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TISSUE AND CELL REACTION OF THE SYNOVIAL MEDIA TO INTRAARTICULAR INJECTION OF POLYMER VISCOPROSTHESIS "NOLTREX" IN EXPERIMENTAL CONDITIONS

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Summary:
Reaction of synovial membrane and cartilage on 1 ml of polymer gel «NOLTREX» injected into the cavity of jumping joint of 20 rabbits was investigated. Biological inertness and safety of noltrex injected into the joint cavity was established.
Keywords: osteoarthritis, osteoarthrosis, synovial fluid, polyacrylamide gel, noltrex.

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INTRODUCTION

Osteoarthritis is one of the most spread diseases in the general structure orthopedic diseases distribution. It is believed that by the age of 60 1/2 of the population suffers from a degenerative- dystrophic disease of large joints [1,5,6,9]. Among the non-operative and minimally invasive methods of osteoarthritis, these are the most widely used: administration of hyaluronic acid salt biopolymer into the joint, oral or parenteral glucosamine administration or chondroitin sulfate, and arthroscopic debridement.

The justification for starting to use hyaluronic acid polymer was the viscosupplementation theory [7]. The fact of the synovial fluid viscosity and hyaline cartilage density reduction in patients with osteoarthritis due to hyaluronan depolymerization and defective synthesis by synoviocytes has been proven [8]. It is reasonable to suggest administering an inert abiotic substance, not prone to hyaluronidase destruction, into the cavity. A viscous polyacrylamide gel (PAA gel) capable of swelling may be such a material [3]. Such gel was developed in 2001 and is produced under the Noltrex trademark. The physical tests have proven that the viscosity of the gel is identical to the natural characteristic of the synovial fluid in a healthy body [4]. Theoretically, the interaction between the synovial fluid and the gel negatively affect the fluid's physical properties without destructive effect from the synovial environment enzymes.

The objective of the work was to study the reaction of the tested animals hock joint tissues to the injection of Noltrex, which is used in orthopedic surgery to compensate for the loss of synovial fluid viscosity in the articulations of patients with osteoarthrosis [2].

MATERIAL AND METHODS

The study was conducted on 20 outbred male rabbits, body weight 3-4 kg. The animals were kept in the kept in the standard vivarium environment, with standard nutrition, all operations were carried out in accordance with the requirements set in the Declaration of Helsinki. 1.0 ml of Noltrex gel was administered into the left hock joint cavity, the left joint was the control one. The articular cavity of the knee joint of a human is 100-150 times larger in terms of volume than the compared rabbit joint, and for this reason, the gel dose is administered into the animals was proportionally 40-60 times higher than that of a human (2.5 ml).

After applying anesthesia (5% ketamine hydrochloride in 50 mg/kg dosage) the joint was punctured with a 23G needle in the corner between the external part of the articular cavity and the patellar ligament. Noltrex was injected into the articular cavity with a syringe from the packaging. The animals were withdrawn from the experiment by thiopental sodium and droperidol overdose after 1, 3, 7 and 14 days, 1, 2, 3, 6, 14 months after the material injection. The general state, behavior, body weight and rectal temperature were controlled in the course of the experiment. Smears of the articular cavity contents (gel and synovial fluid) were taken for cytologic analysis. The smears were fixed in methanol and Giemsa stained, then the proportion of different cell types was calculated. Tissues for histological and histochemical examination were fixed in 70% ethanol, embedded in paraffin; tissue sections 4-5 micron were stained with hematoxylin and eosin, picro-fuchsin solution, toluidine blue, performed the Periodic Acid - Schiff (PAS) reaction and the Brachet reaction for RNA. The results were compared to the control

material of the contralateral joint. The absorption capacity of the original PAA gel samples and the gel removed from the joint by the 6th month after the injection were analyzed with the FTIR spectrometer Magna-IR 750 (Nicolet, USA) with spectral resolution 2 cm^{-1} in the 4000÷400 cm^{-1} wave number region. The specimens were spread on a KBr plate and dried to reduce the water contents in order to avoid water spectrum interference. The resulting spectrum curves were compared among them.

RESULTS AND DISCUSSION

The general state of health of the animals was recovered within 24 hours, loss of weight was 124±16.2 g on average. The weight returned to the original by day 3-4, motor performance did not deteriorate.

Macroscopic examination.

In the first 24 hours after the injection, the articular cavity contained the clear substance, in which the gel and the synovial fluid formed the uniform substrate. The viscosity of this substance was higher than that of synovial fluid in the control joint and was close to that of the intact gel. The synovial membrane structure was normal, without signs of inflammation; the cartilaginous plate did not change. 7-14 and 30 days after the injection the amount of gel decreased and the viscosity of the substance decreased as well. The synovial membrane and the cartilage showed no signs of pathological changes.

Cytologic examination of the intraarticular medium.

In 24 hours the gel in the smear from the experiment joint was a homogeneously metachromatically stained with toluidine blue mass when observed at low magnification and fine-grained mass at high magnification, which means a possible complex formation between the gel and the synovial fluid, as they are not observed separately. The total amount of cells is much higher than in the control materials (16.6 in the field of vision on average as opposed to 2.5 in the control material). The phagocytizing cellular gel was the dominant form (76.3% of the total amount), with vacuoles and minuscule gel inclusions in the cytoplasm. The cellular resorption of the gel was performed by macrophages migrating from the blood and smaller macrophage-derived A-type synoviocytes. Non-phagocytizing synoviocytes, monocyte type macrophages without active phagocytosis and lymphocytes were also found. Neutrophilic leukocytes were more numerous and their percentage was higher than in the control material, but still not more than 3.3%, which means that there was no notable inflammation (**fig. 1**).

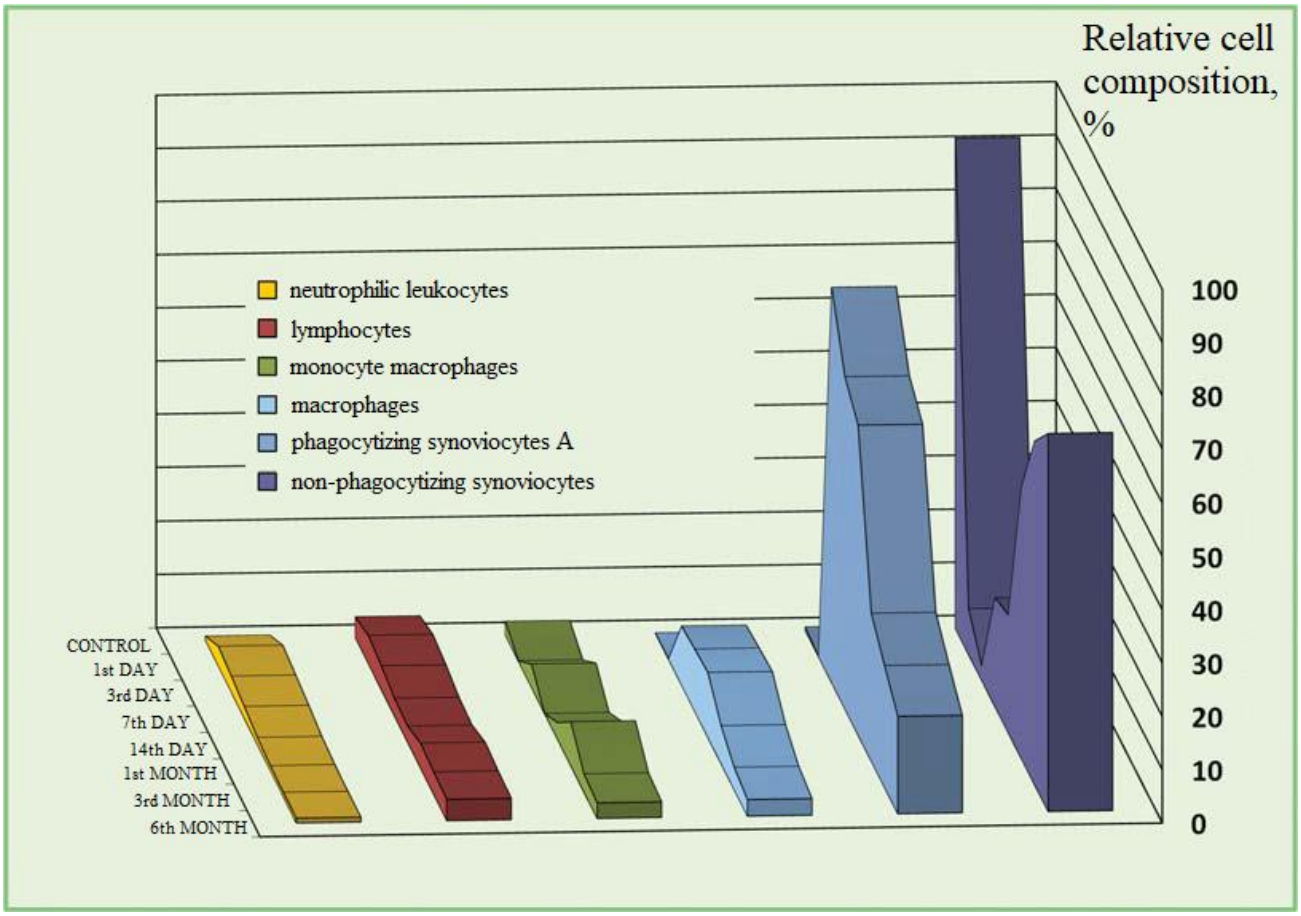


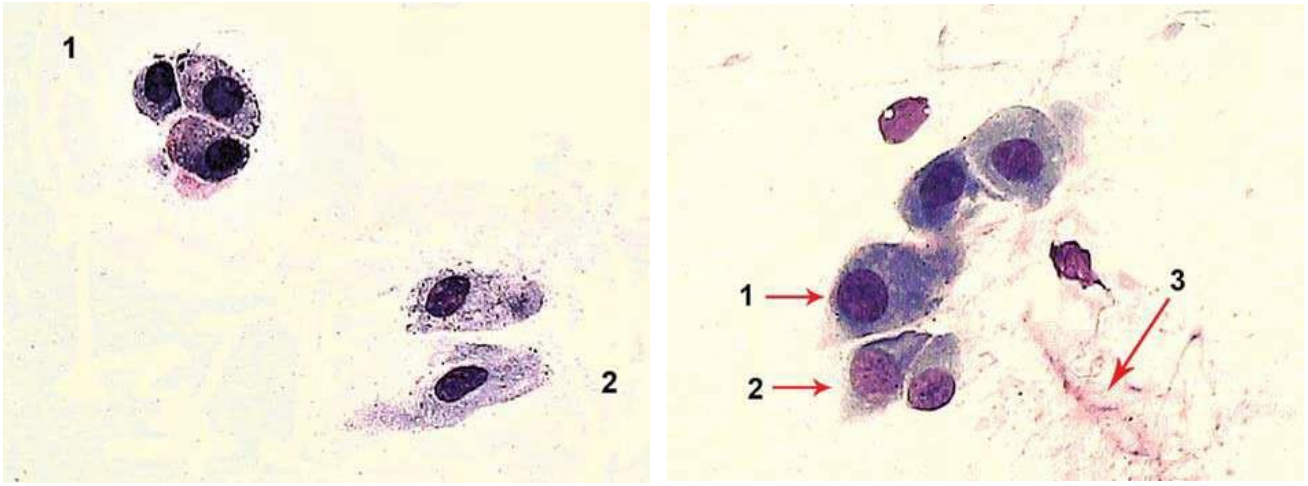
Fig. 1 Synovial fluid cellular composition diagram in the control and experiment joints, over time.

By the 3rd day, the gel's morphology did not change. The total amount of cells grew a little and was 18.4 in the field of vision. The overwhelming amount of the elements (85.8%, out of them 74.2 phagocytizing synoviocytes and 11.6 macrophages) were phagocytizing round or oval cells with round nuclei and large cytoplasm rim with numerous vacuoles. The cells were 1.5- 2 times larger than the synoviocytes in the control material (**fig. 2a**). Non-phagocytizing synoviocytes and inactive monocyte macrophages were fewer. The concentration of lymphocytes and neutrophils did not change much; the inflammation did not become stronger.

By the 7th day, the gel elements in the smear were structurally homogeneous, with vacuolization and fibrillization (beginning of the gel lysis). The total amount of cellular elements was two times lower (8.5 in the field of vision, 62.3% of them were phagocytizing synoviocytes and macrophages). Cellular composition gradually normalized (see **fig. 1**).

On the 14th day, the areas of fine gel vacuolization grew larger, large vacuoles were also observed. A number of active macrophage cells different from phagocytizing synoviocytes (58.1%) grew (12.2%). Separate cells with elongated cytoplasm, similar to fibroblasts, were observed (**fig. 2b**).

After a month the number of cells reduced to 5.4 in the field of vision. Most of them were common synoviocytes (51.2%), which indicated further normalization of the intraarticular medium.



A B

Fig. 2 Synovial fluid cytogram. Giemsa staining ×900.
A – 3rd day: cells phagocytizing the gel - synoviocytes A (1) and large macrophages (2); B - 14th day: large macrophage cells (1), active and inactive synoviocytes (2), remaining vacuolated gel (3).

Neutrophils percentage was only 0.9%, which is comparable with control material figure (0.7%); a number of lymphocytes normalized, amount of phagocytizing synoviocytes dropped (27.8%), same as macrophages (7.1%). 3-6 months after the injection the cellular composition was close to control material figures (see **fig. 1**).

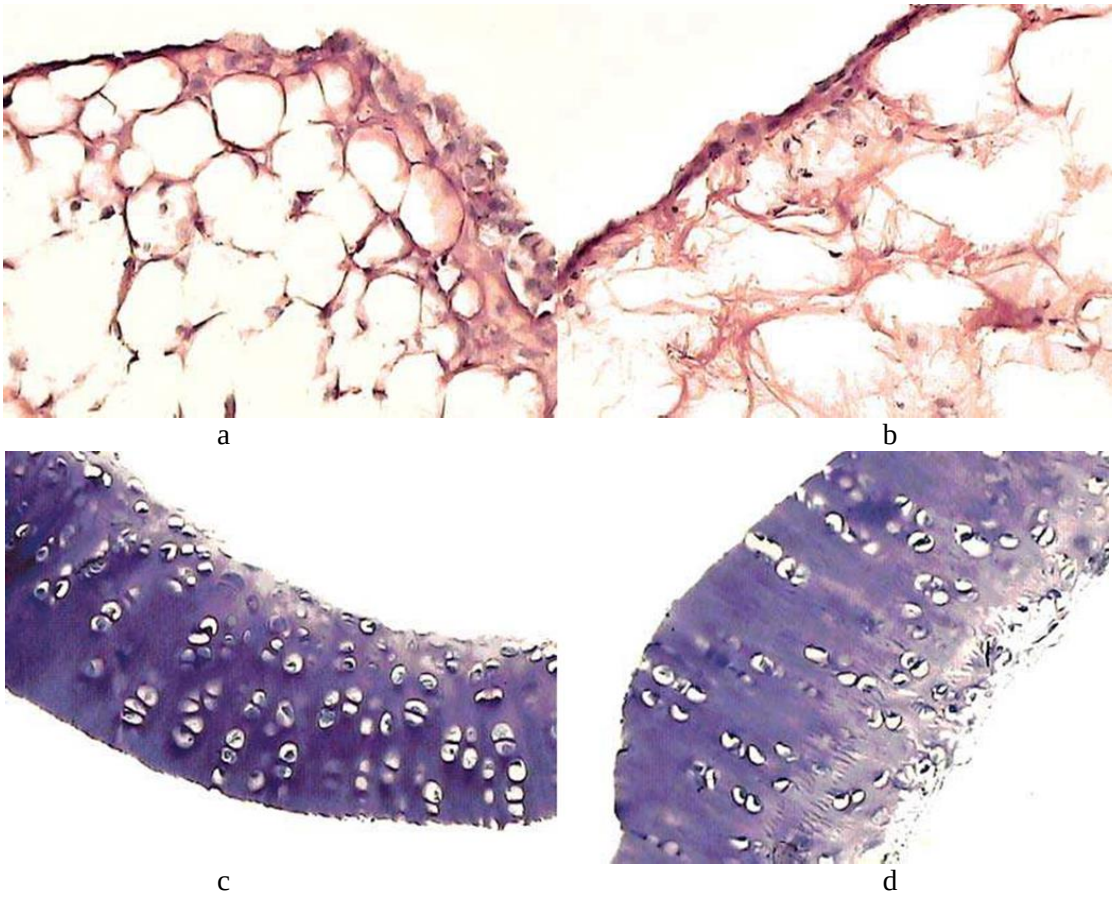
Thus, 1-3 days after the gel was still homogeneous, beginning with the 7th-day partial lysis was observed, which then became more intensive by the 14th day and stopped by month 3-6. Extrapolating the experimental data, we can make a conclusion that in a human body the lysis would, most probably, end between the 7th and the 21st month after the injection (rabbit body metabolism is much faster).

Histological and histochemical analysis of synovial membrane and articular cartilage.

1-3 days after the administration no apparent inflammation was observed in the synovial membrane, and tissue reaction was confined to focal synoviocyte hyperplasia (due to A-cells). On day 3-7 in parts of the animals small areas of the synoviocyte cover layer thickening (**fig. 3a**) were observed, and there the gel was being resorbed without inflammation intensification. No apparent vascular reaction was observed, PAS+ granularity and RNA concentration were higher in synoviocytes, which indicated stronger phagocytic and biosynthesis activity (**fig. 3 a**). The articular cartilage was almost unchanged compared to the control cartilage, no cellular and matrix dystrophy was observed, the glycosaminoglycan concentration was high. On day 14-30 no inflammation was observed, the synoviocyte hyperplasia areas remained in some animals, the synovial membrane structure was in general close to normal (fig. 3b). Cartilage tissue, just as before, was unchanged compared to the control cartilage (see **fig. 3 c, d**).

By the third month in most case, the structure of the synovial membrane returned to normal. 14 months after the injection there were no changes in the membrane compared to control material. The histochemical analysis showed no differences in RNA values and acidic glycosaminoglycan synthesis in synoviocytes compared to the control material, which proves the

ability for synthesis was preserved. The articular cartilage was not different from the control joint cartilage in structure or histochemical properties. The nutrition of the cartilage tissue is partly performed through the diffusion of substances from the synovial fluid, so the absence of dystrophic changes meant that gel injection into the articulation had no effect on the tissue metabolism.



a b c d

Fig. 3 The histological picture of the synovial membrane and articular cartilage tissue. Hematoxylin and eosin staining ×400.
A - day three: local synoviocyte layers thickening due to cellular proliferation, increase in the macrophage A-cell number; B - day 30: singe/double synoviocyte layer, synovial membrane without changes; C - articular cartilage in the experiment: high contents of glycosaminoglycans in the intercellular matrix, no chondrocyte, chondroblast, and matrix dystrophy observed, no difference from the control joint; D - control: cartilage intact.

Infrared spectroscopy of the intact gel and gel removed from the joint.

Figure 4 shows IR specters of the intact and the used gel. The comparison shows differentiating properties in the wave number region 1700÷1500 cm^{-1}, i.e. the vibration bands of amide group "Amide I" and "Amide II". "Amide I" band in the primary and secondary amide specters is in area 1690÷1600 and 1570÷1515 cm^{-1}. After the time spent in the body the intensity of the gel spectral band 1607 cm^{-1} reduced, and the intensity of the spectral band 1542 cm^{-1} increased, which corresponds to the transition of primary amides in the polymer chain to the secondary amides. This fact confirms the formation of a complex between the polymer structure of the PAA gel and the synovial fluid.

CONCLUSIONS

1. Intraarticular injection of Noltrex does not lead to inflammation of the synovial membrane and cartilage tissue dystrophy.
2. Tissue reaction to gel administration into the joint is minimal and includes focal synoviocyte hyperplasia and migration of macrophages and A-type phagocytizing synoviocytes, which gradually resorb the gel, into the articular cavity.
3. The gel forms a complex with the synovial fluid, which does not affect the articular tissue metabolism. Also, Noltrex is the matrix capable to hold the synovial membrane in the articular capsule.
4. Evidence of tissue compatibility and safety of the Noltrex polyacrylamide gel, injected into the articular cavity, has been obtained.
5. Slow gel resorption is an advantage in comparison to quickly eliminated hyaluronic acid salt products and makes PAA gel the option of preference for injection treatment of patients with acute osteoarthrosis.

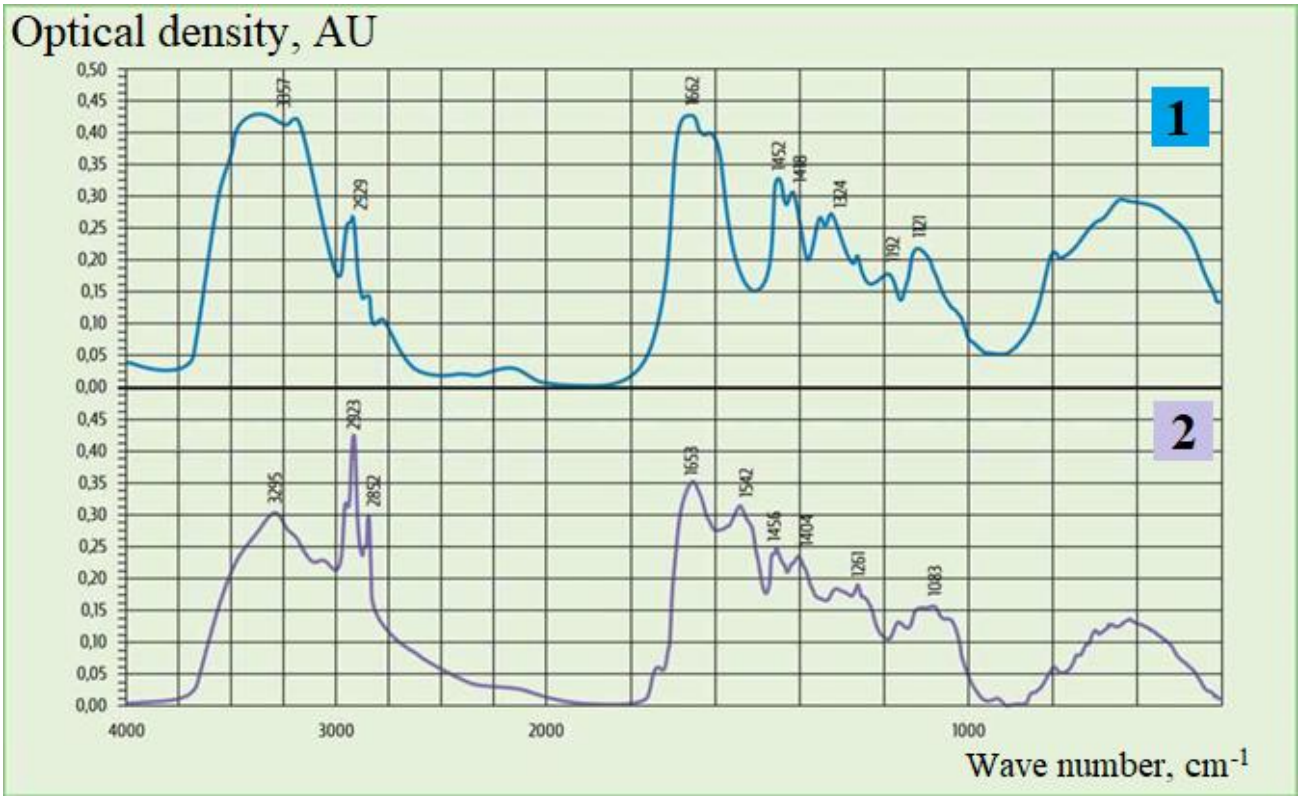


Fig. 4 IR-spectroscopy of the intact gel (1) and the sample extracted from the joint (2).

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