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# ИССЛЕДОВАНИЕ ВЛИЯНИЯ ПЛАЗМЕННОЙ СТРУИ НА РЕГЕНЕРАЦИЮ КОЛЕННОГО ХРЯЩА КРОЛИКА

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***Ключевые слова:*** плазменная струя, коленный сустав, хрящ, соединительная ткань, физиотерапия.
***Keywords:*** plasma jet, knee joint, cartilage, connective tissue, physiotherapy

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**РЕФЕРАТ**
Суставной хрящ биомеханически является соединительной тканью, поэтому отсутствие кровеносных сосудов, нервов, лимфотока и низкий метаболизм вызывают его медленное и отсроченное восстановление. Поэтому очень важно использовать правильные методы лечения для восстановления хряща. Цель исследования-Изучение влияния плазменной струи на регенерацию коленного хряща кролика. Настоящее исследование было проведено на 12 новозеландских белых взрослых кроликах-самцах весом примерно 2,0-2,5 кг. Для подготовки коленного хряща выполняли санацию коленного хряща путем хондрэктомии методом выскабливания во всех трех группах лечения. После трех недель лечения кроликов подвергали эвтаназии, и степень регенерации хряща регистрировали макроскопически и путем записи изображений, а также фиксировали в 10% формалине для гистопатологического исследования. Был выбран метод окрашивания, который обычно использовался в лабораториях патологии. Наблюдение и измерение толщины суставного хряща проводили с помощью глазной сетки. И в конце полученные данные были проанализированы статистически. Результаты, полученные в настоящем исследовании, показали, что использование метода плазменной струи улучшает хрящевую ткань таким образом, что при исследовании гистопатологических срезов было обнаружено, что суставной хрящ в группе плазменной струи вызывает образование волокнистого хряща в месте разрушения суставного хряща, а также применение плазмоструйного воздействия положительно влияет на регенерацию хряща кролика.

## INTRODUCTION

The creation of any cracks and loss of body tissues, whether inside or on the external surface of the body, is referred to as a wound, in other words, any damage to soft tissue that includes skin, muscles, nerves, and blood vessels is called a wound. These injuries may be caused by burns, abrasions and cuts or even diseases such as diabetes or vascular problems. Wounds are classified based on their size, skin penetration and shape[1]. Wound healing and regeneration are natural processes of some body tissues. When the body is injured, a series of complex biological events occur in a coordinated and balanced cascade to restore and repair the damaged area.[2] Simple animals have a high ability to regenerate, because they have large groups of stem cells in their bodies, which, by migrating to the damaged parts, restore the lost tissues.[3] The ability to regenerate is inversely proportional to the complexity of the beast. For example, in many amphibians and water lizards, the ability to repair is much higher than that of mammals.[4] In the formation of cartilaginous tissue, the production and secretion of acidic sugar compounds, including; Glycosaminoglycans such as hyaluronic acid and chondroitin sulfate in the intercellular material (ECM) are one of the most important signs of the differentiation of cartilage cells (chondroblasts). Wound healing is influenced by host factors, wound characteristics and other external factors[5-7] In general, the meaning of wound repair and reconstruction is the regrowth of cells that are similar to the damaged cells in terms of structure and function and replace them. The replacement of the destroyed cells by the proliferation of reserve cells is possible only in tissues whose cells have the ability to divide mitotically.[8, 9] For this reason, regeneration in mammalian tissues has limitations compared to amphibians and some fish. The healing phase usually starts 3 to 5 days after the injury and can last 3 to 4 weeks or more depending on the size of the wound. During healing, proliferation and proliferation mechanisms occur, including fibroplasia, angiogenesis, granulation tissue formation, and epithelial tissue formation.[10-13]

The knee is the largest joint of the body and has hinged movements in the form of bending and opening, as well as rotating movements around the longitudinal axis to some extent. The knee joint is more prone to injury than any other joint in the body, and this is because of the many forces that this joint must endure. While walking, a force equal to 1.5 times the body weight is applied to the knee, while climbing the stairs, a force equal to 3-4 times and when squatting or sitting on two knees, this amount increases to 8 times the body weight. Many bones, ligaments and cartilages are involved in the formation and maintenance of the knee joint. [15 ,14 ,7]Cartilage is a type of specialized connective tissue in which the underlying substance has a hard consistency. This intercellular material has elasticity and gives elasticity to the cartilage and allows the tissue to withstand mechanical pressures without breaking. The main functions of cartilage are to support soft tissues and to provide a sliding area by its smooth surface for joints and thus facilitate the movement of bones. Cartilage is essential for the growth of long bones both before and after birth. Like other connective tissues, cartilage is also composed of filament cells and ground substance [10] Cartilaginous tissue has no blood vessels and supplies its nutrients by diffusion of substances from the capillaries in the perichondrium. Failure of nutrients to reach chondrocytes causes degenerative changes in cartilage, especially in thick cartilage of old animals. Matrix calcification is one of the common consequences of such cartilages. Basically, the degree of repair in each cartilage is different. In addition, in one type of cartilage, this degree is different and depends on the location of the cartilage and the age of the animal. In young cartilage, the perichondrium contains mesenchymal cells. The proliferation and differentiation of these cells causes peripheral growth and proliferation in cartilage cells causes interstitial growth in cartilage. As a result, irritation in hyaline and elastic cartilages for growing animals is associated with repair, but in adults, the perichondrium is not active and its repair power is very limited. In addition, the power of interstitial growth has also been lost, as a result, repair in mature cartilage is generally done by the fibrous connective tissue in the perichondrium or the hard collagen connective tissue of the fascia around the cartilage. Sometimes, the repair tissue may gradually turn into cartilage tissue, but this tissue is probably of fibrocartilage type.[3, 10]

Plasma is a complex collection of electrons, positive and negative ions, charged and neutral atoms and molecules, ultraviolet and heat radiations, electromagnetic fields and free radicals, etc. and usually Due to temperature, electric fields and different types of radiation are detected in it, and when all these things are applied simultaneously on living tissues without causing thermal damage or destructive effects of performance. It affects them biologically and leads to changing their physical and chemical properties. In fact, this device causes changes on the skin membrane by creating an electric flux between the applicator. In the last decade, cold argon plasma was used to remove microorganisms due to the production of ultraviolet rays and plasma ions.[16] Among the advantages of the gaseous form of plasma, it can be mentioned that it can penetrate uneven surfaces and cavities smaller than micrometers, which the use of traditional chemical and fluid techniques had failed in this field. Another advantage of plasma is its ability to be used without contact, without pain, safe and non-invasive, and it also reduces treatment costs in the field of bedsores. The simplicity and flexibility of the cold plasma producing devices along with the treatment of skin diseases has made it a desirable and practical method in wound treatment. Considering that there is no satisfactory method in the field of treating chronic and complex and incurable wounds, new concepts and strategies are urgently needed for faster treatment and healing of these types of wounds. According to the function of cold plasma, by its disinfecting effects and by stimulating the proliferation and migration of skin cells and by activating or inhibiting the integrin receptors on the cell surface or by the effects of angiogenesis, it causes wound healing and also in cases such as covering Implant surface, hemostasis and sterilization of surgical instruments can also be used. According to the laboratory studies, the effect of cold plasma on the growth and differentiation of keratinocytes and fibroblasts has also been realized. Since the cartilage of the nasal septum, the surface of the bone joints and the ventral end of the ribs in humans is of a hyaline type, similar to rabbit ears, therefore, the results of the repair and regeneration of rabbit cartilage by plasma jet can be generalized to humans.[17] Therefore, the purpose of this study was to regenerate rabbit cartilage with plasma jet.

## MATERIALS AND METHOD

The current research was conducted on 12 New Zealand white adult male rabbits weighing approximately 2.5-2.5 kg. Rabbits were kept separately in racks in the animal house under the same conditions and a cycle of 12 hours of light and 12 hours of darkness and at a temperature of 20-25 degrees Celsius.

Cartilage sampling

In order to prepare the knee cartilage, knee cartilage debridement was performed by chondrectomy with scraping method in treatment groups one to three. For the purpose of the mentioned surgery, the treatment groups were anesthetized with the anesthetic ketamine in the amount of 90 mg/kg and xylazine in the amount of 10 mg/kg intraperitoneally. The steps of preparing the surgical surface included complete shaving of the leg, disinfection with betadine and alcohol, and dressing. After scraping, the skin and subcutaneous cartilage were sutured, and oxytetracycline spray and injection were used to prevent possible infections. At this stage, the treatment groups were treated for their own group in 21 days. The first treatment group was subjected to plasma jet radiation daily for 30 seconds. The second treatment group was simultaneously treated with risedronate (0.07 mg/kg/day) and glucosamine (21.5 mg/kg/day). The treatment of the third treatment group was natural without intervention.

Review of cartilage regeneration

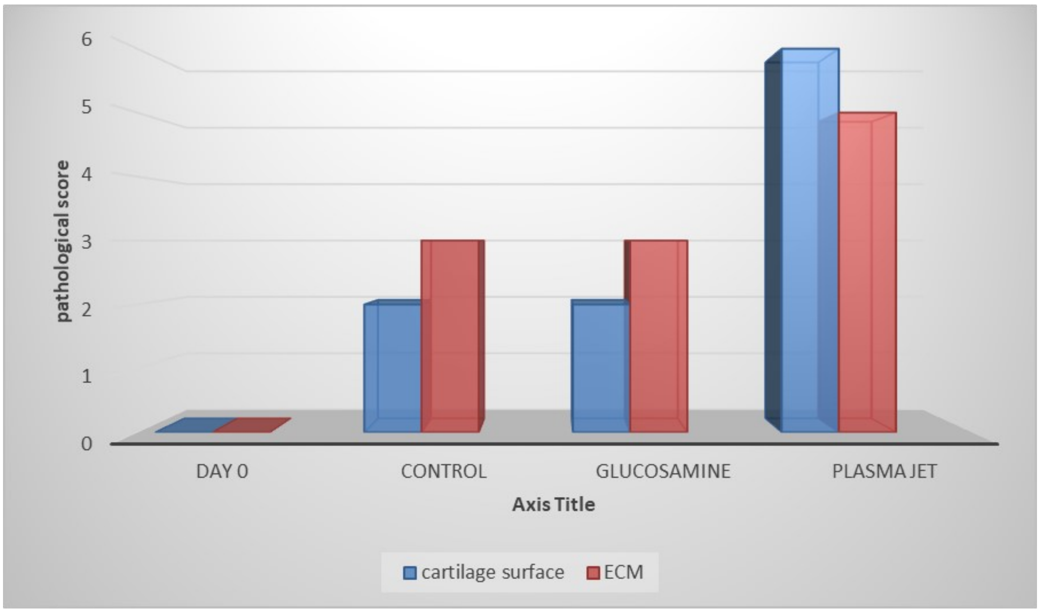
After three weeks of treatment, the rabbits were sacrificed with a high dose of anesthetic at the indicated times. The amount of cartilage regeneration was done macroscopically and by recording the images and they were fixed in 10% formalin for histopathology test. Tissue processing and sectioning (5 microns) were performed. Finally, staining, the staining method that was commonly used in pathology laboratories. Observation and measurement of articular cartilage thickness was done with eye graticule and image j. The data obtained from the present study were evaluated using SPSS version 24 and the obtained data were analyzed using the Tukey test. A significance level of $p<0.05$ was considered.

**RESULTS**

The results obtained in the present study indicated that plasma jet regenerates rabbit cartilage.

**Chart 1**

**Scoring of the studied groups**



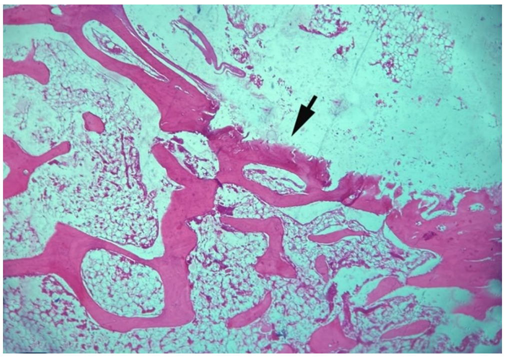



*Figure 1: Cross-section of articular cartilage on day zero, where complete destruction of cartilage (arrow) is observed*

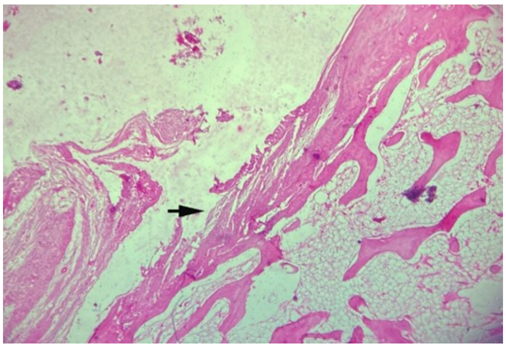


*Figure 2: Cross-section of the articular cartilage in the control group, which can be seen in the place of destruction of the articular cartilage of the fibrotic tissue (arrow).*

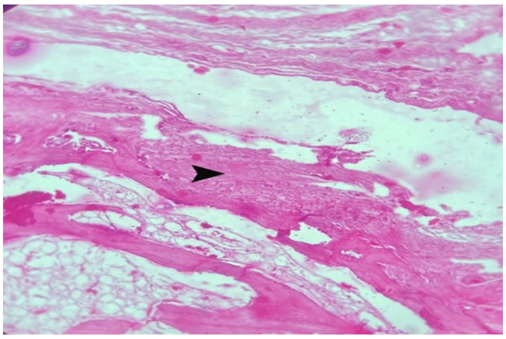


*Figure 3: Cross-section of the articular cartilage in the control group, which can be seen in the place of destruction of the articular cartilage of fibrotic tissue (arrowhead).*

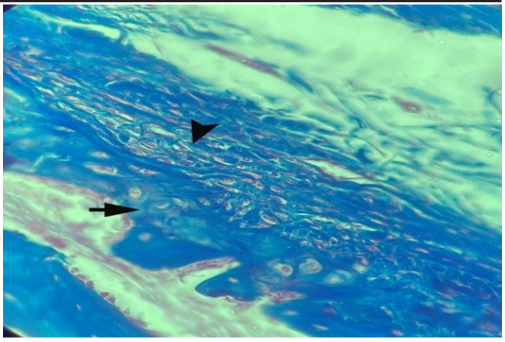


*Figure 4: Cross-section of the articular cartilage in the control group, which can be seen in the place of destruction of the articular cartilage, fibrotic tissue (arrowhead) above the bone below the joint (arrow)*

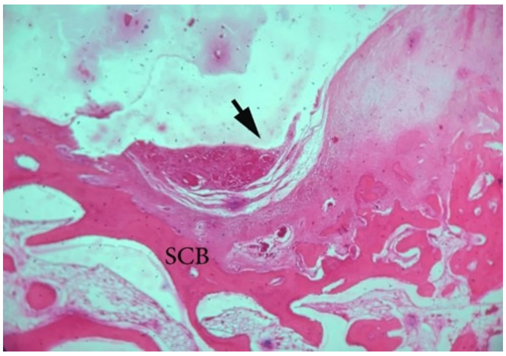



*Figure 5: Cross-section of articular cartilage in the glucosamine group, which is seen above the subarticular bone (SCB), in the place of articular cartilage destruction of fibrotic tissue (arrow).*

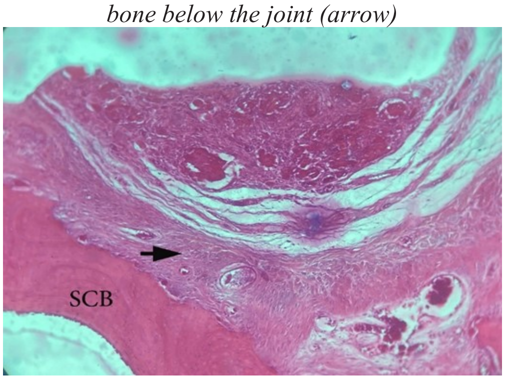



*Figure 6: Cross-section of articular cartilage in the glucosamine group, which is seen above the subarticular bone (SCB), in the place of articular cartilage destruction of fibrotic tissue (arrow).*

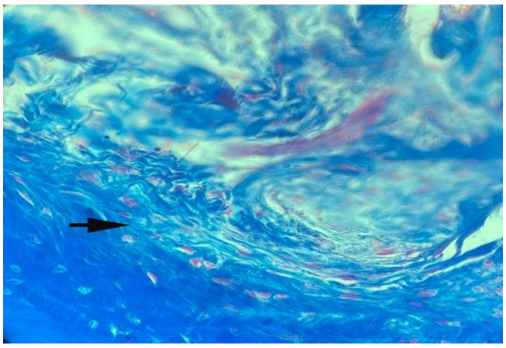


*Figure 7: Cross-section of articular cartilage in the glucosamine group, which can be seen in the place of articular cartilage destruction of fibrotic tissue (arrow)*

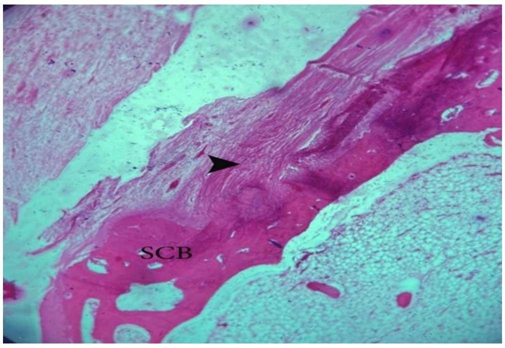



*Figure 8: Cross-section of articular cartilage in the plasma jet group, which can be seen above the subarticular bone (SCB), in the place of articular cartilage destruction, fibrocartilage tissue (arrowhead)*

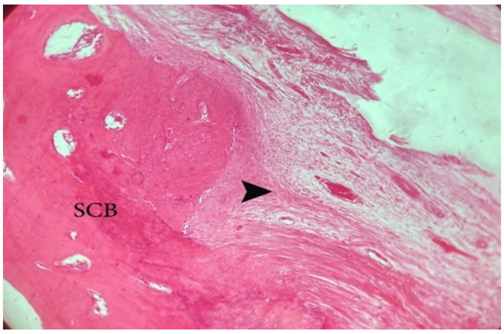



*Figure 9: Cross-section of articular cartilage in the plasmajet group, which can be seen above the subarticular bone (SCB), in the place of destruction of articular cartilage, fibrocartilage tissue (arrowhead)*

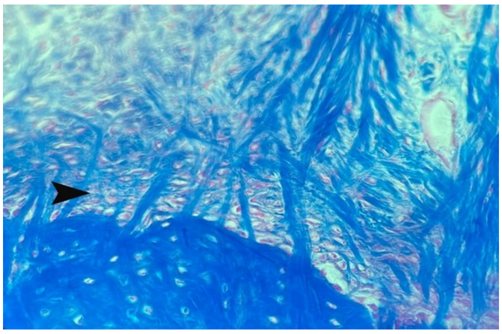


*Figure 10: Cross-section of articular cartilage in the plasmajet group, which can be seen in the place of destruction of articular cartilage, fibrocartilage tissue (arrowhead)*

**DISCUSSION AND CONCLUSION**

Burn wound healing is a dynamic, complex and interactive process, and although superficial burns heal by itself, the process is slow and may lead to many complications, including infection and scar formation.[18, 19] The role of cold plasma and its ionic factors in the burn wound healing process has not been fully determined, but so far, several studies have been conducted on the positive effect of this type of treatment on the wound healing process. Wound healing begins with the inflammation phase of killing bacteria and continues with the proliferation phase of angiogenesis, the formation of collagen, the formation of epithelial tissue and the reduction of the size of the wound, and ends with the destruction of the wound.[2, 20-22] The findings obtained in this research indicated that plasma jet regenerates rabbit cartilage. It is worth noting that so far there have been very limited studies on the effect of plasma jet on rabbit cartilage repair, in this section similar studies will be reviewed.[23] Shimatani et al. (2021) in a study investigating the effect of non-thermal gas discharge plasma under atmospheric pressure on the treatment of rabbit bone defects stated that the improvement of large bone defects was observed by X-ray imaging more than eight weeks after the operation. X -ray results showed a clear difference in new bone occupation of large bone defects among groups with different times of plasma treatment, while new bone occupation was not significant in the control group without treatment. Based on the results of computed tomography analysis at eight weeks, the most successful bone regeneration was achieved using a plasma treatment time of 10 minutes, while the new bone volume was 1.51 times greater than the plasma control group (Shimatani et al., 2021). The results of this study are consistent with the findings of the present study in terms of the effect of the plasma jet method on bone and cartilage tissue repair[23]. Manafi et al. (2012) in a study investigating the effects of platelet-rich plasma on cartilage grafting in rabbits stated that histological findings showed that in intact and crushed cartilage, the addition of platelet-rich plasma led to It increases the regeneration of chondrocytes. In addition, the addition of platelet-rich plasma to the intact cartilage had a significant effect on maintaining the weight and volume of the grafts.[24] Dere Lo et al. (2020) in a study investigating the healing effect of cold plasma on burned skin in adult Syrian mice stated that the macroscopic study of the wound area showed that the treatment of mice with cold plasma reduced the diameter of the burned area. Also, the thickness of the skin layers (corneal layers, epidermis, dermis and hypodermis) in the experimental groups has increased significantly compared to the burn control group, as well as the number and diameter of skin vessels compared to the burn control group. has increased significantly. The area of the wound also decreased significantly compared to the burn control group on the seventh day. The results showed that the cold plasma radiation of helium gas was effective in all indicators of wound healing and its induction at the time of burn can help to accelerate wound healing [25]. Al-Ebrahim et al. (2018) in a study investigating the effect of cold argon plasma at atmospheric pressure on increasing the speed of blood coagulation and healing of full-thickness skin wounds in rats stated that plasma radiation increased the speed of blood coagulation in skin wounds in vivo. According to histopathological findings, the rats in the plasma treatment group showed a significantly better process in the healing of the full-thickness skin wound. Therefore, cold argon plasma can be used to increase the speed of blood coagulation and improve the healing process in skin wounds[26]. On the other hand, research has shown that cold plasma helps to improve and accelerate wound healing without damaging the tissues around the wound. Brun et al. showed in 2012 that cold plasma does not have a negative effect on the surrounding tissue and does not cause damage to these cells[27]. In research that used atmospheric pressure cold plasma for wound healing, it was observed that appropriate doses of cold plasma can inactivate bacteria around the wound and activate fibroblasts in the wound tissue and ultimately lead to wound healing. A higher plasma dose suppresses wound healing due to cell death by apoptosis or necrosis[28]. In another study, researchers conducted on the effect of cold helium gas plasma on the healing of diabetic foot wounds. It was observed that three days after treatment with cold plasma, signs of recovery can be seen, and treatment with this method causes cell proliferation and regrowth of the epidermis, creating the epidermal layer, as well as the formation of granular tissue and the keratin layer. In general, the results obtained in the present study indicated that the use of plasma jet method improves the cartilage tissue in such a way that by examining the histopathological sections, it was determined that the articular cartilage in the plasma jet group caused the formation of fibrocartilage tissue at the site of articular cartilage destruction. In this research, there are 4 groups. The first group with a living sample is called day zero, which is only taken after knee cartilage debridement. The other three groups undergo some kind of treatment for three weeks from the time of knee cartilage debridement to sampling. The findings show that in this treatment period, glucosamine supplements do not make a significant difference in the recovery of joint damage. According to the results obtained in the present study, the use of plasma jet has a positive effect on the regeneration of rabbit cartilage.

## INVESTIGATING THE EFFECT OF PLASMA JET ON RABBIT KNEE CARTILAGE REGENERATION

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### ABSTRACT

Articular cartilage is a connective tissue biomechanically, so the absence of blood vessels, nerves, lymph flow and low metabolism causes its slow and delayed regeneration. Therefore, it is very important to use the correct treatment methods for cartilage healing. Purpose of study: Investigating the effect of plasma jet on rabbit knee cartilage regeneration. The present research was conducted on 12 New Zealand white adult male rabbits weighing approximately 2.0-2.5 kg.

In order to prepare the knee cartilage, its debridement was sanitized by chondrectomy with scraping method in all three treatment groups. After three weeks of treatment, the rabbits were euthanized and the amount of cartilage regeneration was recorded macroscopically and by recording images, and samples were fixed in 10% formalin for histopathology test. The commonly used in pathology laboratories staining method was chosen. Observation and measurement of articular cartilage thickness was done with eye graticule. And at the end, the obtained data were analyzed statistically. The results obtained in the present study indicated that the use of plasma jet method improves the cartilage tissue in such a way that by examining the histopathological sections, it was found that the articular cartilage in the plasma jet group caused the formation of fibrocartilage tissue in the place of injury. Plasma jet has a positive effect on rabbit cartilage regeneration.

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