

Preclinical Trials of Collagen and Hyaluronic Acid Based Gels for Use in Dental Surgery

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Abstract Regenerative medicine is aimed at expanding the possibilities of doctors to stimulate internal regenerative processes and provide artificial materials for tissue and organ repair. The article is devoted to the research of the effectiveness of collagen and hyaluronic acid based gels for application in surgical dentistry. The results of the experiment showed that the introduction of vitreous body and calcium gluconate in combination with collagen increased the regenerative activity of soft gingival tissues. The best results were observed with the combined administration of the preparations, which was characterised by the development of delicate collagen fibres and regenerative process at the injection site. Thus, the results of the study indicate the potential effectiveness of collagen and hyaluronic acid based gels in surgical dentistry to improve the regeneration processes of soft tissues.

Keywords Collagen, Hyaluronic acid, Vitreous body, Standardisation and determination of gel stability, Surgical dentistry

1. Relevance

There are a large number of collagen-containing membranes on the market today. Most of them effectively fulfil barrier functions, but their insufficient thickness does not allow to achieve a significant increase in the volume of soft tissues. In contrast, a collagen matrix with a combined structure helps to fill soft tissue defects during a single operation, even in soft tissue deficiency, but the possibilities of its clinical application are still insufficiently studied.

Due to the wide spread of such diseases as gingival recession, periodontitis, erosive and ulcerative lesions of the oral mucosa, alveolitis, the choice of optimal treatment tactics remains a serious problem. Gels allow to provide a large concentration of drugs directly in the focus of lesions, have optimal rheological properties, provide the most complete and rapid release of the main active substances.

The most promising excipients are collagen and hyaluronic acid, which are characterised by prolonged action, high regenerative properties and indifference, and are widely used in the pharmaceutical industry, in particular for the creation of soft dosage forms [2,8].

There are different types of collagen, depending on location and function. To date, more than 20 types have been classified. Type I-IV collagens are the most abundant in the human body. Type I collagen is the main component of several commercially available collagen membranes (CMs),

developed as an evolution of barrier membranes first introduced in the mid-1980s. The principle of so-called directed tissue regeneration was based on the membrane's ability to exclude epithelial and connective cells to enhance the ability of damaged periodontal tissue to regenerate to form new bone, periodontal ligament and cementum.

The mould-forming ability of collagen is multifaceted. In the form of solutions it can be the basis of ointments, liniments, prolonger of liquid dosage forms (drops, injection solutions), thickener, etc. When solutions are air-dried, transparent films are formed, which are used as an independent dosage form. After freezing and lyophilic drying of solutions, highly porous structures (sponges) are obtained, the density of which depends on the initial concentration of collagen and other ingredients [6,7]. Spray drying of solutions or dispersion of dry films, sponges and other products yields powders that can be used as independent dosage forms and as excipients in the manufacture of other drugs.

The vitreous body is composed of 90% hyaluronic acid and 10% water. The vitreous body (stroma vitreum) is formed by a thin network of interwoven fibres of different forms of collagen protein (vitreous-specific forms of collagens are also called vitrosins). The spaces between the fibres are filled with fluid (humor vitreus). This structure of the vitreous body gives it the character of a gelatinous mass [3]. Hyalocyte cells are present in the vitreous body, mainly located at its borders. These cells are involved in the secretion of hyaluronic acid, collagens and soluble proteins of the vitreous body, and also form hemidesmosomes, which provide attachment of the vitreous body to the retinal lining membrane. By origin,

most hyalocytes are macrophages [4,5].

Hyaluronic acid is also widely used in the pharmaceutical and cosmetic industries. Hyaluronic acid has a transport function - the polymeric network that hyaluronic acid forms on the surface of the skin allows the bioactive substances in various preparations to linger longer on it, increasing the likelihood that they will penetrate into the tissues. Hyaluronic acid has a high water-holding capacity - one hyaluronic acid molecule binds up to 5000 water molecules. The absorbed water is retained inside in the form of a gel and does not evaporate even when the humidity of the surrounding air decreases. We have developed several gel models using different excipients [1,2]. In this connection, the urgent task is to further study the properties of materials allowing to obtain a sufficient volume of attached keratinised gingiva, as well as to develop techniques contributing to the reduction of traumatism and the volume of surgical interventions.

The aim of the research: Studying the influence of collagens of domestic production on the soft tissues repair by experimental way on animals.

2. Materials and Methods of Research

The collagen "Bioplant" developed in the Institute of Biochemistry named after academician A.S.Sadikov was used to achieve the set goal. Vitreous body (90% hyaluronic acid and 10% water) and calcium gluconate solution (10% 10 ml) were used as a solvent.

5%, 10% and 15% solutions for injection with collagen were prepared.

The technological process of gel preparation consisted of several stages: The first stage involved the preparation of drug substances and materials.



Figure 1. A. Primary state of collagen. B. Collagen mixed with vitreous (in mushy form)



Figure 2. Collagen dissolved in the vitreous and placed in a 35-degree bath

The result of the first stage is the obtained mushy base (Fig. 1), which was obtained by dissolving the preparation "Bioplant" in the vitreous body in the volume of 0.5 ml, 1 ml and 2 ml. The solution was then placed in a 35°C water bath for 5 minutes. The obtained gel was a homogeneous mass of white colour with a weak characteristic odour (Fig. 2).

The most optimal gel model turned out to be model No. 2 (1 ml of vitreous body diluted in 10% collagen solution), which by its consistency is convenient for placing in the periodontal pocket and has the property of a longer effect on the lesion focus. Other compositions were not suitable in their consistency because they were liquefied or too thick, which was inconvenient for application, and also the system was subjected to delamination and lost its structural and mechanical properties [3].

Injection of biomaterials into the body was carried out on white male rats, body weight 180-220 g, the observation process lasted for 7 and 14 days, after which morphohistological examination was performed.

The injection site was shaved, treated twice with alcohol. The injected collagen solution was heated in a bath of warm water at 35°C before injection, the drug was drawn into syringes of 0.1 and 0.2 ml each. The animals were divided into 3 groups and 4 subgroups of 5 animals each, by random sampling method:

Group 1 - intact;

2nd group (n = 10):

- subgroup A (n = 5) - animals were injected collagen in vitreous solution - 0.1 ml into the soft tissue thickness of the gingiva on the right oral side of the NA;
- subgroup B (n = 5) - collagen in vitreous body solution - 0.2 ml was injected into the gingival soft tissue thickness on the right side from the oral side of the NA;

Group 3 (n = 10):

- subgroup A (n = 5) - animals were injected collagen in calcium gluconate solution - 0.1 ml into the soft tissue thickness of the gingiva on the right side from the oral side of the NA;
- subgroup B (n = 5) - animals were injected with collagen in calcium gluconate solution - 0.2 ml into the gingival soft tissue thickness on the right side from the oral side of the NA.

Collagen was injected into the gingival thickness by tunnelling method. The animals were kept under normal vivarium conditions (Fig. 3).

10 animals each were removed from the experiment on days 7 and 14. Soft tissue samples of 1.0 x 1.0 cm were obtained from the gingival area into which collagen was injected and further placed in a fixative 10% formalin solution. The specimens were then loaded for 1-2 hours in a thermostat at 37°C and 57°C for impregnation for 1 hour, after solidification paraffin blocks were cut out. Serial slices were made from the prepared blocks. The slices were coated with protein and rolled on a spirit lamp. The cut out materials were mounted on glasses and stained with haematoxylin and eosin. The finished preparations were viewed under a BIO BLUE binocular microscope c adapter, Euromex Microscopen BV camera.

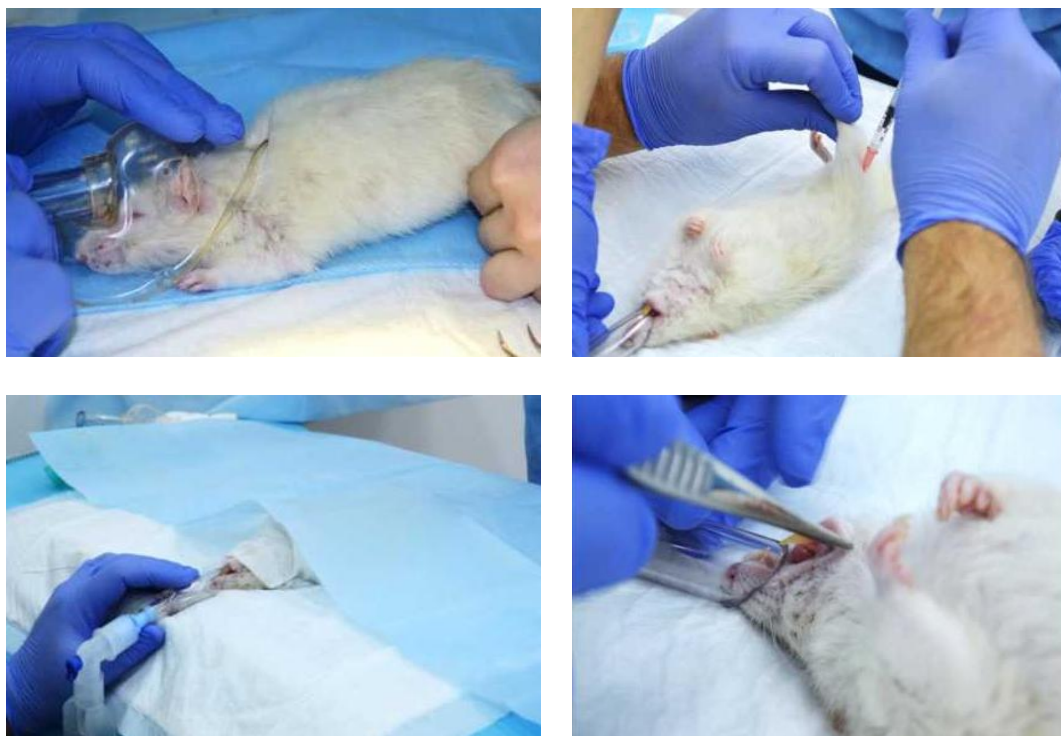


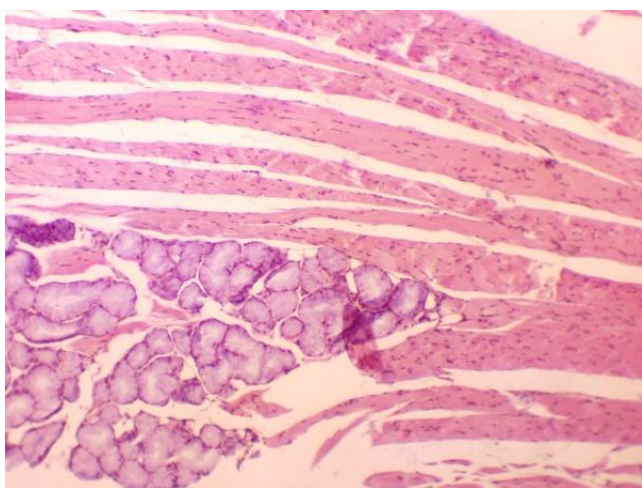
Figure 3. Experiments were performed on male rats under aseptic conditions

3. Results and Discussion

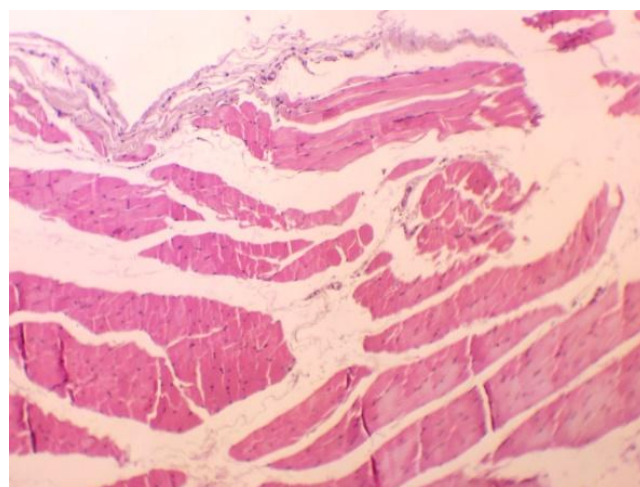
I. Day 7 of the experiment.

1). Intact group.

The results of histological examination of soft tissues on the 7th day of the experiment in the intact control group showed the absence of signs of focal changes and other pathological processes. In fragments of muscle tissue transverse striation and nuclei were preserved, only in intermuscular layers swelling was determined. Fragments of the mucous membrane and salivary gland were also without visible changes (Fig. 4 A and B).



A



B

Figure 4. A and B Intact group of animals. Soft tissue is represented by muscle fibres and a fragment of salivary gland. Swelling of intermuscular layers is noted. Uv. ob. 10x10. Staining: haematoxylin and eosin

2). The second group (collagen + vitreous body).

In subgroup A of the second group of experimental animals, where collagen in 0.1 ml vitreous body solution was injected, an area of longitudinally arranged thin fibrous collagenous structure was noted in the thickness of muscle tissue, the fibres had a homogeneous structure. In some cases, there were layers of densely adjoining collagen fibres, where their transverse striated striation was clearly defined (Fig. 5).

In subgroup B, where 0.2 ml of collagen in vitreous body solution was injected, the development of immature granulation

tissue with enhanced angiogenesis was observed. Newly formed vessels were located among loose and delicate collagen fibres (Fig. 6 A and B).

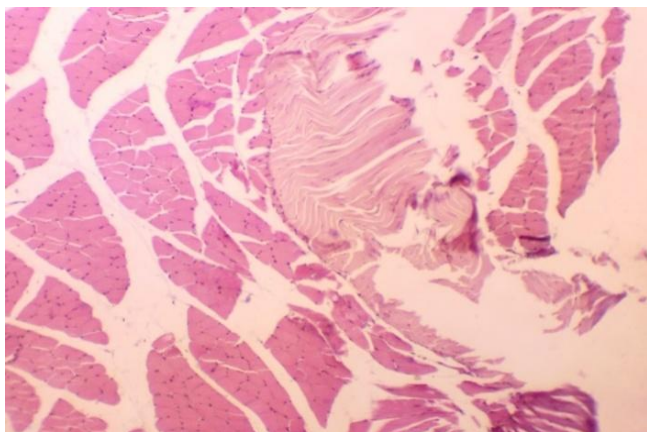
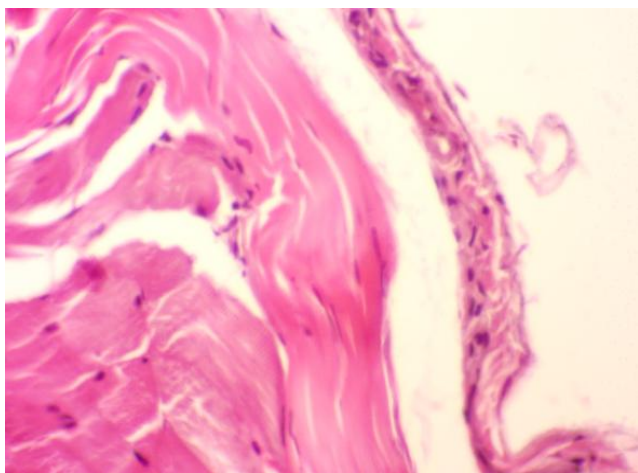
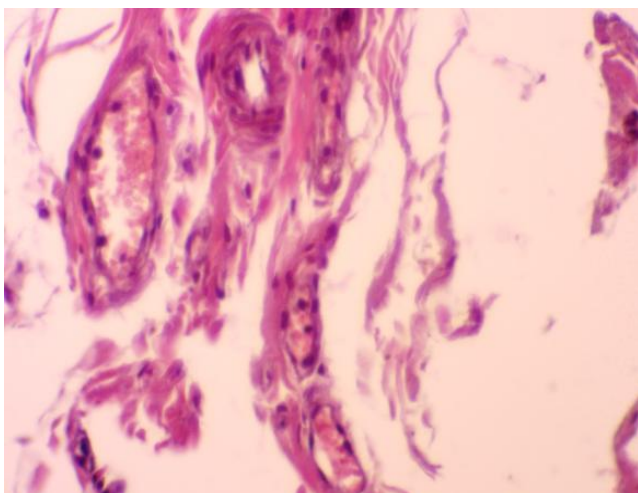


Figure 5. 0.1 ml of vitreous with collagen. An area of thin fibrous collagen structure among the muscle tissue. Eq. vol. 10x10. Staining: haematoxylin and eosin



A



B

Figure 6. 0.2 ml of vitreous with collagen. A. Mature fibrous tissue with elements of maturing connective tissue. Eq. vol. 4.0x10; B. Enhanced angiogenesis, newly formed capillaries among loose and delicate collagen fibres. Uv. ob. 10x10. Staining: haematoxylin and eosin.

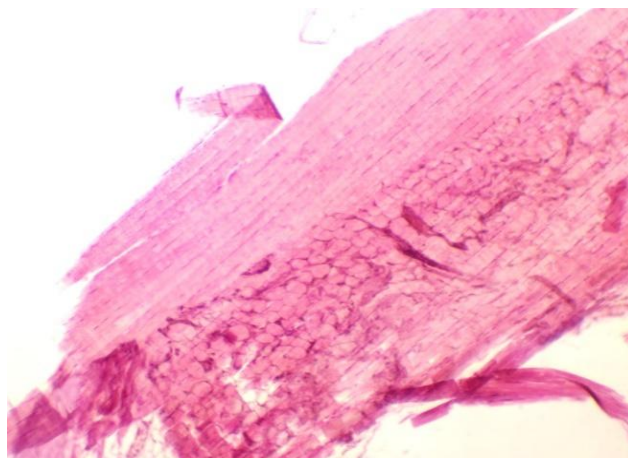


Figure 7. 0.1 ml of Ca gluconate solution with collagen. Fragmentation in the form of clumps of calcium salts, adjacent tissue consists of dense collagen fibres, transverse striation is clearly visible. Uv. ob. 10x10. Staining: haematoxylin and eosin

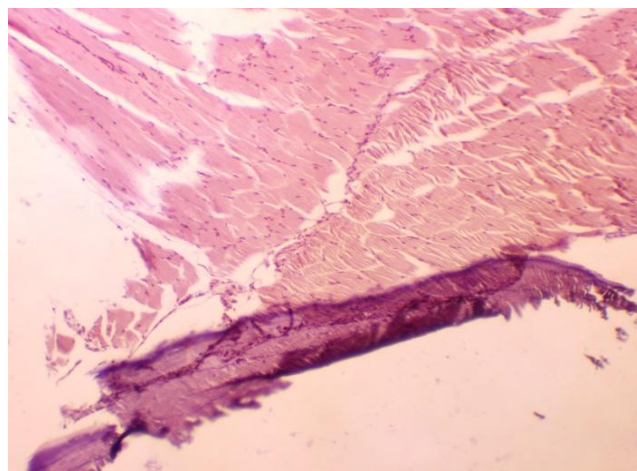


Figure 8. 0.1 ml of Ca gluconate solution with collagen, same other section. Muscle fibrous tissue with preservation of transverse striation and nuclei. A band of dark purple calcium salt deposition in the lower segment. Uv ob. 10x10. Staining: haematoxylin and eosin

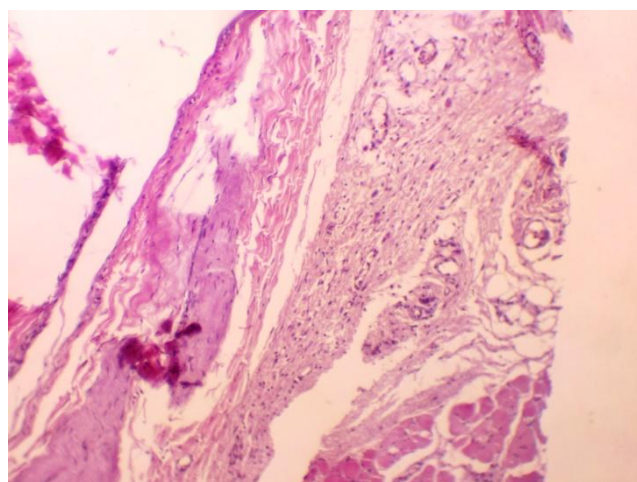


Figure 9. 0.2 ml of Ca gluconate solution with collagen. Foci of calcium salt deposition and cellular-fibrous immature connective tissue with the presence of small blood vessels are visible among the homogeneous structures. In the lower corner a fragment of muscle tissue. Uv. ob. 10x10. Staining: haematoxylin and eosin

3). The third group (collagen + Ca gluconate)

In subgroup A of the third group of animals, which were injected with collagen in calcium gluconate solution 0.1 ml, the results of histological examination were characterised by fragmentation in visualised clumps of calcium salts among fibrous connective tissue, transverse striation was clearly visible. Muscular fibrous tissue sections were without signs of destruction and necrosis. In the lower segment there was a linear deposition of calcium salts of dark purple colour in the form of a stripe. And in subgroup B (0.2 ml of calcium gluconate solution with collagen), insignificant foci of calcium salts deposition were detected in the layers of soft tissue, which is represented by fibres of connective, muscular tissue and fatty fibre (Fig. 7-9.).

II. Day 14 of the experiment

1). Intact group

Histological analysis of soft tissues on day 14 of the experiment in the control group revealed no signs of focal changes and other pathological processes. The structure of transverse striated fibres and the integrity of nuclei were preserved in the muscle tissue samples, only in the intermuscular layers swelling was noted. Fragments of salivary gland and mucous membrane are also unchanged.

2). The second group

In subgroup A of the second group of experimental animals, when vitreous body was injected into the soft tissues of the gingiva in the volume of 0.1 ml, the preparations were characterised by signs of encapsulation around the foreign body. In some areas there was a partial dissolution of the substance replaced by fibrous connective tissue, small newly formed blood vessels with a thickened wall were detected under high magnification of the lens (Fig. 10-11.).

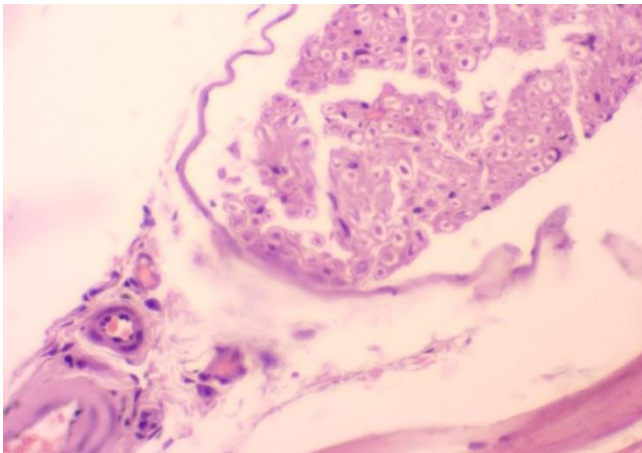


Figure 10. 0.1 ml of vitreous with collagen. A section of soft tissue separated by a fibrous capsule included in the vitreous cavity. Haematoxylin and eosin staining. Eq. 10x10

In subgroup B, where 0.2 ml of vitreous body was injected, under low magnification of the objective lens interstitial swelling was noted in the soft tissue of the gingiva, under high magnification of the objective lens in some samples the ingrowth of loose connective tissue into the area of homogeneous

structure was noted. In this case, the strengthening of the regeneration process through organisation was noted (Fig. 12A and B).

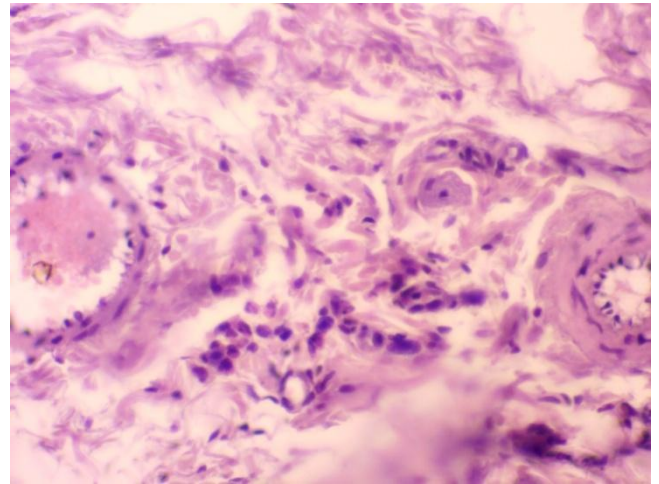
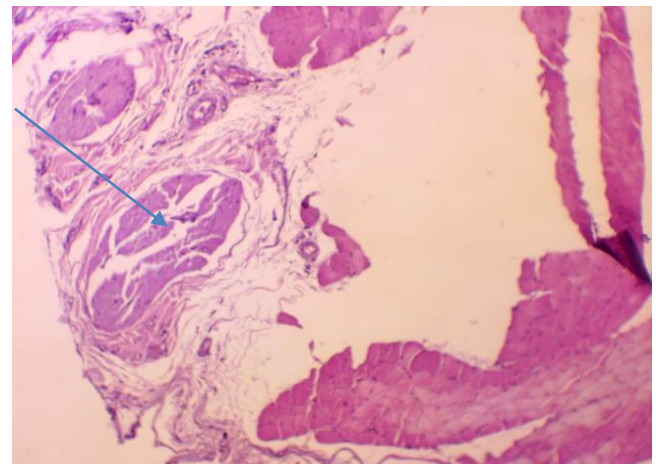
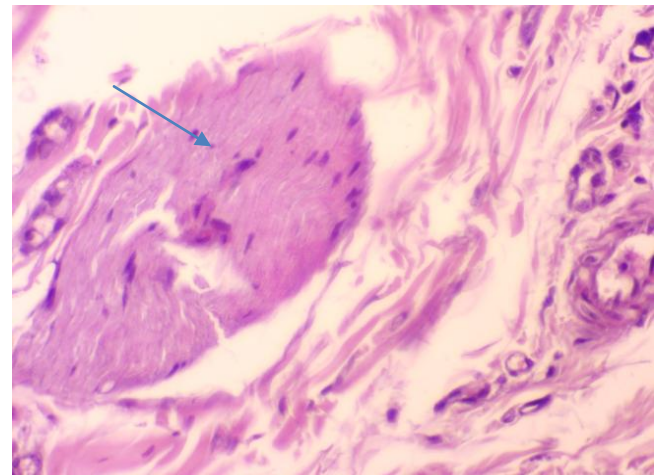


Figure 11. 0.1 ml of vitreous with collagen. Areas of partial dissolution of the substance and its replacement by fibrous connective tissue. Haematoxylin and eosin staining. Eq. 10x10



A



B

Figure 12. 0.2 ml of vitreous with collagen. A. Interstitial swelling of soft tissue B. Ingrowth of loose connective tissue into the area of homogeneous structure. Haematoxylin and eosin staining. Eq. vol. 10x10

Slit-shaped blood vessels, fatty fibre, muscle fibres were found among the connective tissue elements in the preparations (the arrow indicates the area of the formed collagen structures on the cross section during experimental injection of collagen into the gingival tissue) (Fig. 12).

3). The third group

The results of histological examination of soft tissues in subgroup A when collagen was injected in calcium gluconate solution 0.1 ml indicated the absence of visible focal changes. Fibres of connective and muscular tissue with transversely striated striation, round-shaped nuclei, small blood vessels with narrowed lumen were visualised, layers of adipose tissue were represented by lipocytes of the same size. (Fig 13).

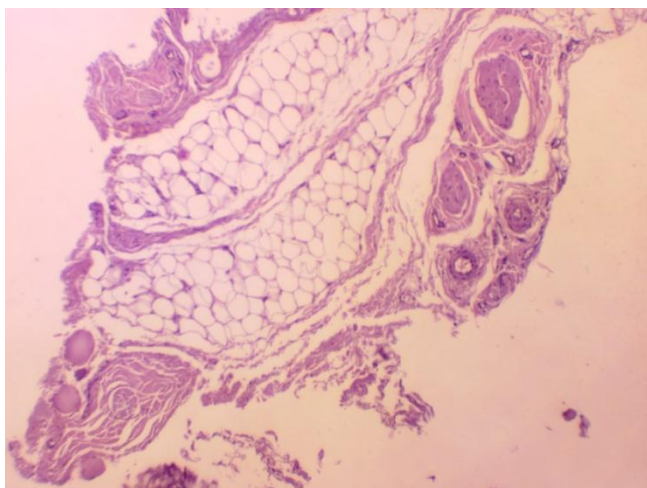


Figure 13. 0.1 ml calcium gluconate solution with collagen. Muscle, fibrofibrous tissue fragments and fatty fibre without visible changes. Haematoxylin and eosin staining. ob. 10x10.

In the subgroup B after combined injection of 0.2 ml of calcium gluconate with collagen in the soft tissue there was an overgrowth of rough fibrous connective tissue with residual structures in the form of basophilic-coloured clumps. Partial resorption and organisation of calcium gluconate were noted. Fatty fibre was unchanged. (Figures 14,15)

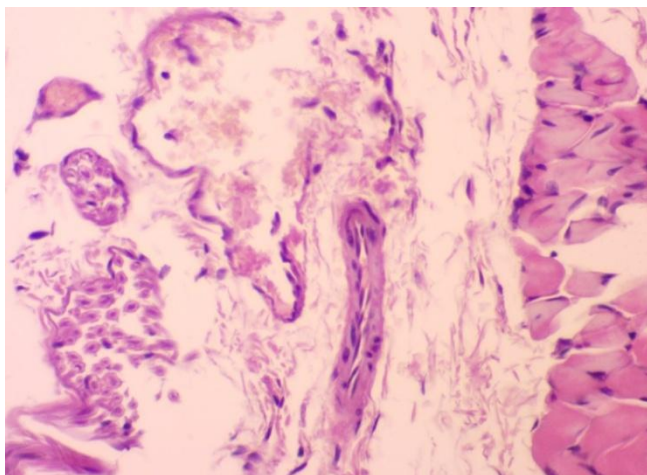


Figure 14. 0.2 ml calcium gluconate solution with collagen. A section of formed collagen structures on a cross section. Haematoxylin and eosin staining. 10x10

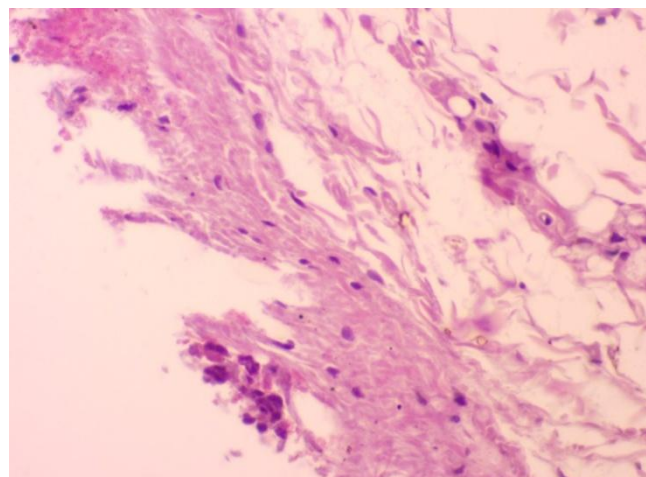


Figure 15. 0.2 ml calcium gluconate solution with collagen. Overgrowth of coarse fibrous connective tissue with residual structures in the form of basophilically stained clumps. Haematoxylin and eosin staining. Eq. vol. 10x10

4. Conclusions

The obtained results of the experimental study of the soft tissues of the gingiva, after the introduction of various wound-healing substances in the form of collagen together with vitreous body and calcium gluconate testified to the enhancement of regenerative activity especially on days 7, 14. Soft tissue in this case was characterised by resorption of collagen and calcium gluconate replaced by scar tissue. Residual structures with basophilia in the form of clumps were also revealed. At collagen injection the regeneration processes were characterised by moderate organisation, and at vitreous body injection signs of encapsulation.

Thus, the most optimal results of the experiment were observed in animals that were injected with collagen in vitreous body solution and were characterised by the development of delicate collagen fibres, which indicated the regenerative process at the site of drug injection.

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