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# POSSIBILITIES OF OBTAINING AND USING HYDROGEL-BASED BIOMATERIALS FOR REGENERATION OF HUMAN BONE TISSUE

*V.E. Dubrov, E.S. Klimashina, I.M. Scherbakov, G.A. Shipunov, V.I. Putlayev, P.V. Evdokimov, A.A. Tikhonov, S.V. Gulko, D.A. Zyuzin*

Lomonosov Moscow State University, Moscow, Russian Federation

Substitution of defects in various tissues, especially bone tissues, is a major challenge in modern medicine. There is currently no universal method of filling defects which has no drawbacks. Hydrogels are one of the promising groups of alloplastic materials. At present, you can obtain materials with various biological properties like natural extracellular matrix using various methods of chemical and physical modification. These biomaterials can be used as a means of delivering stem cells and bioactive substances to the defect zone. This literature review is devoted to the various aspects of preparation and use of hydrogel-based biological materials.

*Keywords: hydrogels, tissue regeneration, tissue engineering.*

Replacement of bone tissue defects is an urgent problem in modern traumatology and orthopedic oncology [1–4]. Bone defects can form from open bone fractures, fractures with compressed spongy bone tissue, formation of bone cysts, as well as a result of surgical treatment (tumor removal, resection of false joints, osteomyelitis areas, and bone osteotomy) [5]. Some defects are filled independently in the process of reparative regeneration. However, filling does not occur when a defect reaches a critical value [6]. Doctors have several ways of replacing bone defects, but each has its own drawbacks. Autologous spongy bone tissue is limited in the volume of the donor zone, it does not have mechanical strength. Moreover, cosmetic and pain problems often arise in the donor region [7]. Transplantation of bone blocks on vascular pedicle is complex, requires special equipment for the operating room and staff training. It cannot be a routine and generally accessible method [8]. Distraction osteogenesis requires prolonged use of external fixation apparatus, patient and staff discipline, and may be accompanied by purulent-septic complications [9]. The use of cadaveric bone comes with a risk of infection of the patient. Besides, there are many problems involved in taking, processing, and sterilizing the material [10]. Similar problems arise when using specially treated animal bone. Synthetic materials are free from many drawbacks – they are not limited in volume, materials can be created with specified mechanical properties, there is no risk of infection transmission, and biological modification over a wide range is possible [11].

In general, ideally optimized materials for replacement of bone defects should meet the following requirements: 1) no cytotoxicity and immunogenicity in order to avoid inflammation; 2) osteoinductive properties (ability to stimulate differentiation of surrounding progenitor cells to osteoblasts); 3) osteoconductive properties (ability of a material to be a three-dimensional matrix for germination of blood vessels and tissue elements due to the corresponding pore size and associated porosity, i.e., to simulate a natural extracellular matrix to ensure cell adhesion and proliferation); 4) possible presence of osteogenic properties (ability to be a medium for placement of osteoblast progenitor cells); 5) biodegradability (possibility of decomposition by endogenous enzymes or by hydrolysis simultaneously with the substitution process to create sufficient space for formation of a new bone); 6) structural stability and mechanical strength, which can be used to correct defects in the loaded zone and prevent denaturation during sterilization [4, 12–16].

## DIVERSITY AND OBTAINING VARIOUS TYPES OF HYDROGELS

Hydrogels are one of the promising groups of alloplastic materials that can meet all the above properties.

A hydrogel is a three-dimensional network of hydrophilic polymers that can swell in water and hold different amounts of water (almost 100%) or biological fluids, while maintaining its structure and properties of a solid [17].

Hydrogels were first reported in Germany at the end of the 19th century [18]. Their biomedical use was dis-

**Corresponding author:** Vadim Dubrov. Address: 27, Lomonosov av., Moscow, 119991, Russian Federation.
Tel. (985) 764-52-89. E-mail: vduort@gmail.com

cussed in Czechoslovakia in the late 1950s after publication of the works of professors Wichterle and Lim [19], who studied materials based on synthesized poly-2-hydroxyethyl methacrylate (polyHEMA), which was later used in the manufacture of contact lenses.

Based on origin of polymers, hydrogels can be subdivided into natural, synthetic and semi-synthetic, or mixed [15, 20].

The most commonly used natural materials include polypeptides (collagen, gelatin and fibrin) and polysaccharides (hyaluronic acid, chitosan, alginate and chondroitin sulfate) [21–23]. The main advantages of such materials are their low cytotoxicity, high biocompatibility and biodegradability, which is facilitated by *in vivo* enzymes. However, their main disadvantage is the difficulty of controlling mechanical properties and swelling.

Among synthetic substances, biodegradable polymers with controlled microstructure and mechanical properties, such as polyhydroxyethylmethacrylate (polyHEMA), polyethylene glycol (PEG) and its derivatives acrylates (PEGDA – diacrylate, PEGDMA – dimethacrylate), poly(N-isopropylacrylamide) (PNIPAm), polyvinyl alcohol (PVA), polyglycolic acid (PGA) and poly(lactic-co-glycolic acid), polyacrylic acid (PAA), polyacrylamide (PAM), etc. are more often used [15, 22, 24, 25]. Synthetic polymers have long shelf life without the risk of increase in immunogenicity. In addition, they can be produced in large volumes. In turn, the use of synthetic monomers allows you to set and control the mechanical strength and elasticity of hydrogels, biodegradation, biological and chemical behavior in the body. The main challenge in this case is the choice of biocompatible and non-toxic monomers, their polymers, as well as polymerization initiators [20].

Due to the indicated limitations of synthetic hydrogels, various combinations of natural and synthetic hydrogels with the best, according to some authors, biological and mechanical properties, such as chitosan-PEG, collagen-poly(N-isopropylacrylamide) and chitosan-poly(vinyl alcohol) are used in biotechnology [26–28].

Polymeric hydrogels are obtained via polymerization reaction, initiated by radiation (electron beam, gamma radiation, x-ray or ultraviolet radiation), changes in pH, temperature or by chemical reactions (click chemistry, disulfide crosslinking, enzyme-mediated crosslinking, Michael reaction, Schiff base cross-linking, ionic crosslinking, self-assembly) [17]. Traditional approaches to preparation of porous hydrogels include leaching of porous material, gas formation, lyophilization, and electrospinning [22, 29–31].

Despite advances in production of porous hydrogels, these methods could not provide precise control of pore size and spatial location of pores. Recently, more advanced additive technologies, such as stereolithography, 3D printing, and microfluidics, have been used to develop complex porous microarchitectures [22, 32–34].

## METHODS OF MODIFYING HYDROGEL PROPERTIES

Various chemical and physical modifications are used to control the biological properties of hydrogels. They include choosing the composition of monomers, changing the degree of polymer crosslinking, constructing different architectures using 3D printing, introducing various functional groups and nanoparticles that change the properties of the whole composite.

Selection of monomers determines the production of hydrogels capable of carrying out a sol-gel phase transition when heated to body temperature. That is why hydrogels can be introduced into the body in liquid form, that is, in a minimally invasive way. Such hydrogels include, for example, poly(N-isopropylacrylamide) (PNIPAm), hydrogels that become soluble in water at a temperature below 32 °C and are reversibly converted into gel form when heated above 32 °C [35]. Thermo-sensitive injection composite materials with improved mechanical properties and biological activity can be obtained by adding to PNIPAm other functional components, such as PEG, poly(N,N-dimethylacrylamide), and poly(2-hydroxyethyl methacrylate) [36]. Currently, TSV Gel (OsteoBiol, Italy) is commercially available, which is a mixture of animal collagen and gel-forming synthetic copolymer Poloxamer 407. This drug exists in liquid form at temperatures below 8 °C, and begins to turn into a gel-like state at temperatures above 13 °C. This allows it to fill defects of complex shape [37].

The monomers selected determine the rate and conditions of degradation of hydrogels in the body. A group of scientists led by S.P. Zustiak et al. synthesized hydrolytically degradable PEG hydrogel, composed of PEG vinyl sulfone (PEG-VS) cross-linked with PEG-diester-dithiol. Degradation time and the mechanical properties of this hydrogel can be controlled by altering parameters such as distance between thiol and ester group in the cross-linker, molecular weight and polymer density [38].

Various chemical and physical methods for crosslinking polymers are important aspects of hydrogel synthesis, allowing to vary physical characteristics. Using chemical crosslinking, more stable hydrogels with enhanced mechanical properties are built through formation of strong covalent bonds [39]. A physical compound results from non-covalent interaction, such as van der Waals forces, hydrogen bonds, hydrophobic bonds and electrostatic forces [40]. Consequently, the mechanical strength of physically bonded hydrogels is relatively lower than covalently bonded ones, but they decompose more easily in the body. Chemically bound hydrogels may be less compatible with tissues due to the potential

cytotoxicity of residual polymerization initiators and organic solvents, as well as delayed degradation [41].

It should be considered that the mechanical properties of hydrogels can affect cell differentiation by various mechanotransduction pathways through the tension and integrity of actin cytoskeleton, nuclear mechanics, and integrin-mediated adhesion and signaling [42–44]. Early studies using 2D substrates suggested that rigid hydrogels promote osteogenic differentiation, while compliant hydrogels enhance neuro- and adipogenic differentiation progenitor cells [42, 45]. For example, Huebsch et al. investigated the effect of stiffness on cells grown on alginate hydrogels. The study demonstrated that osteogenic differentiation of mesenchymal stem cells was stimulated by growing cells on 3D matrices with 11–30 kPa rigidity, while adipogenic differentiation was enhanced with a gel rigidity of 2.5–5 kPa [45]. It has also been shown that rapidly relaxing hydrogels promote spreading, proliferation, and osteogenic differentiation of mesenchymal stem cells [46].

By changing photopolymerization conditions, it is possible to change such hydrogel characteristics as stiffness and viscoelastic properties. In S. Yang et al., gel stiffness and spatial organization were monitored on a photodegradable hydrogel matrix using lithographic masks and photographic coating of soft and hard regions on a micrometer scale [47]. Results showed that the cells had a large area and elongated morphologies with increasing hard areas on the hydrogel substrate. In addition, regular patterns with high stiffness enhanced osteogenic differentiation of mesenchymal stem cells compared to randomized patterns. Exact spatial control of the mechanical properties of a hydrogel can mimic the gradually varying stiffness of the interface between soft and hard tissue, such as "ligament-bone" or "tendon-bone" [48].

Free diffusion in the thickness of hydrogels is limited. So, porosity is a decisive physical factor for facilitating transport of nutrients and oxygen [49]. Absence of anastomoses and blood perfusion can delay tissue regeneration due to difficulties in cell migration and proliferation [50]. In addition, by altering the size and 3D organization of the pore system, you can create biophysical signals that regulate cellular behavior by simulating physical features at the micro and nanoscale [51].

By creating porous materials in the form of frames of various architectures, you can reach a compromise between the permeability of the material and its strength. Due to some topological optimization features, either cellular porous structures [52] or 3D periodic minimal surfaces are selected to find an optimal geometry [53, 54].

If the pore size is too large, the cells recognize their contact surface as 2D and become more susceptible to the influence of surface properties of the material, such as stiffness. When cells migrate through a smaller porous structure, the speed and efficiency of migration are more dependent on 3D geometry. Consequently, different pore sizes are required depending on the 3D geometry and properties of the frame materials and cell types [51, 55]. For example, mesenchymal stem cells in scaffolds migrated further when the pore diameter (12 μm) was relatively similar to the cell size than when the pore size was small (7 μm) or large (17 μm) [56]. Fibroblast migration rate decreases as the pore size of hydrogels increases across a range from 90 to 150 μm [57]. According to literature sources, there are various optimal pore sizes of implants for induction of regeneration of various types of tissues: 5 μm pore diameter for vascularization [58], 5–15 μm for fibroblast ingrowth [59], 20–125 μm for regeneration of adult skin [60] and 100–350 μm for bone regeneration [61].

One example of altering the properties of hydrogels by introducing functional groups is the creation of materials whose swelling depends on the pH of the environment. This is achieved by incorporating carboxyl groups into the starting monomers. Ionization/deionization of these groups induces swelling/deswelling depending on the pH of the medium [62]. In an alkaline medium, carboxyl groups are ionized and repel each other, leading to hydrogel swelling. In an acidic environment, COOH groups are protonized with charge loss and hydrogel deswelling with water release. The clinical significance of this fact is that such scaffolds can selectively deliver biomolecules to defect sites where the environment is more acidic, for example, in ischemia or inflammation. Based on this, a team of scientists led by Matsusaki prepared a pH-sensitive semi-interpenetrating polymer network like heterogels composed of γ-PGA (polyglutamic acid) and sulfonated γ-PEG [62]. Hydrogels modified in this way swell/deswell depending on pH conditions, while the sulfonic acid groups can increase proton concentration. As a result, growth factors, such as fibroblast growth factor-2 (FGF-2), are released as the surrounding acidity increases. These pH-sensitive hydrogels can be used to fill defect sites in inflammation or ischemia – these areas have comparatively acidic pH (<6.5) compared to surrounding tissues [36].

Alginate is widely used as a hydrogel crosslinked via ionic interactions due to its high biocompatibility and ease of gel formation [63, 64]. Alginate hydrogels are obtained through a combination of solutions of alginate with calcium chloride, in which $Ca^{2+}$ ions bind to hyaluronate blocks of alginate chains. However, after crosslinking, limited release of $Ca^{2+}$ ions from these hydrogels is accompanied by slow degradation of the material, which reduces the viability of hydrogel-encapsulated cells [65]. To solve this problem, Z. Wu et al. increased the ability of calcium-crosslinked alginate to decompose by adding sodium citrate, whose citrate ion can chelate to calcium ions in a hydrogel. By controlling the mole ratio of sodium citrate/sodium alginate, decay of 3D-printed

alginate hydrogel was regulated, which contributed to high viability and proliferation of cells introduced into the hydrogel [66].

By adding various functional groups even to natural polymers, one can significantly increase the affinity of hydrogels for water and various protein compounds. Widespread use of PEG in the medical field is based on its inherent biocompatibility and ease of control of physical and chemical properties. However, unmodified PEG hydrogels are inert and adsorb limited amount of proteins. In addition, many cell types cannot attach to PEG hydrogels or have low viability during encapsulation internally [67]. To overcome this limitation, arginyl-glycyl-aspartic acid (RGD peptide), which is a natural component of the collagen molecule, is attached to this type of hydrogels [14]. Additional substrates covalently bind to the components of the hydrogel using enzymes or factor XIII transglutaminase, which acts as a catalyst. Hydrogels modified in this way demonstrate a higher cell density than PEG hydrogels without RGD peptides due to faster penetration of mesenchymal stem cells into the material structure [68].

Another problem that could be solved by adding auxiliary substances to hydrogels is excessive shrinkage of the material when saturated with water. High degree of shrinkage can lead to a mismatch in size between the implant and tissues [69]. For example, collagen, which is the main component of connective tissue and is widely used in biomedical engineering, has low stability and can shrink severely after immersion in liquid. This limits its use for tissue regeneration [14, 70]. To address this problem, aminated bioactive glass particles were included in collagen, which formed strong chemical bonds between positively charged amine groups and negatively charged carboxyl groups of collagen. Mesenchymal stem cells, cultured in such a hydrogel, had a higher viability and a more diverse morphology than when using pure collagen [70].

The low mechanical strength of hydrogels may limit their use in regenerative engineering of supporting tissues. Since the very high density of hydrogel networks is accompanied by lower diffusion rate, the mechanical properties of hydrogels can also be modulated by including various nanomaterials [22, 71, 72]. For example, to create composite materials for bone tissue regeneration, such nanoparticles as calcium phosphates [73] and silicates [74] are introduced in hydrogels. This increases their mechanical strength and osteogenic properties. For example, introduction of hydroxyapatite particles increases the elastic modulus, ultimate deformation, and strength of the composite by up to 15% compared to empty hydrogel [75]. Studies of the physical properties of hydrogels filled with various types of calcium phosphates suggest that strength properties (compressive and tensile strength) can be highly dependent on the type of calcium phosphate used [64, 76].

Among the variety of calcium orthophosphates, hydroxyapatite is a classic and most used component in the creation of bioimplants both as the main phase [77] and as coatings or an additional bioresistive phase [14, 78, 79]. However, low resorbability of hydroxyapatite, due to the calcium/phosphorus ratio (Ca/P ratio = 1.67) [80], makes it necessary to look for a replacement for hydroxyapatite. Alternatively, calcium phosphates with a lower Ca/P ratio or their mixtures are proposed: brushite, monetite and calcium pyrophosphate (Ca/P = 1), octalcium phosphate (Ca/P = 1.33), tricalcium phosphate (Ca/P = 1.5) [23, 76, 80, 81]. These phosphates belong to the class of acid phosphates (hydrophosphates). *In vivo* studies have suggested that the biodegradation rate of alginate-based composite materials decreases depending on the type of calcium phosphate: maximum for octacalcium phosphate, less for tricalcium fascate and significantly less for carbonate hydroxyapatite [64]. Upon dissolution (resorption), they create slightly acidic pH values in the environment, which leads to partial dissolution (etching) of hydroxyapatite crystals of the surrounding bone tissue. When morphogenetic bone proteins and other bioactive factors are adsorbed on their surface [82], they can transit to a dissolved state, which locally creates higher concentration of bioactive substances and starts a chain of biological processes, as a result of which bone tissue forms in this place.

## APPLICATION OF HYDROGEL-BASED BIOMATERIALS

Research on creation and use of hydrogels is still largely at the preclinical stage of development. However, there are reports on the first real applications of the above principles in creation of artificial organs.

N. Kang et al. (2016) described a method for creating cell-loaded hydrogel composites (fragments of the bones of the cranial vault and lower jaw, auricular cartilage and a fragment of the skeletal muscle) using integrated 3D printer created by the authors for printing organs and tissues. Here, multicomponent composition of the hydrogel was used. The mechanical basis of the hydrogel was polycaprolactone (PCL), whose pores were filled with a less mechanically strong (but more compatible with cells) composition of gelatin, fibrinogen, hyaluronic acid and glycerol. The cell component was lines of fibroblasts and myoblasts, chondrocytes and human amniotic stem cells. One of the features of the created materials – a microchannel system that permeates the entire structure and provides cell nutrition throughout its thickness. During implantation of the obtained materials in laboratory animals, it was shown that the cells included in the material begin to differentiate, grow and

synthesize their own surrounding matrix at the site of the absorbable hydrogel [83].

In domestic literature sources, there are examples of creation of hydrogel-based nanocomposite materials loaded with stem cells [84]. In [22], the authors describe the creation of a hydrogel matrix based on poly(L-lactide) obtained by imprint lithography, which was populated with mesenchymal stem cells. *In vitro* studies showed that stem cells differentiated along the osteogenic pathway, with good adhesion to the resulting material and high survival.

In another work [85], a preclinical *in vivo* study of a fibrin hydrogel-based composite material with tricalcium phosphate inclusions and loaded with mesenchymal stromal stem cells was performed on a model of a critical defect in the femoral epiphysis of a rabbit. This scaffold was shown to be able to transfer living stem cells while maintaining their regenerative potential and the potential for bone tissue replacement. However, the negative effect of fibrin hydrogel on the osteoconductive properties of ceramics in the composite was shown.

In *in vivo* study by Petrov et al. [86] on an animal model of a bone defect, the biological properties of biomaterial based on demineralized bovine bone collagen and hyaluronic acid and chondroitin sulfate were studied on animal model of bone defect. Histological examination showed faster and more complete replacement of ileal defect with newly formed bone tissue when using biomaterial compared with the control group.

Literature sources describe the preparation and study of alginate-based composite hydrogel materials in laboratory animals (mice and rats) with the addition of gelatin, as well as octacalcium phosphate (OCP) and tricalcium phosphate (TCP) crystals. Here, it is shown that addition of gelatin and calcium crystals helps to increase strength and porosity. It was suggested that three-component hydrogels using OCP have better osteoconductive properties and faster bone formation [2].

National literature sources describe successful clinical applications of a combination of spongy pelvic autologous bone and commercially available collagen-based hydrogels (SFERO®gel LONG, Russia) to replace critical defects of the femur and tibia in humans. At the same time, authors point to the role of hydrogel in maintaining the regenerative process launched by autologous bone [9].

## CONCLUSION

This literature review shows that hydrogels are presently a very diverse class of compounds, both in chemical composition and in chemical, physical, and biological properties. Such a variety seems promising in terms of creation of biomedical materials that can effectively replace the natural structures of the body. Various methods for modifying the properties of hydrogels provide opportunities for adapting them to specific clinical situations in order to meet the needs of a customized approach in modern medicine. Despite an understanding of the general principles of creating an "ideal" tissue-engineering design for bone defect replacement, real life samples that meet all the requirements of efficiency and safety have not yet been obtained. Further research is needed to create composite materials for effective replacement of large volumes of lost bone tissue, which would allow for full and quick restoration of body functions.

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## REFERENCES

1. *Anastasieva EA, Sadovoy MA, Voropaeva AA, Kirilova IA*. Reconstruction of Bone Defects after Tumor Resection by Auto- and Allografts (Review of Literature). *Travmatologiya i ortopediya Rossii [Traumatology and Orthopedics of Russia]*. 2017; 23 (3): 148–155. (in Russian). doi: 10.21823/2311-2905-2017-23-3-148-155.
2. *Karalkin PA, Sergeeva NS, Komlev VS, Sviridova IK, Kirsanova VA, Akhmedova SA et al*. Biocompatibility and osteoplastic properties of mineral polymer composite materials based on sodium alginate, gelatin, and calcium phosphates intended for 3D-printing of the constructions for bone replacement. *Genes and Cells*. 2016; 11 (3): 94–101.
3. *Karyakin NN, Gorbatov RO, Novikov AE, Niftullaev RM*. Surgical treatment of patients with tumors of long bones of upper limbs using tailored 3D printed bone substitute implants. *Genij ortopedii*. 2017; 23 (3): 323–330. doi 10.18019/1028-4427-2017-23-3-323-330.
4. *Popkov AV*. Biocompatible implants in traumatology and orthopaedics (A review of literature). *Genij ortopedii*. 2014; 3: 94–99.
5. *Fernandez de Grado G, Keller L, Idoux-Gillet Y, Wagner Q, Musset AM, Benkirane-Jessel N et al*. Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. *J Tissue Eng*. 2018; 9: 2041731418776819. doi: 10.1177/2041731418776819.
6. *Wang W, Yeung KWK*. Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioact Mater*. 2017; 2 (4): 224–247. doi: 10.1016/j.bioactmat.2017.05.007.
7. *Dau M, Ganz C, Zaage F, Frerich B, Gerber T*. Hydrogel-embedded nanocrystalline hydroxyapatite granules (elastic blocks) based on a cross-linked polyvinylpyrrolidone as bone grafting substitute in a rat tibia model. *Int J Nanomedicine*. 2017; 12: 7393–7404. doi: 10.2147/IJN.S142550.
8. *Douglas AJ, Kyzas PA*. A new autologous block-bone prefabricated flap concept based on the supraclavicular artery island flap (SCAIF) for reconstruction of a

neo-mandibular osteoradionecrosis (ORN) defect, IDEAL Stage 1 report. JPRAS Open 2017; 12: 19–24. doi: 10.1016/j.jpra.2016.11.002.
9. *Kryukov EV, Brizhan' LK, Khominets VV, Davydov DV, Chirva YuV, Sevastianov VI et al.* Clinical use of scaffold-technology to manage extensive bone defects. *Genij ortopedii*. 2019; 25 (1): 49–57.
10. *Gut G, Marowska J, Jastrzebska A, Olender E, Kamiński A*. Structural mechanical properties of radiation-sterilized human Bone-Tendon-Bone grafts preserved by different methods. *Cell Tissue Bank*. 2015; 17 (2): 277–287. doi: 10.1007/s10561-015-9538-1.
11. *Ferracini R, Martínez Herreros I, Russo A, Casalini T, Rossi F, Perale G*. Scaffolds as Structural Tools for Bone-Targeted Drug Delivery. *Pharmaceutics*. 2018; 10 (3): 122. doi: 10.3390/pharmaceutics10030122.
12. *Rehmann MS, Kloxin AM*. Tunable and dynamic soft materials for three-dimensional cell culture. *Soft Matter*. 2013; 9 (29): 6737–6746. doi: 10.1039/C3SM50217A.
13. *Bai X, Gao M, Syed S, Zhuang J, Xu X, Zhang XQ*. Bioactive hydrogels for bone regeneration. *Bioact Mater*. 2018; 3 (4): 401–417. doi: 10.1016/j.bioactmat.2018.05.006.
14. *Fatkhudinova NL, Vasilyev AV, Bukharova TB, Osidak EO, Starikova NV, Domogatsky SP et al.* The prospects of collagen as a basis for curable and activated osteoplastic materials. *Stomatologiya*. 2018; (6): 78–83. https://doi.org/10.17116/stomat20189706178.
15. *Kuznetsova DS, Timashev PS, Bagratashvili VN, Zagaynova EV*. Scaffold- and Cell System-Based Bone Grafts in Tissue Engineering (Review). *Sovremennye tehnologii v medicine*. 2014; 6 (4): 201–212.
16. *Sadovoy MA, Larionov PM, Samokhin AG, Rozhnova OM*. Cellular Matrices (Scaffolds) for Bone Regeneration: State of the Art. *Hir Pozvonoc*. 2014; (2): 79–86.
17. *Barbucci R.* Hydrogels. Milano: Springer; 2009. doi: 10.1007/978-88-470-1104-5.
18. *Bemmelen JM.* Zeitschr f Chem und Ind der Kolloide (1907) 1: 213. [In Deu]. doi: 10.1007/BF01830147.
19. *Wichterle O&L, [Iacute] M.D.* Hydrophilic Gels for Biological Use. *Nature*. 1960; 185: 117–118 doi: 10.1038/185117a0.
20. *Gibbs DMR, Black CRM, Dawson JI, Oreffo ROC*. A review of hydrogel use in fracture healing and bone regeneration. *Journal of Tissue Engineering and Regenerative Medicine*. 2016; 10 (3): 187–198. doi: 10.1002/term.1968.
21. *Annabi N, Tamayol A, Uquillas JA, Akbari M, Bertassoni LE, Cha C et al*. 25th anniversary article: Rational design and applications of hydrogels in regenerative medicine. *Adv Mater*. 2013; 26 (1): 85–123. doi: 10.1002/adma.201303233.
22. *Tereshchenko VP, Larionov PM, Kirilova IA, Sadovoy MA, Mamonova EV*. Materials and methods of bone tissue engineering. *Hir Pozvonoc*. 2016; 13 (1): 72–81. . doi: http://dx.doi.org/10.14531/ss2016.1.72–81.
23. *Gurin AN, Komlev VS, Fedotov AYu, Berkovsky AA, Mamonov VE, Grigoryan AS*. Comparative study of osteoplastic materials based on chitosan, alginate or fibrin with tricalcium phosphate. *Stomatologiya*. 2014; 93 (1): 4–10. ISSN 0039-1735.
24. *Shi K, Wang YL, Qu Y, Liao JF, Chu BY, Zhang HP et al*. Synthesis, characterization, and application of reversible PDLLA-PEG-PDLLA copolymer thermogels *in vitro* and *in vivo*. *Sci Rep*. 2016; 6: 19077. doi: 10.1038/srep19077.
25. *Vo TN, Ekenseair AK, Spicer PP, Watson BM, Tzouanas SN, Roh TT et al*. *In vitro* and *in vivo* evaluation of self-mineralization and biocompatibility of injectable, dual-gelling hydrogels for bone tissue engineering. *J Control Release*. 2014; 205: 25–34. doi: 10.1016/j.jconrel.2014.11.028.
26. *Das D, Ghosh P, Ghosh A, Haldar C, Dhara S, Panda AB et al*. Stimulus-responsive, biodegradable, biocompatible, covalently cross-linked hydrogel based on dextrin and poly (N-isopropylacrylamide) for *in vitro*/*in vivo* controlled drug release ACS Applied Materials & Interfaces 2015; 7 (26): 14338–14351 doi: 10.1021/acsami.
27. *Barnes AL, Genever PG, Rimmer S, Coles MC*. Collagen, Äìpoly (N-isopropylacrylamide) hydrogels with tunable properties. *Biomacromolecules*. 2016; 17: 723–734 doi: 10.1021/acs.biomac.5b01251.
28. *Truong VX, Ablett MP, Gilbert HT, Bowen J, Richardson SM, Hoyland JA et al*. *In situ* forming robust chitosan-poly (ethylene glycol) hydrogels prepared by copper-free azide–alkyne click reaction for tissue engineering. *Biomaterials Science*, 2014; 2 (2): 167–175. doi: 10.1039/C3BM60159E.
29. *Sundaramurthi D, Krishnan UM, Sethuraman S*. Electrospun Nanofibers as Scaffolds for Skin Tissue Engineering. *Polymer Reviews*. 2014; 54: 348–376. doi: 10.1080/15583724.2014.881374.
30. *Hasan A, Memic A, Annabi N, Hossain M, Paul A, Dokmeci MR et al*. Electrospun scaffolds for tissue engineering of vascular grafts. *Acta Biomater*. 2013; 10 (1): 11–25. doi: 10.1016/j.actbio.2013.08.022.
31. *Wade RJ, Bassin EJ, Gramlich WM, Burdick JA*. Nanofibrous hydrogels with spatially patterned biochemical signals to control cell behavior. *Adv Mater*. 2015; 27 (8): 1356–1362. doi: 10.1002/adma.201404993.
32. *Jiang T, Deng M, James R, Nair LS, Laurencin CT*. Micro- and nanofabrication of chitosan structures for regenerative engineering. *Acta Biomater*. 2014; 10: 1632–1645. doi: 10.1016/j.actbio.2013.07.003.
33. *Ma S, Yu B, Pei X, Zhou F*. Structural hydrogels. *Polymer*. 2016; 98: 516–535. doi: 10.1016/j.polymer.2016.06.053.
34. *Cui H, Zhu W, Nowicki M, Zhou X, Khademhosseini A, Zhang LG*. Hierarchical Fabrication of Engineered Vascularized Bone Biphasic Constructs via Dual 3D Bioprinting: Integrating Regional Bioactive Factors into Architectural Design. *Adv Healthc Mater*. 2016; 5 (17): 2174–2181. doi: 10.1002/adhm.201600505.
35. *Dai Y, Ma PA, Cheng Z, Kang X, Zhang X, Hou Z et al*. Up-conversion cell imaging and pH-induced thermally controlled drug release from NaYF4: Yb3+/Er3+ hydrogel core-shell hybrid microspheres. *ACS nano*. 2012; 6: 3327–3338. doi: 10.1021/nn300303q.

36. *Kirkland SE, Hensarling RM, McConaughy SD, Guo Y, Jarrett WL, McCormick CL*. Thermoreversible hydrogels from RAFT-synthesized BAB triblock copolymers: steps toward iomimetic matrices for tissue regeneration. *Biomacromolecules*. 2007; 9: 481–486. doi: 10.1021/bm700968t.
37. *Sampas CT, Philbrook M, Seedling A, McPherson J*. Thermo-sensitive bone growth compositions. JP Application 2016514030. May 19, 2016.
38. *Zustiak SP, Leach JB*. Hydrolytically degradable poly (ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. *Biomacromolecules*. 2010; 11: 1348–1357. doi: 10.1021/bm100137q.
39. *Wang H, Heilshorn SC*. Adaptable hydrogel networks with reversible linkages for tissue engineering. *Adv Mater*. 2015; 27 (25): 3717–3736. doi: 10.1002/adma.201501558.
40. *Hennink W, Van Nostrum CF*. Novel crosslinking methods to design hydrogels. *Adv Drug Del Rev*. 2012; 64: 223–236. doi: 10.1016/S0169-409X(01)00240-X.
41. *Bae KH, Wang L-S, Kurisawa M*. Injectable biodegradable hydrogels: progress and challenges. *J Mater Chem B*. 2013; 1: 5371–5388. doi: 10.1039/C3TB20940G.
42. *Engler AJ, Sen S, Sweeney HL, Discher DE*. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006; 126: 677–689. doi: 10.1016/j.cell.2006.06.044.
43. *Ehrbar M, Sala A, Lienemann P, Ranga A, Mosiewicz K, Bittermann A et al*. Elucidating the role of matrix stiffness in 3D cell migration and remodeling. *Biophys J*. 2011; 100: 284–293. doi: 10.1016/j.bpj.2010.11.082.
44. *Zhang Y, Gordon A, Qian W, Chen W*. Engineering nanoscale stem cell niche: direct stem cell behavior at cell-matrix interface. *Adv Healthc Mater*. 2015; 4: 1900–1914. doi: 10.1002/adhm.201500351.
45. *Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA et al*. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat Mater*. 2010; 9: 518–526. doi: 10.1038/nmat2732.
46. *Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC et al*. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater*. 2016; 15: 326–334. doi: 10.1038/nmat4489.
47. *Yang C, DelRio FW, Ma H, Killaars AR, Basta LP, Kyburz KA et al*. Spatially patterned matrix elasticity directs stem cell fate. *Proc Natl Acad Sci USA*. 2016; 113 (31): E4439–E4445. doi: 10.1073/pnas.1609731113.
48. *Seidi A, Ramalingam M, Elloumi-Hannachi I, Ostrovidov S, Khademhosseini A*. Gradient biomaterials for soft-to-hard interface tissue engineering. *Acta Biomater*. 2011; 7: 1441–1451. doi: 10.1016/j.actbio.2011.01.011.
49. *Jain RK, Au P, Tam J, Duda DG, Fukumura D*. Engineering vascularized tissue. *Nat Biotechnol*. 2005; 23: 821–823. doi: 10.1038/nbt0705-821.
50. *Chen X, Aledia AS, Ghajar CM, Griffith CK, Putnam AJ, Hughes CC et al*. Prevascularization of a fibrin-based tissue construct accelerates the formation of functional anastomosis with host vasculature. *Tissue Eng. Part A*. 2008; 15: 1363–1371. doi: 10.1089/ten.tea.2008.0314.
51. *Peyton SR, Kalcioglu ZI, Cohen JC, Runkle AP, Van Vliet KJ, Lauffenburger DA et al*. Marrow-derived stem cell motility in 3D synthetic scaffold is governed by geometry along with adhesivity and stiffness. *Biotechnol Bioeng*. 2011; 108: 1181–1193. doi: 10.1002/bit.23027.
52. *Bauer J, Hengsbach S, Tesari I, Schwaiger R, Kraft O*. High-strength cellular ceramic composites with 3D microarchitecture. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111: 2453–2458. doi: 10.1073/pnas.1315147111.
53. *Kapfer SC, Hyde ST, Mecke K, Arns CH, Schröder-Turk GE*. Minimal surface scaffold designs for tissue engineering. *Biomaterials*. 2011; 32 (29): 6875–6882. doi: 10.1016/j.biomaterials.2011.06.012.
54. *Dubrov VE, Klimashina ES, Scherbakov IM, Shipunov GA, Putlayev VI, Evdokimov PV et al*. The experimental evaluation of the properties of the 3D-porous bone substitute based on calcium phosphate on the model of monocortical diaphyseal rat femur's defect. *Bulletin of Experimental Biology and Medicine*. 2019; 167 (3): 377–380. [In Russ, English abstract].
55. *Charras G, Sahai E*. Physical influences of the extracellular environment on cell migration. *Nat Rev Mol Cell Biol*. 2014; 15: 813–824. doi: 10.1038/nrm3897.
56. *Overstreet DJ, Huynh R, Jarbo K, McLemore RY, Vernon BL*. *In situ* forming, resorbable graft copolymer hydrogels providing controlled drug release. *J Biomed Mater Res. Part A*. 2013; 101: 1437–1446. doi: 10.1002/jbm.a.34443.
57. *Harley BA, Kim HD, Zaman MH, Yannas IV, Lauffenburger DA, Gibson LJ*. Microarchitecture of three-dimensional scaffolds influences cell migration behavior via junction interactions. *Biophys J*. 2008; 95 (8): 4013–4024. doi: 10.1529/biophysj.107.122598.
58. *Brauker JH, Carr-Brendel VE, Martinson LA, Crudele J, Johnston WD, Johnson RC*. Neovascularization of synthetic membranes directed by membrane microarchitecture. *J Biomed Mater Res*. 1995; 29: 1517–1524. doi: 10.1002/jbm.820291208.
59. *Klawitter J, Hulbert S*. Application of porous ceramics for the attachment of load bearing internal orthopedic applications. *J Biomed Mater Res*. 1971; 5: 161–229. doi: 10.1002/jbm.820050613.
60. *Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF*. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proc Natl Acad Sci USA*. 1989; 86 (3): 933–937. PubMed PMID: 2915988.
61. *Annabi N, Nichol JW, Zhong X, Ji C, Koshy S, Khademhosseini A et al*. Controlling the porosity and microarchitecture of hydrogels for tissue engineering. *Tissue Eng Part B Rev*. 2010; 16 (4): 371–383. doi: 10.1089/ten.TEB.2009.0639.
62. *Matsusaki M, Akashi M*. Novel functional biodegradable polymer IV: pH-sensitive controlled release of fibroblast growth factor-2 from a poly (γ-glutamic acid)-sulfonate matrix for tissue engineering. *Biomacromolecules*. 2005; 6: 3351–3356. doi: 10.1021/bm050369m.

63. *Kinoshita K, Iwase M, Yamada M, Yajima Y, Seki M*. Fabrication of multilayered vascular tissues using microfluidic agarose hydrogel platforms. *Biotechnol J*. 2016; 11: 1415–1423. doi: 10.1002/biot.201600083.
64. *Sergeeva NS, Komlev VS, Sviridova IK, Kirsanova VA, Akhmedova SA, Shanskiy YaD et al*. Some physicochemical and biological characteristics of 3D printed constructions based on sodium alginate and calcium phosphates for bone defects reconstruction. *Genes and Cells*. 2015; 10 (2): 39–45.
65. *Gao C, Liu M, Chen J, Zhang X*. Preparation and controlled degradation of oxidized sodium alginate hydrogel. *Polym Degrad Stab*. 2009; 94: 1405–1410. doi: 10.1016/j.polymdegradstab.2009.05.011.
66. *Wu Z, Su X, Xu Y, Kong B, Sun W, Mi S*. Bioprinting three-dimensional cell-laden tissue constructs with controllable degradation. *Sci Rep*. 2016; 6: 24474. doi: 10.1038/srep24474.
67. *El-Fiqi A, Lee JH, Lee EJ, Kim HW*. Collagen hydrogels incorporated with surface-aminated mesoporous nanobioactive glass: improvement of physicochemical stability and mechanical properties is effective for hard tissue engineering. *Acta Biomater*. 2013; 9: 9508–9521. doi: 10.1016/j.actbio.2013.07.036.
68. *Hersel U, Dahmen C, Kessler H*. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials*. 2003; 24: 4385–4415. doi: 10.1016/S0142-9612(03)00343-0.
69. *Sargeant TD, Desai AP, Banerjee S, Agawu A, Stopek JB*. An *in situ* forming collagen-PEG hydrogel for tissue regeneration. *Acta Biomater*. 2012; 8: 124–132. doi: 10.1016/j.actbio.2011.07.028.
70. *El-Fiqi A, Lee JH, Lee EJ, Kim HW*. Collagen hydrogels incorporated with surface-aminated mesoporous nanobioactive glass: improvement of physicochemical stability and mechanical properties is effective for hard tissue engineering. *Acta Biomater*. 2013; 9: 9508–9521. doi: 10.1016/j.actbio.2013.07.036.
71. *Gaharwar AK, Mihaila SM, Swami A, Patel A, Sant S, Reis RL et al*. Bioactive silicate nanoplatelets for osteogenic differentiation of human mesenchymal stem cells. *Adv Mater*. 2013; 25: 3329–3336. doi: 10.1002/adma.201300584.
72. *Shin SR, Bae H, Cha JM, Mun JY, Chen YC, Tekin H et al*. Carbon nanotube reinforced hybrid microgels as scaffold materials for cell encapsulation. *ACS Nano*. 2011; 6 (1): 362–372. doi: 10.1021/nn203711s.
73. *Zhao L, Weir MD, Xu HH*. An injectable calcium phosphate-alginate hydrogel-umbilical cord mesenchymal stem cell paste for bone tissue engineering. *Biomaterials*. 2010; 31 (25): 6502–6510. doi: 10.1016/j.biomaterials.2010.05.017.
74. *Xavier JR, Thakur T, Desai P, Jaiswal MK, Sears N, Cosgriff-Hernandez E et al*. Bioactive nanoengineered hydrogels for bone tissue engineering: a growth-factor-free approach. *ACS nano*. 2015; 9: 3109–3118. doi: 10.1021/nn507488s.
75. *Gaharwar AK, Dammu SA, Canter JM, Wu CJ, Schmidt G*. Highly Extensible, Tough and Elastomeric Nanocomposite Hydrogels from Poly(ethyleneglycol) and Hydroxyapatite Nanoparticles. *Biomacromolecules*. 2011; 12 (5): 1641–1650. doi: 10.1021/bm200027z.
76. *Tikhonov AA, Kukueva EV, Evdokimov PV, Klimashina ES, Putlyaev VI, Shcherbakov IM et al*. Synthesis of substituted octacalcium phosphate for filling composite implants based on polymer hydrogels produced by stereolithographic 3D printing. *Inorganic Materials*. 2018; 54 (10): 1062–1070. doi: 10.1134/S0020168518100175.
77. *Bose S, Tarafder S*. Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. *Acta Biomater*. 2011; 8 (4): 1401–1421. doi: 10.1016/j.actbio.2011.11.017.
78. *Dapporto M, Sprio S, Fabbi C, Figallo E, Tampieri A*. A novel route for the synthesis of macroporous bioceramics for bone regeneration. *Journal of the European Ceramic Society*. 2016; 36 (9): 2383–2388. doi: 10.1016/j.jeurceramsoc.2015.10.020.
79. *Gazhva JV, Bonartsev AP, Mukhametshin RF, Zharkova II, Andreeva NV, Makhina TK et al*. *In vivo* and *in vitro* Development and Study of Osteoplastic Material Based on Hydroxyapatite, Poly-3-Hydroxybutyrate and Sodium Alginate Composition. *Sovremennye tehnologii v medicine*. 2014; 6 (1): 6–13. ISSN 2076-4243.
80. *Dorozhkin SV*. Calcium orthophosphate bioceramics. *Eurasian Chemico-Technological Journal*. 2010; 12 (3–4): 247–258. doi: 10.1016/j.ceramint.2015.08.004.
81. *Sergeeva NS, Komlev VS, Sviridova IK, Kirsanova VA, Akhmedova SA, Kuvshinova EA et al*. *In vitro* Evaluation of the Composite Alginate – Calcium Phosphate Materials for Prototyping Technologies in Bone Defects Substitution. *Vestnik travmotologii i ortopedii im. N.N. Priorova*. 2015; (1): 28–34. ISSN 0869-8678.
82. *Tozzi G, De Mori A, Oliveira A, Roldo M*. Composite Hydrogels for Bone Regeneration. *Materials* (Basel). 2016; 9 (4): 267. doi: 10.3390/ma9040267.
83. *Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A*. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol*. 2016; 34: 312–319. doi: 10.1038/nbt.3413.
84. *Larionov PM, Sadovoy MA, Samokhin AG, Rozhnova OM, Gusev AF, Prinz VYa et al*. Creation of tissue-engineered living bone equivalent and prospects for its application in traumatology and orthopaedics. *Hir Pozvonoc*. 2014; (3): 77–85.
85. *Mamonov VE, Chemis AG, Komlev VS, Berkovskiy AL, Golubev EM, Proskurina NV et al*. Biologic Characteristics of Bone Substituting Tissue Engineering Construction Based on Calcium Phosphate Ceramics, Autologous Mesenchymal Stromal Cells and Fibrin Hydrogel. *Vestnik travmatologii i ortopedii imeni N.N. Priorova*. 2015; (4): 52–59. doi: 10.32414/0869-8678-2015-4-52-59.
86. *Petrov IYu, Larionov EV, Ippolitov YuA, But LV, Petrov AI*. Morphohistochemical studies of osteoplastic material based on hyaluronic acid, hondroitinsulfate and undermineralaized bone collagen for bone defects recovery in experiment. *Journal of new medical technologies*. 2018; 12 (3): 41–46. doi: 10.24411/2075-4094-2018-16038.

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