th>DAY 3</th>
<th>DAY 10</th>
<th>DAY 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Walking</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Running</td>
<td>—</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Hind limb standing</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td>Stair climbing</td>
<td>—</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

From above result, Sub group B rats performed gross motor activities better than Sub group A rats from 10th day onwards.

On 30th day, Sub group B rats performed gross motor activities almost similar to normal rats.

HISTOLOGY RESULTS

Table 4: Results of Control group (Normal rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 30</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Well defined lacunae with mature chondrocytes were seen, Interterritorial matrix and territorial matrix was seen clearly and homogeneously stained. (Fig.1)</td>
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</tr>
<tr>
<td>(Normal rats )</td>
<td></td>
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</tbody>
</table>

Figure 1

Table 5: Results of Experimental group - Sub group A (Rats with articular cartilage damage deprived of BMSCs treatment)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>Chondrocytes were destroyed. Irregularities in matrix was observed (Fig.2)</td>
<td>Proliferation of chondrocytes was absent. Inflammatory cells started to appear in matrix. (Fig.3)</td>
<td>Many inflammatory cells were seen in matrix, chondrocytes were very few and marked matrix irregularity was observed. (Fig.4)</td>
</tr>
<tr>
<td>(Sub group A rats)</td>
<td></td>
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http://www.ijre.org
Inflammatory cells seen in matrix of articular cartilage.
Proliferation of chondrocytes were not seen.

Many inflammatory cells were seen in matrix
Chondrocytes were very few
Marked matrix irregularity was observed.

**Experimental group- Sub group B**

**Table 6: (Rats with articular cartilage damage treated with BMSCs)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group (Sub group A rats)</td>
<td>Chondrocytes were destroyed. Irregularities in matrix was observed</td>
<td>Proliferation of chondrocytes with homogeneous matrix seen. Few inflammatory cells also observed</td>
<td>Well defined lacunae with mature chondrocytes seen, Interterritorial matrix and territorial matrix was seen with some irregularity</td>
</tr>
</tbody>
</table>

**Figure 5 (3rd day image)**
Figure 6  (10th day image)

Proliferation of chondrocytes were seen clearly

Figure 7  (30th day image)

- Well defined lacunae with mature chondrocytes was seen
- Interterritorial matrix and territorial matrix was seen with some irregularity

DISCUSSION:
In this study experimental group rats were compared with control group rats under following parameters like gross motor activities (walking, running, hind limb standing and stair climbing), histology study on 3rd, 10th and 30th day. Based on the performance of rats, gross motor activities were evaluated by grading. On comparing the result on 3rd day both sub group A and B rats did not perform gross motor activities. But on 10th day sub group A rats performed walking and stair climbing mildly, running and stair climbing was absent. It may be because of pain. On the other hand by 10th day sub group B rats performed all gross motor activities moderately. This may be due to rapid healing property of articular cartilage by bone marrow mesenchymal stem cells. By comparing 10th day results of both sub group A and sub group B rats, performance of rats treated with bone marrow stem cells was moderate. This implies BMSCs has rapid potential to reduce pain by its anti inflammatory property and by its differentiation property it was converted to chondrocytes and healed the damaged articular cartilage.

By 30th day sub group A rats dint perform gross motor activities. But on the same day sub group B rats performed functional activities (gross motor activities) almost similar to control group rats.3rd day histology report of articular cartilage for both sub groups (A and B) shows damaged articular cartilage and irregularity in matrix, this was the reason for pain so both sub group rats dint perform gross motor activities. On 10th day sub group A rats micro anatomy report of articular cartilage shows inflammatory cells and no new chondrocytes were seen. Damage of articular cartilage was not repaired. Since the under lying pathology was not repaired rats cant do functional activities well. But in sub group B rats, active proliferation of chondrocytes observed and homogeneous matrix seen. Because, BMSCs injected in sub group B rats differentiated into chondrocytes and matrix components. Though articular cartilage in sub group B rats is healing better than sub group A rats by 10th day, sub group A rats performed gross motor activities moderately.

On 30th day sub group A histology report revels many inflammatory cells and negligible amount of chondrocytes and marked irregularity of matrix, this made rats not to perform functional activities. On other hand 30th day histology report of sub group B rats revels well defined lacunae with mature chondrocytes, Interterritorial matrix and territorial matrix was seen with some irregularity. Rats of sub group B performed gross motor activities almost similar to control group rats because underlying pathology has recovered. Even on 30th day of Computed tomography results of sub group A rats, articular cartilage was not healed. But articular cartilage of sub group B
healed moderately on 10th day itself and by 30th day marked healing was observed due to the effect of BMSCs. Mauro Krampera et al (2006) stated that Allogeneic goat BMSCs heals damaged medial meniscus and anterior cruciate ligament structurally in 10 goats. David et al (2005) stated that BMSCs transplant heal articular cartilage damage but thinning occurs later. These researchers confirmed the healing of articular cartilage through histology study but in this study functional status of healed articular cartilage was assessed with structural back ground (Histology study).

CONCLUSION:
Articular cartilage is a type of hyaline cartilage and devoid of blood and nerve supply.

Though articular cartilage is devoid of nerve supply, damage of it is not sensed by patients until damage reaches sub chondral bone which has ample of nerve endings. On the other hand articular cartilage is not having the property of self healing because of devoid of blood supply. So once articular cartilage damage reaches sub chondral bone it starts heals by replacement of articular cartilage by fibro cartilage. Fibro cartilage cannot provide firm structural and flexible support as hyaline cartilage, so again it will get damage on weight bearing.

Articular cartilage damage is seen more common in osteoarthritis (OA), a degenerative condition occurs mainly due to wear and tear of articular cartilage and also in various knee pathology. People with OA become disabled due to pain and deformity developed at later stage of this disease. To overcome all these problems regeneration of articular cartilage by BMSCs in the damaged area of it gives a great permanent remedy for people suffering from articular cartilage disease. Rosa McCarty et al (2005) stated that BMSCs transplantation lead to chondrogenesis in damaged cartilage. Ali Mobasheri et al (2011) stated that BMSCs may used as treatment option for Arthritis. So BMSCs may be used as a treatment option for articular cartilage damage in OA.

References
5. Rosa McCarty, David Leavesley and Paul Simmons, Application of Mesenchymal Stem Cells for Repair and Regeneration of Cartilage and Bone, Vol 36 No 1 April 2005