



## Therapeutic Potential of Hyaluronic acid hydrogel /Bone marrow stem cell-conditioned medium on Articulating Surfaces Associated with Induced Arthritis in Rabbit's TMJ

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### KEYWORDS

TMJ, osteoarthritis, hyaluronic acid hydrogel, bone marrow stem cell, articulating surfaces.

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### ABSTRACT

**Aim:** To assess hyaluronic acid hydrogel /bone marrow stem cell-conditioned medium (MSC-CM with HA hydrogel) on articulating surfaces associated with induced arthritis in rabbit's TMJ. **Subjects and methods:** , fifty rabbits were employed. A technique to induce arthritis was performed on forty rabbits using a double IA injection of CFA. There were made five groups of rabbits. 10 rabbits in Group I (the normal group) and 10 in Group II (the arthritic group) did not receive any treatment, respectively. Ten animals each got a bilateral IA injection of 0.2 ml of Group III hyaluronic acid hydrogel.10 rabbits each got a bilateral IA injection of 0.2 ml of Group IV and bone marrow stem cell condition media.10 rabbits were given bilateral IA injections of 0.2ml of a combination therapy (hyaluronic acid hydrogel and bone marrow stem cell condition medium (Group V). After the fourth week of injection-based therapy, all rabbits will be anesthetized then euthanized. Dissection, decalcification, and preparation of the mandible for Masson's Tri-chrome staining. **Results:** Those different treatment modalities responded to osteoarthritis in different ways, with MSC-CM with HA hydrogel being regarded as the most effective treatment of induced temporomandibular joint osteoarthritis among the tested treatment modalities. **Conclusion:** It was possible to conclude, within the limitations of the current study, MSC-CM with HA hydrogel being regarded as the most effective treatment of induced temporomandibular joint osteoarthritis.

### INTRODUCTION

The group of degenerative diseases known as osteoarthritis (OA) predominantly impacts the joints. Chronic discomfort, synovitis, and a slow deterioration of cartilage are its distinguishing features. (Poole, 2012) Depending on how serious the problem is, arthritis may be treated with invasive, minimally invasive, or non-invasive procedures. (Murphy et al., 2013)

A novel idea called MSC-Condition medium would use an acellular derivative of MSC to replace MSC effects in regenerative medicine.

Because it is possible to employ commercial, allogeneic human MSC in clinical studies, it could reduce the cost and increase the safety of MSC therapy. (Katagiri *et al.*, 2016) The conditioned medium is created by growing MSC under various circumstances, and it has the ability to repair tissue without the need for additional MSC implantation. (Meiliana *et al.*, 2019) Because it contains a lot of growth factors, angiogenic factors, hormones, and cytokines, CM made by cultivating MSC for a few days has a lot of promise. When MSC are supplied to living recipients, those bioactive chemicals are regarded as the primary mechanism for tissue regeneration. (Katsuda *et al.*, 2013)

A naturally occurring substance of joint tissues, hyaluronic acid (HA), or intra-articular hyaluronic acid, is continuously released into the synovial fluid where it acts as a lubricant, anti-inflammatory, and painkiller. As a result of research showing decreased HA molecular weight and concentration in arthritic joints, injectable preparations known as visco-supplementation were created. (Marshall, 2000) Intra-articular HA is employed in two separate treatment plans for TMJ problems. It is used as an addition to arthrocentesis, a surgical treatment where the joint is flushed to eliminate inflammatory mediators, as well as alone for visco-supplementation. Both treatments aim to lessen inflammation and improve the function of TMJ. (Mountziaris *et al.*, 2009)

## MATERIALS AND METHODS

### Animals and experimental design

In this investigation, fifty rabbits were employed. A technique to induce arthritis was performed on forty rabbits using a double IA injection of CFA. There were made five groups of rabbits. 10 rabbits in Group I (the normal group) and 10 in Group II (the arthritic group) did not receive any treatment, respectively. Ten animals each got a bilateral IA injection of 0.2 ml of Group III hyaluronic acid hydrogel. 10 rabbits each got a bilateral IA injection of 0.2 ml of Group IV and bone marrow stem cell

condition media. 10 rabbits were given bilateral IA injections of 0.2ml of a combination therapy (hyaluronic acid hydrogel and bone marrow stem cell condition medium (Group V). After the fourth week of injection-based therapy, all rabbits will be anesthetized then euthanized. Dissection, decalcification, and preparation of the mandible for Masson's Tri-chrome staining.

### Preparation and collection of BMSCs-CM

Cryopreserved cell line with cell density 106 from the Nile Centre for Experimental Research, Mansoura, Egypt, was used to produce BMSCs. At passage 3 and after BMSCs reached 70-80% confluence, the growth medium was removed, the cells washed twice with phosphate buffer saline (PBS), the culture medium was replaced with serum-free MEM, then the culture flasks were incubated for 72 h before collecting and centrifuging the medium at 1500 rpm for 5 min at 4°C and the supernatants was collected and centrifuged again at 3000 rpm for 3 min at 4 oC. Then, the resulting supernatants was filtered through a 0.22- $\mu$ m filter unit and stored in small tubes at -80 oC until used in the experiments. A biological safety cabinet (class II A2, UNIL@B) was used to carry out these steps aseptically.

## RESULTS

### Masson's Tri-chrome stain results.

**Group I:** It demonstrates how collagen fibers in condylar cartilage were arranged normally, increased the lacuna around them, and decreased away from them. Positively stained mean% area of 18.83 was used to detect these features.

**Group II:** Positively stained mean% area of 4.87 significantly decreased, and collagen fibers were disorganized.

**Group III:** Compared to the arthritic group, this group displayed an increase in the collagen fibers' positively stained mean% area 6.14 and normal distribution surrounding cell lacunae.



**Group IV:** this group had a significantly significant increase in the collagen fibers positively stained mean% area 12.21 with a normal organization around cell lacunae.

**Group V:** this group displayed a significantly significant increase in the collagen fibers that were favorably stained on a mean percentage area basis (12.29).

#### Comparison between the studied groups regarding Masson's Trichrome stain.

Trichrome	N	Mean	Std. Deviation	Post hoc LSD test			
				P1	P2	P3	P4
<b>Group I</b>	10	18.83	2.30	-	-	-	-
<b>Group II</b>	10	4.87	0.250	≤0.001	-	-	-
<b>Group III</b>	10	6.14	1.05	≤0.001	0.053 (NS)	-	-
<b>Group IV</b>	10	12.21	1.32	≤0.001	≤0.001	≤0.001	
<b>Group V</b>	10	12.29	1.38	≤0.001	≤0.001	≤0.001	0.09 (NS)
<b>ANOVA test</b>			F=155.33, p≤0.001				

Notes: Data are Mean and Standard deviation SD). Test of significance is One-Way ANOVA. Post-Hoc Tukey HSD tests showed a statistically significant difference between the tested groups.

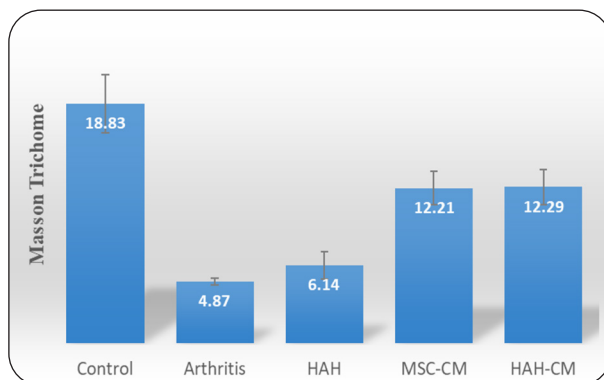


Fig. (1) Bar chart representing descriptive statistics for Masson's Trichrome stain among the studied groups

#### DISCUSSION

TMJ arthritis causes swelling, discomfort, mobility restrictions, and stiffness. 70% of the population reports having TMJ problems (*Tanaka E, 2008*). regenerative medicine and Tissue engineering (RM- TE) may be able to replace the TMJ's damaged tissues and help the joint regain its function. (*Salash J.R., 2016*).

In this study, arthritic group sections had abnormal bone trabeculation. This result in agreement with *Xu et al. (Xu et al., 2016)*. *G. S. El-Tanbouly et al* also explained that there were adaptive changes in subchondral bone in CFA induced arthritis group. (*El-Tanbouly and Abdelrahman, 2022*). Also, *Sabine Kuchler-Bopp et al.* detected a loss of the TMJ cartilage fibrous layer due to erosive alterations that mostly affected the surface areas of the articular cartilage in arthritis. (*Kuchler-Bopp et al., 2020*).

In Hyaluronic acid hydrogel treated group there was an improvement in fibrous layer, condylar cartilage thickness and bone trabecular volume. These findings are in agreement with **Suwannaloet et al.** who observed that HA has anti-inflammatory, and chondroprotective effects and it is effective in cartilage repair and osteochondral defect that made in knee joint of rabbit (*Suwannaloet et al., 2012*). According to **Elmorsy S et al.** Cross-linked HA has a substantially longer half-life in the joint than normal HA, leading to persistent benefits in joint lubrication and a strengthened chondroprotective impact. (*Elmorsy et al., 2014*).

In BMSC -CM treated group as comparison to the arthritic non-treated group, there was a considerable increase in the condylar cartilage thickness with practically normal bone trabeculae organization. These results are consistent with **chen w et al.** who explained that intraarticular injection of MSCs-CM showed nearly normal appearance of chondrocytes as CM reduced the apoptosis of chondrocytes under OA environment and it also protected the microarchitecture of subchondral bone. (*Chen et al., 2019*).

In BMSC-CM with HA hydrogel treated group there was an increase in fibrocartilaginous layer thickness, a decrease in disc thickness, an increase in condylar cartilage thickness. This group has a better regenerative ability compared to HA hydrogel alone and MCS-CM alone. Similar to our findings **Arifka M et al.** observed that Cytokines/chemokines, as well as anti- and/or pro-inflammatory molecules, are released in response to the implantation of SC-CM into the HA hydrogel (*Arifka et al., 2022*).

**Köhnke R et al.** reported that the combination of HA and mesenchymal stromal cells develop cartilage healing in a rabbit model with TMJ OA (*Köhnke et al., 2021*). In a beagle-dog model, **Li et al.** indicated that the combination of mesenchymal bone marrow stromal cells (BMSCs) and HA had a greater therapeutic effect than HA alone. (*Li et*

*al., 2018*). In addition, **Chiang et al.** observed that as compared to HA injections administered individually, intra-articular injections of allogeneic MSCs suspended in HA significantly reduced the progression of osteoarthritis in rabbits. (knee). (*Chiang et al., 2016*).

The positive mean% area and collagen fiber arrangement in the arthritic non-treated group in the current study were significantly lower than in the negative control group, according to the results of MTC staining. This is consistent with the findings of **Chabaud et al.** who found that IL17 produced by T cells as a result of arthritis inhibited the synthesis of cartilage proteoglycan and caused its breakdown both in vivo and ex vivo. (*Koshy et al., 2002*).

The HA hydrogel treated group showed a moderate increase in the collagen fibers positively stained mean % area the size of chondrocyte was relatively smaller than a normal one in the mid zone. These results are in agreement with **Chung JY et al.** who found that HA hydrogel has a beneficial effect on cartilage regeneration in vivo. In the MSC-CM treated group showed a highly significant increase in the collagen fibers positively stained mean % area with normal arrangement around cell lacunae relative to arthritic group. (*Chung et al., 2014*). These results are consistent with **Marlina M. et al.** who clarified that by blocking matrix degradation enzymes, the growth factors in the SMMSCs-CM play a chondroprotective role that reduces hypertrophic markers and promotes chondrogenesis. (*Marlina et al., 2021*).

In MSC-CM with HA hydrogel showed the most developed form of cartilaginous tissue repair in terms of cellular organization, shape, quantity, creation of lacunae, and articular contour. These outcomes are consistent with **Chiang E. R. et al.** (*Chiang et al., 2016*) who discussed that intra-articular injection of HA combined with MSCs treated cartilage in knee joint of rabbits with induce OA.



## CONCLUSION

It was possible to conclude, within the limitations of the current study, that different treatment modalities responded to osteoarthritis in different ways, with MSC-CM with HA hydrogel being regarded as the most effective treatment of induced temporomandibular joint osteoarthritis among the tested treatment modalities.

## RECOMMENDATION

More research must be done on the treatment of osteoarthritis with various doses that must then be tried on humans. In order to treat temporomandibular joint osteoarthritis, it is being investigated whether combining various therapy modalities will be more effective.

## DISCLOSURE

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. There is no Conflict of Interest

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## القدرة العلاجية للجل المائي لحمض الهيالورونيك/الوسط المغذي المستخلصة من الخلايا الجذعية لنخاع العظام علي الاسطح المفصالية للفك العظمي في الارانب المصابة بالالتهاب

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### الملخص :

**الهدف:** تقييم وسط مكيف الخلايا الجذعية لحمض الهيالورونيك / هيدروجيل نخاع العظم (MSC-CM مع HA هيدروجيل) على الأسطح المفصالية المرتبطة بالتهاب المفاصل المستحث في المفصل الفكي الصدغي للأرانب.

**المواد والاساليب:** تم استخدام خمسين أرنباً. تم إجراء تقنية للحث على التهاب المفاصل على أربعين أرنباً باستخدام حقنة IA مزدوجة من CFA. كانت هناك خمس مجموعات من الأرانب. 10 أرانب في المجموعة الأولى (المجموعة الطبيعية) و10 في المجموعة الثانية (المجموعة المصابة بالتهاب المفاصل) لم يتلقوا أي علاج على التوالي. حصلت عشرة حيوانات على حقنة IA ثنائية لكل منها 0.2 مل من هيدروجيل حمض الهيالورونيك المجموعة الثالثة. حصلت كل أرانب على حقنة IA ثنائية قدرها 0.2 مل من المجموعة الرابعة ووسائط حالة الخلايا الجذعية لنخاع العظم. تم إعطاء 10 أرانب حقن IA ثنائية بمقدار 0.2 مل من العلاج المركب (حمض الهيالورونيك هيدروجيل ووسط حالة الخلايا الجذعية لنخاع العظم (المجموعة الخامسة). بعد الأسبوع الرابع من العلاج القائم على الحقن. سيتم تخدير جميع الأرانب ثم القتل الرحيم. تشريح وإزالة الكلس وإعادة الفك السفلي لثلاثي ماسون - صبغة الكروم.

**النتائج:** استجاب طرق العلاج المختلفة لالتهاب المفاصل العظمي بطرق مختلفة. حيث يعتبر MSC-CM مع هيدروجيل HA العلاج الأكثر فعالية لالتهاب المفاصل العظمي الفكي الصدغي بين طرق العلاج التي تم اختبارها.

**الخلاصة:** في حدود الدراسة الحالية. يعتبر MSC-CM مع HA هيدروجيل العلاج الأكثر فعالية لالتهاب المفاصل العظمي الفكي الصدغي المستحث.

**الكلمات المفتاحية:** مفصل الفك السفلي, هشاشة العظام, هيدروجيل حمض الهيالورونيك, الخلايا الجذعية لنخاع العظم, الأسطح المفصالية.