

AUTOLOGOUS BLOOD COMPONENTS AND THEIR PERFORMANCE IN SCAFFOLDES MADE BY ORIGINAL TECHNOLOGY

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Relevance. It is believed that when scaffolds fill in bone defects after removal of jaw cysts or revision of inflammatory foci, a new bone grows from the periphery to the center, replacing the graft and at the same time changing its properties. Delivery of all materials necessary to start the reparative process begins with the contact area of the bone bed and scaffold. The properties of the material for making the scaffold and its hydrophilicity determine its ability to incorporate chemical and cellular material from surrounding tissues.

Scaffold properties can be programmed. The properties of the fluid conductor from the wall of the bone defect to the implanted material are also important. The use of autologous blood components for this role becomes attractive.

Objective: to study the composition of autologous blood components prepared using simplified technology, and their interaction with scaffolds of the original design in the treatment of patients with destructive periodontitis and jaw cysts.

Materials and methods. Microscopic examination of the components of autologous blood and their ability to penetrate into the scaffold during surgical preparation.

Results. Microscopic examination of autologous blood after centrifugation showed a platelet count in plasma similar to the norm, increased in the liquid from the gel, as well as their presence in the gel itself and in the gel membrane. A small increase in the number of leukocytes compared to the norm is determined in the liquid obtained from the gel. In the remaining blood components, the number of leukocytes in our micropreparations is not determined. The proposed original scaffold with initial cell sizes of 10 µm during immersion in the liquid components of autologous blood showed great absorption potential against the background of the possibility of increasing its porosity.

Conclusions. The proposed simple technique for obtaining the components of autologous blood allows you to create such an environment on the border of the «bone bed-scaffold», where platelets, white blood cells and biochemical blood components are concentrated in the required quantity. On the other hand, the proposed original design of the scaffold allows, in the process of filling it with a mixture of the liquid components of autologous blood, an increase in cell sizes, which leads to an increase in the absorption volume and contact area. Both of these circumstances make it possible to achieve the necessary incorporation of biological material already before scaffold is introduced into bone defects.

Key words: reparative bone regeneration, scaffold, blood components.

Relevance. One of the most important problems of modern reconstructive surgery of the maxillofacial region is the restoration of bone tissue. At the same time, preference is given to one-stage, highly effective, low-cost, safe methods, allowing to achieve the fastest anatomical and functional and social rehabilitation [10].

Reparative bone tissue regeneration is an incredibly complex and multi-component process. The role of surgery in this case is to create the most favorable conditions for the body to implement its regenerative program at each stage. The reparative regeneration of each type of tissue has unique features, but it always starts from the following processes: destruction of damaged cells and structures, including introduced ones, and proliferation of viable cells in the area of the defect.

If at the first stage blood and lymph leak from damaged vessels, then a fibrin-blood clot becomes

a naturally necessary substrate for proliferation and differentiation of cellular material with the subsequent formation of new bone tissue. The shape and localization of the bone regenerate corresponds to the shape and localization of the fibrin-blood clot. During the first 12 to 24 hours, blood flows from damaged vessels and the bone defect is filled with blood.

After a while, a blood clot forms, which consists mainly of platelets and fibrin fibers, which form a kind of network. Subsequently, this network is a biological matrix for the migration and proliferation of osteogenic cells. During the first hour after the formation of a blood clot, its retraction sets in, the clot contracts. Platelet compression occurs. They are activated and burst. Platelet activation is their degranulation. A-granules carrying growth factors are released from the protoplasm of platelets.

Vascular-platelet hemostasis, capable of independently stopping bleeding from microcirculatory vessels with low blood pressure and including the following main successive phenomena: reflex spasm of the vessel, platelet adhesion to exposed collagen of the vascular wall, their aggregation (gluing). Platelet aggregation is accompanied by the destruction of their membranes and the release of new factors that continue the process of thrombosis and trigger the mechanisms of inflammation and proliferation.

Next, there is a migration and proliferation of mesenchymal cells into the fibrin matrix from the side of intact tissues with the formation of blood vessels and the differentiation of cells in the direction of bone diaphoron. Cell adhesion occurs on fibrin jumpers with a longitudinal orientation parallel to fibrin fibers. With hypoxia (with a limited number of capillaries), anaerobic glycolytic metabolism prevails, which is characteristic for connective tissue with a low decay energy. Finally, with euxia (i.e., good capillary supply), oxybiosis, which provides bone formation, dominates.

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Platelet products are of great interest in tissue engineering. This is due to the high content of a complex of biologically active substances in them that can cause a number of cellular processes, as well as with pro-angiogenic potential. Materials based on blood components have high biological activity, which

ultimately determines the regenerative potential of scaffolds when implanted into damaged tissues.

Thus, blood components, in particular blood plasma, platelet-rich blood plasma, individual proteins, platelets, platelet lysate, and growth factors are promising and sought-after materials in tissue engineering [3,11,13,14,15,16].

There are proteins in the blood plasma that can form nanofibrous polymers and have active centers of interaction with the integrins of the cells, which remain available after polymerization. Blood plasma and platelet-rich plasma contain many nutrients and growth factors, the presence of which can determine the normal functioning of cells in the structure of the scaffold. The use of blood plasma, rather than pure fibrinogen, as a material for fibrin scaffolds, to a certain extent, can improve its mechanical properties due to its natural components.

When using platelet-rich plasma, it must be taken into account that all cell elements are degranulated within the first 3-5 days, and the natural initial activity of the cells runs out within 10 days. Not only platelet-rich plasma can be prepared from autologous blood and used as a valuable additions to bone materials, but also to prepare gels of various densities and membranes. Gels and membranes can give additional properties to both the bone materials used in this area and to stabilize the initially loose plastic material. They can also serve as a rapidly resorbable biological barrier.

True, membranes themselves, made of platelet-rich plasma, do not represent a barrier that can withstand the germination of the epithelium, but can be used as insulating membranes.

PRF represents a new step in the therapeutic concept of platelet gel [7]. Unlike other platelet concentrates, the manufacture of this material does not require any gelling agent, which gives it superiority over PRP. In PRF, platelets and released cytokines accumulate. The harmony between cytokines and their supporting fibrin matrix is much more unique than any other constant.

Fibrin gel can be used to accelerate wound healing, bone tissue regeneration, graft stabilization, wound sealing and effective hemostasis. Since the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration [8, 9].

During the production of PRF, in addition to platelets, other cellular elements, such as white blood cells, are activated. White blood cells have a great influence on the biological nature and properties of platelet concentrates, not only because of their immune and antibacterial potential, but also because these cells play a decisive role in wound healing [10]. They are capable of releasing three pro-inflammatory cytokines (IL-1 β , IL-6, and TNF α), an anti-inflammatory cytokine (IL-4), and an angiogenesis promoter (VEGF), and therefore perform important functions such as anti-infective effect, immune regulation and the ability to secrete cytokines.

The presence of leukocytes in moderation in platelet concentrates are important participants in the healing process. PRF is also able to regulate inflammation and stimulate the immune process, since the fibrin network stimulates the migration of neutrophils, modulates phagocytosis and promotes enzymatic degradation of immune cells. An important role in the mechanism of bone tissue regeneration belongs to monocytes and lymphocytes, the change in the number, ratio and functional state of which leads to a change in systemic and local inducers and modulators of bone formation, including the production of interleukins.

In the mechanism of bone resorption, macrophage activation is of great importance with increased production of IL # 1 and TNF, which activate osteoclasts and initiate an inflammatory reaction, resulting in bone destruction. This suggests that the resorption and regeneration of bone tissue have similar mechanisms of immune regulation [1].

The vast majority of autologous blood preparation methods described today (enriched plasma, platelet concentrates, fibrin gels, etc.) reflect the development and marketing of many commercial procedures that are designed to concentrate platelets and suspend them in plasma or fibrin structures of different densities. All these methods are generally grouped under the general term «platelet-rich plasma.»

All procedures for preparing preparations from autologous blood involve the collection of various volumes of venous blood, the use of various centrifugation techniques, the addition of different reagents and fragmentation of the finished product with its mechanical or other processing. The exact composition of the final material and, therefore, its potential effectiveness, can vary widely. For example, some PRP products contain white blood cells, while others do not. In some methods, exogenous thrombin or calcium chloride is added to activate platelets or to initiate a coagulation cascade. Finally, differences in the initial volume of whole blood used, as well as in the efficiency of platelet recovery, vary markedly among PRP methods, which leads to a significant (3-27 times) increase in the concentration and availability of growth factors. All PRP products are not the same and their application does not have universal indications. The success or failure of each specific PRP product or product associated with PRP is associated with specific local situations. Most authors consider it necessary to obtain a large concentration of platelets in a small plasma volume [4, 5, 6, 10, 12, 16]. We doubt this view.

Purpose of the study. The aim of our study was to study the composition of the components of autologous blood after centrifugation (plasma, liquid from the gel, gel and the membrane from the gel) and the possibility of their use in scaffold technology in the restoration of bone tissue after removal of benign neoplasms of the jaw. Attention was focused on the determination of platelets and leukocytes - the main cellular participants in the

initial stage of reparative osteogenesis. The main criteria in this case were: technological simplicity of preparing blood components, non-use of chemical reagents, low cost of the procedure and the possibility of quickly filling scaffolds and the recipient bed with the components of the autoblood.

MATERIALS AND METHODS

1. We used the original material for scaffold, consisting of calcium phosphates (from tricalcium phosphates in pure form to hydroxyapatite in pure form) of biological and synthetic origin and their mixtures in different molar compositions, as well as solid solutions of tricalcium phosphate-hydroxyapatite. Scaffolds are made using the high-temperature ($T \sim 2300$ K) photon and laser-stimulated nanocomposite crystallization process from the stoichiometric composition of calcium phosphates to a predetermined one.

In a crystalline nanocomposite solid solution of tricalcium phosphate-hydroxyapatite with a microporous structure was about $10\text{ }\mu\text{m}$, while contacting with the liquid, component resorption with different kinetics can occur, which is accompanied by the growth of micropores to 20-30 or more microns. This leads to an increase in the contact area of the nanocomposite with the liquid, as a result of which the concentration of resorbed elements in the liquid (in our case, in the blood plasma, where $3.5\text{ }\mu\text{m}$ platelets and lymphocytes from $4.5\text{--}6\text{ }\mu\text{m}$ to $20\text{ }\mu\text{m}$ were), can also increase.

2. Blood sampling was carried out from the ulnar vein in a volume of 10 ml and without any additives it was centrifuged for 12 minutes at 3000 rpm. The components for microscopic examination were separated into plasma, liquid from the gel by mechanical compression, an impression of the gel on a glass slide and a membrane.

3. Microscopic evaluation of blood components was carried out on a Konus Biorex 3 40-1000 microscope. The materials were applied to a glass slide, fixed with a Main-Grunwald solution and stained with a Romanovsky solution.

4. Platelet counts in the resulting fluids were performed on an Advia 60 analyzer, Siemens Healthcare Diagnostics Inc. USA The number of leukocytes was assessed as “not detected”, “insignificant” and “sufficient”, while focusing on the normal blood picture.

RESULTS AND DISCUSSION

The count of platelets in the plasma when counting on the analyzer was from $197 \cdot 10^9$, and in the liquid after isolation from the gel it was up to $458 \cdot 10^9\text{ }\mu\text{l}$ (which is more than the physiological norm), while about $50 \cdot 10^9\text{ }\mu\text{l}$ of erythrocytes remained in the erythrocyte mass.

During microscopic examination of blood plasma (the first series of glasses) after centrifugation (Fig. 1, 2), platelets occupied the entire viewing field with single copies or small groups. Attempts to detect white blood cells were practically unsuccessful.

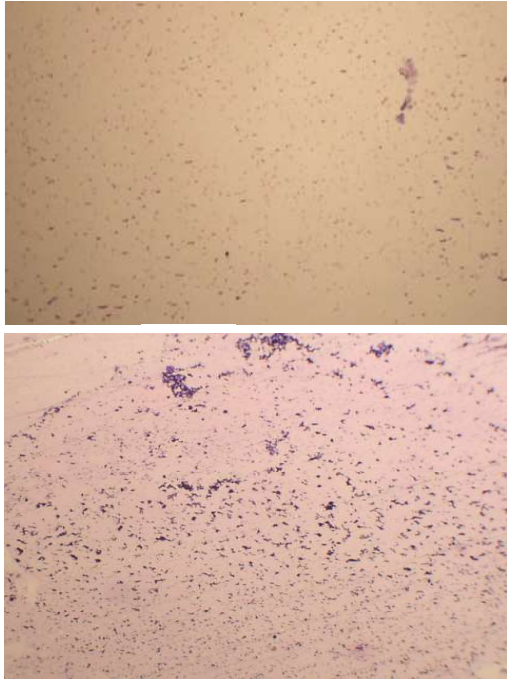


Fig. 1, 2. 100x. A significant number of platelets, other cellular material was not detected

Under microscopy of the second series of glasses (liquid from the gel), the platelet count was significantly larger (Fig. 3) and they were located mainly in groups of different sizes. In the same series, a sufficient number of different forms of white blood cells were revealed (Fig. 4, 5)

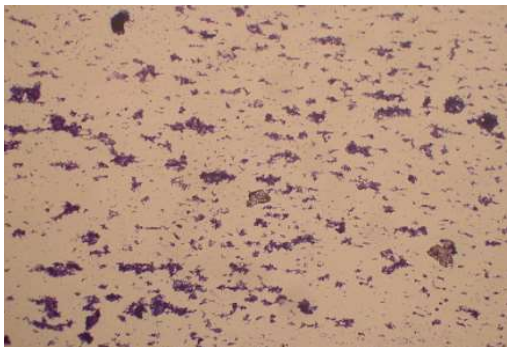


Fig. 3. 100x. Single and platelet groups in liquid from the gel



Fig. 4. 100x. A sufficiently large number of white blood cells in liquid from the gel.

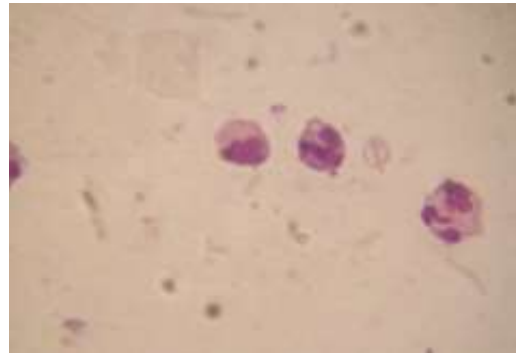


Fig. 5. 1000x. Several white blood cells from previous photo

Microscopic examination of a glass smeared with a thin layer of gel (Fig. 6, 7), it was possible to determine the longitudinally located fibrils of the gel and single and platelets going in this direction and collected in small groups; practically no leukocytes were found in the preparations.

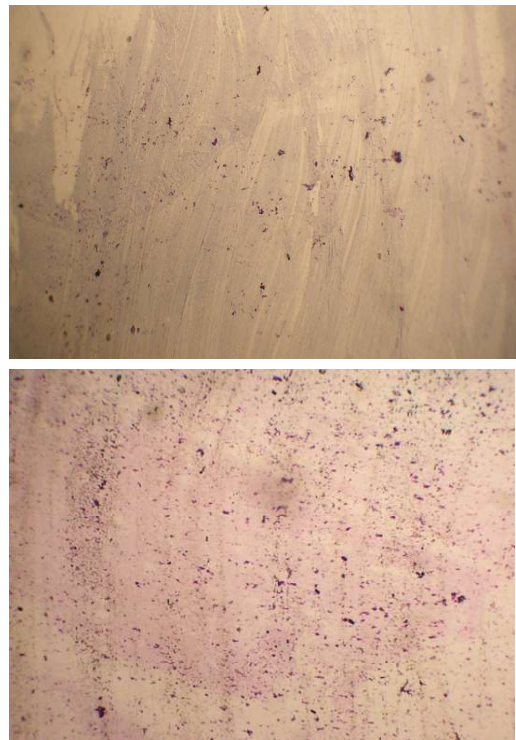


Fig. 6, 7. 100x. Parallel-directed fibrils (resembling carpal smears) are clearly defined, and single or small platelet groups following in their direction are not detected; no cellular material was detected

Microscopy of the fourth series of glasses – gel membranes obtained by finger compression to obtain a dense translucent plate – showed their inhomogeneous density and degree of saturation with stop solution and dye. Nevertheless, single and platelet-forming groups were randomly located in them. No other cellular material was detected (Fig. 8, 9).

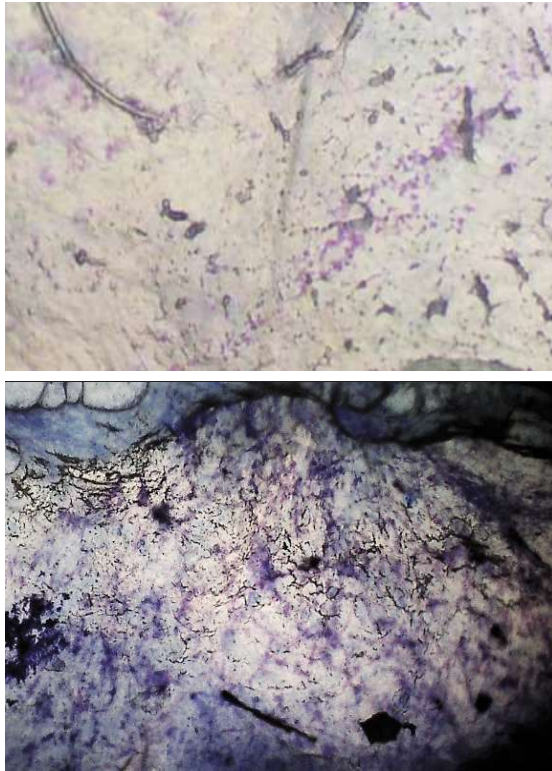


Fig. 8, 9. 100x. Against the background of a translucent membrane preparation, randomly dispersed platelets are determined. There is no other cellular material

Thus, the dynamics of the number of cellular components of the blood after centrifugation and separation into individual components (Fig. 10) has led to the fact that platelets are present in all four series of drugs, but their greatest number (above the physiological norm) is concentrated in the liquid from the gel. As for the leukocyte material, its obvious increase (compared with the physiological norm) is observed in the liquid from the gel. Attempts to detect any forms of white blood cells in plasma, gel typos, and gel membranes were unsuccessful.

The assessment of the possibility of saturating scaffolds with blood components took about 15-20 minutes (this was the time provided for by the protocols of most of our surgical interventions). In this study, 2 types of scaffolds of the original preparation were used: denser with extremely small cells and scaffolds with a microporous structure of about 10 μm (Fig. 11, 12, 13).

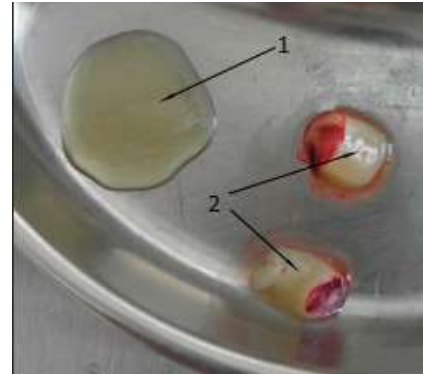


Fig. 11. Plasma (1) and gel (2) after centrifugation



Fig. 12. Small cell scaffold (1) and large cell scaffold (2) in a mixture of plasma and liquid from a gel. Membranes 3 after gel compression.

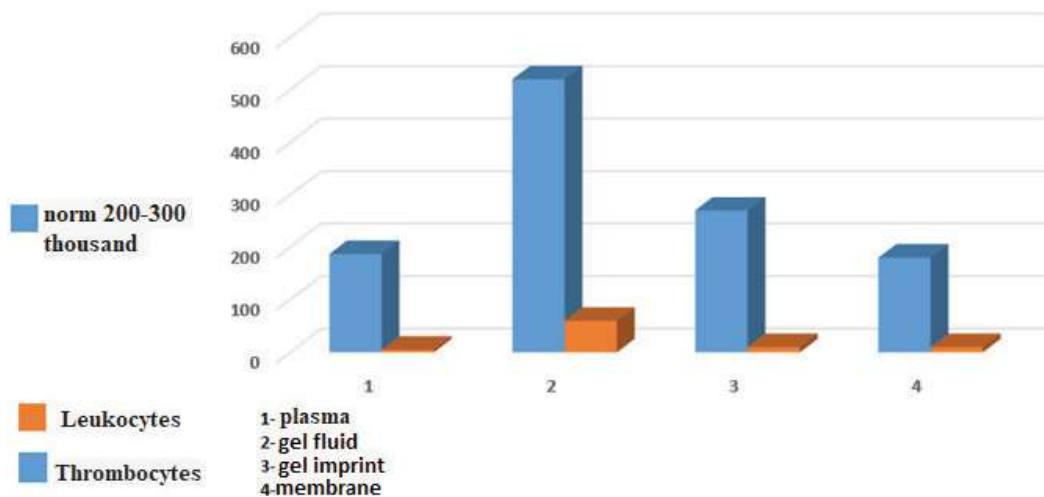


Fig. 10. Plot distribution of platelets and leukocyte group in the preparations after microscopy



Fig. 13. Gel (2) with large cells absorbs the liquid part of the blood, unlike gel (1) with small cells. Membranes (3) after gel compression.

After blood sampling in patients, plasma (1) was extracted from the tube and the gel (2) was separated from the blood clot with scissors. Subsequently, liquid was released from it and mixed with plasma. It was in this mixture that a very dense scaffold (1) was immersed in Fig. 12 and around it a scaffold with greater porosity (2). Both materials were mixed with the liquid for 15 - 20 minutes, the membranes after squeezing the gel (3) were in the form of thin translucent plates. After mixing (Fig. 13), the hard scaffold did not absorb the liquid part of the blood (1), and the scaffold with large cells (2) was saturated with a mixture of plasma and liquid from the gel and became smaller. The gel membranes (3) remained unchanged.

In more detail, the saturation test of a scaffold with large cells is as follows (Fig. 14).

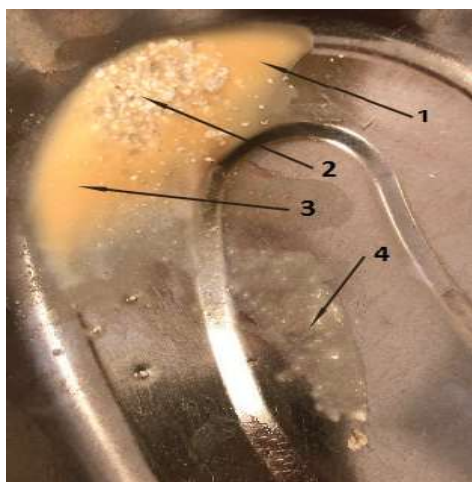


Fig. 14. Large cell scaffold (1, 3) in small fractions and in larger fractions (2). Small cell scaffolds (4) do not absorb liquid even after grinding

The scaffold (1, 3) after 15 minutes of contact with the mixture of plasma and liquid from the gel becomes soft and can be turned into an almost liquid state (in the form of milk), which can be used to fill the gaps between the incongruent surfaces of the recipient bed (wall of the

bone defect, tooth root, etc.) and made large fragments of the implanted material.

Larger scaffolds (2) adsorb liquid material well – they become colored similarly to plasma and gel liquid. An attempt to saturate a scaffold with a weakly squeezed gel, even if there is a small amount of liquid in it (4), is unsuccessful – gel fibers prevent the penetration of the material into the pores of the scaffold.

Thus, a test for the possibility of penetration of autologous components after centrifugation showed that the liquid from the gel and blood plasma are (and their mixture especially!) The ideal material for placement in a scaffold with 10 μm pores. It should be remembered that the cells of our original scaffold immediately after that increase to larger sizes – 30-40 or more microns.

Together with the non-cellular composition of the liquid material (proteins, fats, carbohydrates, minerals, signaling molecules, etc.), the first cell delegation (platelets and leukocytes) easily gets into scaffolds – this is important at the first early stage of reparative osteogenesis. Gel from autologous blood, liquid or with a slight squeeze, can, in our opinion, be used for more specific indications – parietal hemostasis, retention of the material, etc. The widespread use of membranes after more severe mechanical processing of the gel has very limited (protective, mechanical, hemostatic) application, in addition, the body must spend time and resources on its disposal and replacement.

CONCLUSIONS

Our morphological studies suggest that a simple, effective and cheap way to obtain autologous blood components has real clinical potential when used in maxillofacial surgery and dentistry. All the materials obtained have different purposes. The mixture of plasma and liquid from fibrin gel contains a natural or a slightly increased number of white blood cells and platelets. This mixture effectively penetrates the scaffold developed by us with variable cell geometry, which ensures the platelet and leukocyte culture before the material is introduced into bone defects. A mixture of plasma and liquid from a gel with small fragments of a scaffold fills the contact space between the incongruent surfaces of the recipient bed and large fragments of the scaffold. A fibrin gel with various degrees of fluid loss is useful for hemostasis on the wound surfaces of the recipient bed and for holding volumes of the scaffold in different geometric shapes. We consider this direction promising and will continue to work.

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КОМПОНЕНТИ АУТОЛОГІЧНОЇ КРОВІ ТА ЇХ ЕФЕКТИВНІСТЬ ПРИ ВИКОРИСТАННІ СКАФФОЛДІВ ОРИГІНАЛЬНОЇ КОНСТРУКЦІЇ

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Актуальність. Вважається, що після заповнення скаффолдами кісткових дефектів після видалення кіст щелеп або ревізії вогнищ запалення, нова кістка росте від периферії до центру, заміщаючи трансплантат і в той же час змінюючи його властивості. Доставка всіх матеріалів, необхідних для початку репаративного процесу, починається з області контакту кісткового ложа і скаффолда. Властивості матеріалу для виготовлення скаффолда і його гідрофільність визначають його здатність включати хімічний і клітинний матеріал з навколишніх тканин. Властивості скаффолдів можуть бути запрограмовані. Властивості провідної рідини від стінки кісткового дефекту до імплантованого матеріалу також важливі. Використання компонентів аутологічної крові для цієї ролі стає привабливим.

Мета: вивчити склад компонентів аутологічної крові, приготовленої за спрощеною технологією, і її взаємодію із скаффолдами оригінальної конструкції при лікуванні хворих з деструктивними пародонтитами та кістами щелеп.

Матеріали та методи. Мікроскопічне дослідження компонентів аутологічної крові та їх здатності проникати у скаффолди при хірургічній підготовці.

Результати. Мікроскопічне дослідження аутологічної крові після центрифугування показало, що кількість тромбоцитів в плазмі близька до норми, проте її кількість збільшилася в рідині з гелю. Тромбоцити також присутні в самому гелі і в гелевій мембрані. Збільшення кількості лейкоцитів у порівнянні з нормою визначається в рідині, отриманій з гелю. В інших компонентах аутологічної крові кількість лейкоцитів в наших мікропрепаратах не визначалася. Пропонований оригінальний скаффолд з початковими розмірами комірок 10 мкм при зануренні в рідкі компоненти аутологічної крові показав великий потенціал її поглинання на фоні можливості збільшення його пористості.

Висновки. Запропонована проста методика отримання компонентів аутологічної крові, компоненти якої дозволяють створити таке середовище на кордоні «кісткове ложе-скаффолд», де тромбоцити, лейкоцити і біохімічні компоненти крові концентруються в необхідній кількості. З іншого боку, пропонована оригінальна конструкція скаффолда дозволяє при заповненні його сумішшю рідких компонентів аутологічної крові збільшувати розміри осередків, що призводить до збільшення обсягу поглинання і площі контакту. Обидві ці обставини дозволяють досягти необхідного включення біологічного матеріалу ще до того, як скаффолд буде введений в кісткові дефекти.

Ключові слова: репаративна регенерація кістки, скаффолд, компоненти аутокрові.

КОМПОНЕНТЫ АУТОЛОГИЧНОЙ КРОВИ И ИХ ЭФФЕКТИВНОСТЬ ПРИ ИСПОЛЬЗОВАНИИ СКАФФОЛДОВ ОРИГИНАЛЬНОЙ КОНСТРУКЦИИ

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Актуальность. Считается, что, когда скаффолды заполняют костные дефекты после удаления кист челюсти или ревизии очагов воспаления, новая кость растет от периферии к центру, замещая трансплантат и в то же время изменяя его свойства. Доставка всех материалов, необходимых для начала репаративного процесса, начинается с области контакта костного ложа и скаффолда. Свойства материала для изготовления скаффолда и его гидрофильность определяют его способность включать химический и клеточный материал из окружающих тканей. Свойства скаффолдов могут быть запрограммированы. Свойства проводящей жидкости от стенки костного дефекта к имплантированному материалу также важны. Использование компонентов аутологичной крови для этой роли становится привлекательным.

Цель: изучить состав компонентов аутологичной крови, приготовленных по упрощенной технологии, и их взаимодействие со скаффолдами оригинальной конструкции при лечении больных с деструктивным пародонтитом и кистами челюстей.

Материалы и методы. Микроскопическое исследование компонентов аутологичной крови и их способности проникать в скаффолды при хирургической подготовке.

Результаты. Микроскопическое исследование аутологичной крови после центрифугирования показало, что количество тромбоцитов в плазме, близкое к норме, увеличилось в жидкости из геля, тромбоциты также присутствовали в самом геле и в гелевой мембране. Увеличение количества лейкоцитов по сравнению с нормой определяется в жидкости, полученной из геля. В остальных компонентах крови количество лейкоцитов в наших микропрепаратах не определялось. Предлагаемый оригинальный скаффолд с начальными размерами клеток 10 мкм при погружении в жидкие компоненты аутологичной крови показал большой потенциал ее поглощения на фоне возможности увеличения его пористости.

Выводы. Предлагаемая простая методика получения компонентов аутологичной крови позволяет создать такую среду на границе «костное ложе-скаффолд», где тромбоциты, лейкоциты и биохимические компоненты крови концентрируются в необходимом количестве. С другой стороны, предлагаемая оригинальная конструкция скаффолда позволяет при заполнении его смесью жидких компонентов аутологичной крови увеличивать размеры ячеек, что приводит к увеличению объема поглощения и площади контакта. Оба эти обстоятельства позволяют достичь необходимого включения биологического материала ещё до того, как скаффолд будет введен в костные дефекты.

Ключевые слова: репаративная регенерация кости, скаффолд, компоненты аутокрови.