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The use of three-dimensional bioprinting for skin regeneration and wound healing (literature review)

Barsuk I.A.[1], Golovko K.P.[1, 2], Alexandrov V.N.[1, 3], Khasanov A.R.[1], Edgeev N.I.[4], Galiullin R.I. [4]

[1] *S.M. Kirov Military Medical Academy*
6, Akademika Lebedeva Str., Saint Petersburg, 194044, Russian Federation

[2] *Saint Petersburg State University*
7–9, Universitetskaya Embankment, Saint Petersburg, 199034, Russian Federation

[3] *Saint Petersburg State Pediatric Medical University*
2, Litovskaya Str., Saint Petersburg, 194100, Russian Federation

[4] *Branch No. 4 of Naval Clinical Hospital No. 1469*
22, Matrosa Ryabinina Str., Murmansk region, Zaozersk, 184310, Russian Federation

ABSTRACT

Three-dimensional (3D) bioprinting is rapidly proliferating across many medical disciplines and is making strides towards manufacturing intricate human organs for clinical application. One of the most promising areas in 3D bioprinting is development of bioinks with certain composition and designed properties.

The aim of this systematic review was to assess current biomedical research evidence regarding the efficacy of 3D bioprinting for skin regeneration and wound healing. A comprehensive search for all applicable original articles was conducted according to pre-established eligibility criteria. The study employed PubMed, Web of Science, Scopus, Medline Ovid, and ScienceDirect databases.

Of the retrieved articles, eighteen satisfied the inclusion criteria, while twenty-three were excluded. A total of 159 animals that had wound defects were considered in all animal-based research. Collagen and gelatin hydrogels were the most commonly employed bioinks. In relation to cellular composition, allogeneic fibroblasts and keratinocytes were predominant. The observation period ranged from one day to six weeks. Complete wound closure was achieved within 2–4 weeks in most animal studies. *In vitro* and *in vivo* animal studies have shown a positive effect of printed bioengineered constructs in accelerating wound healing. Notably, the research where bioprinting was performed directly in the wound *in situ* was of particular interest. Further studies are required to enhance the tissue bioprinting technique to address skin wound healing in animal models. The utilization of standardized parameters may pave the way for human clinical studies.

Keywords: 3D bioprinting, bioinks, biopolymers, wound healing, skin regeneration, wound dressings

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✉ *Barsuk Ilya A.,* barsuk20220@gmail.com

Использование трехмерной биопечати для регенерации кожи и заживления ран (обзор литературы)

Барсук И.А.[1], Головко К.П.[1, 2], Александров В.Н.[1, 3], Хасанов А.Р.[1], Едгеев Н.И.[4], Галиуллин Р.И.[4]

[1] Военно-медицинская академия (ВМА) им. С.М. Кирова
Россия, 194044, г. Санкт-Петербург, ул. Академика Лебедева, 6

[2] Санкт-Петербургский государственный университет (СПбГУ)
Россия, 199034, г. Санкт-Петербург, Университетская наб., 7/9

[3] Санкт-Петербургский государственный педиатрический медицинский университет (СПбГПМУ)
Россия, 194100, г. Санкт-Петербург, ул. Литовская, 2

[4] Филиал № 4 1469-го военно-морского клинического госпиталя
Россия, 184310, г. Заозерск, ул. Матроса Рябинина, 22

РЕЗЮМЕ

Трехмерная биопечать в настоящее время применяется в самых разных областях медицины, являясь движущей силой многих медицинских исследований. Эти исследования способствуют продвижению в область персонализированной медицины, включающих печать сложных человеческих органов для их использования в клинической практике. Одним из ведущих направлений в продвижении трехмерной биопечати является разработка биочернил определенного состава с заданными свойствами.

Цель настоящего систематического обзора состоит в анализе данных современных биомедицинских исследований, касающихся оценки эффективности использования трехмерной биопечати для регенерации кожи и заживления ран. Всеобъемлющий поиск всех релевантных оригинальных статей выполнили на основе заранее определенных критериев приемлемости. Поиск проводили с использованием платформ PubMed, Web of Science, Scopus, Medline Ovid и ScienceDirect.

В результате сужения области поиска из 2 256 статей отобрали 18, полностью соответствовавших критериям включения. Во все отобранные исследования было включено 159 животных с раневыми дефектами. В качестве биочернил чаще всего использовали коллагеновые и желатиновые гидрогели. В части клеточного компонента превалировали аллогенные фибробласты и кератиноциты. Период наблюдения колебался от 1 сут до 6 нед. В большинстве включенных исследований на животных полное закрытие раны достигалось через 2–4 нед.

Результаты как *in vitro*, так и *in vivo* показали положительное влияние напечатанных биоинженерных конструкций на ускорение заживления ран. Особый интерес представляет исследование, где биопечать выполняется непосредственно в ране *in situ*.

Проведенное исследование позволяет сделать вывод о необходимости отработки технологии биопечати тканей для лечения кожных ран на животных моделях с использованием стандартизированных параметров, чтобы открыть двери для клинических испытаний на людях.

Ключевые слова: 3D-биопринтинг, биочернила, биополимеры, заживление ран, регенерация кожи, раневые повязки

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Tissue injury is a significant medical problem, accounting for approximately half of the global annual health care expenditures [1]. Wound healing is a complex multistep process aimed at protecting and regenerating the damaged tissue area [2]. To avoid the development of adverse outcomes, it is essential to provide patient care and utilize appropriate dressings throughout this process. Although traditional wound coverings (e.g., gauze, lint, plasters, and bandages) protect the wound from contamination, these dressings require frequent changing to avoid infection and maceration of neighboring tissues. Additionally, they tend to adhere to the wound, making replacement traumatic and painful [3].

Additive manufacturing technologies offer a method for rapid wound healing, thereby avoiding common complications, such as wound contractures and scar formation [4]. Three-dimensional (3D) bioprinting is one of the emerging adaptive manufacturing technologies aimed at utilizing biocompatible materials, together with living cells and growth factors, to mimic and repair the extracellular matrix of human organs [5]. This approach allows for layer-by-layer printing of flexible hydrogel constructs by converting a digital computer-aided design (CAD) model into complex 3D structures [6].

The characteristics of the product obtained by 3D bioprinting are almost completely determined by the properties of the bioinks used. In this regard, bioinks are a key defining component of 3D bioprinting [7, 8].

In traditional 3D printing, ink is fed to the molding process as a melt at a high temperature (for plastics, ceramics, and alloys). However, such conditions are unacceptable for bioinks, which must meet high biocompatibility requirements to promote cell growth, be mechanically stable, and guarantee shape retention of the printed construct [9]. A number of parameters have a significant impact on high functional integrity of bioinks. These include cell load parameters (e.g. cell type, cell density, and incubation period), physicochemical properties (e.g. shear thinning, viscosity, degree of crosslinking, and gelation time), and printing parameters (e.g. nozzle temperature and diameter, feed rate, and printing duration) [10, 11]. Furthermore, the selection of cell type and source is of paramount importance to prevent immune rejection following implantation. Primary skin cells, including keratinocytes, melanocytes, and fibroblasts, can be properly isolated from donor skin and subsequently co-cultured during skin bioprinting [12, 13].

A variety of natural and synthetic polymeric hydrogels are utilized for bioink production [14]. Hydrogels are a class of cross-linked polymeric substances that are capable of absorbing and retaining a considerable amount of water. They are capable of absorbing water up to 1,000 times their original weight without dissolution [15]. It makes them an optimal choice for encapsulated cells due to their high permeability to oxygen, nutrients, and other water-soluble compounds. The ability of cells within the hydrogel to migrate and bind to each other through the porous network [16] is a key property that has enabled hydrogels to become one of the main materials for 3D bioprinting [17, 18].

Despite the lack of mechanical stability, 90% of the polymers used in bioprinting are derived from natural sources [19]. Natural-based biopolymers exhibit a number of advantages over synthetic biopolymers, due to their high similarity to the composition of the human extracellular matrix. This allows them to mimic the native cell microenvironment, facilitating cell attachment, proliferation, migration, and differentiation [20–22].

Following the widespread adoption of 3D bioprinting in the early part of the last decade, there was a clear need to identify printable biocompatible polymers that would enable the technology to be used in medicine. According to a citation report, the application of 3D bioprinting for wound healing and skin regeneration commenced in 2012, utilizing collagen bioinks. The number of studies in this field reached 12 in 2017 and 19 in 2019, with approximately 70 published studies by mid-2020. The majority of these studies employed natural-based polymers as the primary component of the bioinks.

The use of natural polymers in the manufacture of wound care products has been the subject of debate among researchers. While many of the drawbacks associated with these polymers have been identified and potential solutions proposed, no explicit agreement or decision has been reached.

The objective of this systematic review was to evaluate the efficacy of bioprinting using natural polymer-based bioinks as skin substitutes for skin tissue regeneration and wound healing. In addition to reporting the biological properties of bioprinted constructs in *in vitro* and *in vivo* studies, this review also provides recommendations for the use of such constructs in practice.

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [18]. To identify relevant digital records from five electronic databases – RINC, PubMed, Web of Science, Scopus, and ScienceDirect – a comprehensive search strategy was employed.

The search query consisted of 18 terms, comprising two sets. The first set included "skin," "skin regeneration," "skin tissue engineering," "wound healing," "wound," "burns," and "wound." The second set included "3D bioprinting," "3D printing," "3D cell printing," "3D printing," "bioprinting," "3D scaffold," and "3D prototyping." This query was aimed at identifying 3D bioprinted skin substitutes as potential wound healing or skin regeneration agents.

The titles and abstracts of all identified records were pre-screened for potentially relevant research. Included entries were further screened by reading full texts to ensure eligibility. To be included, an article had to meet the following criteria: use of natural-based bioinks; actual *in vitro* or *in vivo* study; scaffold obtained by 3D bioprinting; original article written in Russian or English The following criteria were employed to exclude articles: isolated articles that consider the theoretical possibility of using 3D bioprinting; articles that describe synthetic bioinks; articles pertaining to chronic wounds; systematic and descriptive reviews, interpretations, case series, guidelines, and technical reports.

The following data were extracted from the included studies: 1 – information about the study (authors, year of publication, study design, database, and journal name); intervention details (biomaterials and cells used, gelation time, printing temperature, crosslinking materials and techniques, and printing methods); outcome details (rheological, mechanical, and biological characteristics, construct shape accuracy, and wound healing time).

The initial search yielded 4,345 articles, but after removing duplicates, 2,566 articles were selected for review. Following the screening of titles and abstracts, 2,499 records were excluded as they did not meet the inclusion criteria, leaving 18 articles for review. The main parameters identified for evaluation were as follows: study design, bioprinting and polymer crosslinking method, bioink base material, cellular component of bioinks, cell viability level after the printing process, and animals used for the experiment. The study characteristics and results are summarized in Table.

Table

Brief description and results of the selected studies

Study design	Bioinks	Cells / animals used	Conclusion	References
In vitro	Collagen – chitosan blend	NIH 3T3	When printing with inks of varying collagen / chitosan ratios, the optimal ink delivery rate was found to lie between 0.19 µl / s and 0.42 µl / s	[23]
In vitro	CNF/ GelMA	NIH 3T3	The CNF/GelMA scaffolds exhibited no cytotoxicity and demonstrated favorable cytocompatibility with 3T3 mouse fibroblasts	[27]
In vitro	Sulfated and rhamnose-rich XRU	HDFs	*In vitro* testing of the XRU hydrogel with human dermal fibroblasts (HDFs) revealed that the material exhibited high biocompatibility with a high cellular density and the capacity to promote active cell proliferation and attachment	[24]
In vitro	Suspension dSIS	HDFs	The dSIS scaffold developed in the study may be a promising candidate for the treatment of skin defects. Its high precision and high swelling ratio make it an attractive option for this purpose	[28]
In vitro	Viscoll native collagen	NIH 3T3	Viscoll advanced bioink permits the fabrication of intricate geometries without the necessity of chemical or photocrosslinking, thus ensuring the maintenance of the specified shape	[29]

End of table

Study design	Bioinks	Cells / animals used	Conclusion	References
In vitro	Alginate / Gelatin	AECs and WJMSCs	The human AECs demonstrated a superior epithelial cell phenotype, while the WJMSCs exhibited enhanced angiogenic and fibroblastic potential	[30]
In vitro and *in vivo*	BCNFs, SF/ Gelatin	L929 fibroblasts and 12 nude mice	The introduction of bacterial cellulose nanofibers had a minimal impact on the printing parameters of composite bioinks. The obtained data demonstrated that the porous structure exhibited favorable properties for nutrient supply to the forming tissues following *in vivo* implantation	[37]
In vivo	Fibrinogen, thrombin and collagen type I	HDFs, HEK 293 cells, and 36 female nude mice, along with six pigs	The use of three-dimensional *in situ* bioprinting of autologous cells was found to accelerate wound healing by approximately three weeks compared to other treatments	[35]
In vitro	CNF	HDFs	The utilization of a matrix generated through 3D printing, in contrast to 2D frames, facilitated accelerated cell proliferation, a crucial element in the process of rapid wound healing	[31]
In vitro	Sodium alginate / Gelatin	HDFs	The EDC-CaCl2 solution demonstrated enhanced cellular proliferation and was deemed more suitable for use as a dermal replacement	[32]
In vitro	Collagen	NIH 3T3, Vero cell line	The micro- and macropore structure of fibrillar collagen promoted high cell attachment and proliferation at 37 °C	[33]
In vitro and *in vivo*	S-dECM	HDFs, HEK 293 and 8 male BALB/ cA-nu nude mice	The fabricated S-dECM bioink demonstrated no cytotoxicity and exhibited high biocompatibility comparable to native type I collagen. The 3D-printed constructs with S-dECM bioink exhibited accelerated wound closure, neovascularization, and reliable blood flow at the implantation site	[38]
In vitro	Alginate / honey	NIH 3T3	The incorporation of approximately 1–2% honey into the bioprinted alginate resulted in enhanced cell proliferation without a significant impact on printability	[34]
In vitro	Gelatin	HDFs	The growth rate of HDFs was approximately 14% higher in G8–G12 gelatin scaffolds than in G6 gelatin scaffolds. The mechanical properties of the scaffolds are strongly dependent on the pore size	[25]
In vitro and *in vivo*	SS / GelMA	L929 fibroblast lineage, HDFs, HaCaT and 21 female Sprague Dawley rats	The incorporation of silk sericin (SS) into the matrices was demonstrated to facilitate cell growth in HDFs. The study also indicated that SS/GelMA is an appropriate substrate for cell cultures in human keratinocytes (HaCaT), as high cell viability was maintained even after seven days	[39]
In vitro and *in vivo*	G-SF-SO_3-FGF2	HDFs/ 36 male Sprague Dawley rats	The administration of 100 ng / ml FGF2 resulted in a 40% increase in the proliferation rate of the cells in question. The sulfated SF-coated scaffold facilitated cell adhesion, proliferation, and growth. The FGF2 growth factor enhanced re-epithelialization and also stimulated blood vessel formation and the expression of several relevant markers	[40]
In vivo	Gelatin – alginate	40 female mice	The use of gelatin – alginate has been demonstrated to reduce wound bleeding subsequent to implantation. Furthermore, the scaffold has been shown to facilitate granulation tissue maturation and wound healing	[36]
In vitro	Collagen	HDF и HEK 293	The study demonstrated that fibroblasts and keratinocytes can be printed in a sequential, layer-by-layer manner, resulting in dermo- and epidermal-like layers. The 3D printing technique offers a high degree of control over the shape and quality of the resulting engineered skin tissues	[26]

Note. NIH 3T3 – mouse embryonic fibroblast line; CNF – cellulose nanofibrils; GelMA – gelatin methacrylate; XRU – xylorhamno-uronic acid; HDFs – human dermal fibroblasts; dSIS – suspension of decellularized small intestinal submucosa; AECs – amniotic epithelial cells; WJMSCs – Wharton's jelly-derived mesenchymal stromal stem cells; BCNF – bacterial cellulose nanofibers; SF – silk fibroin; EDC –N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; Vero – cell lines from kidney epithelium taken from an African green monkey; S-dECM – extracellular matrix of cutaneous origin; HEK 293 – cell line derived from human embryonic kidneys; SS – silk sericin; HaCaT – human keratinocyte cell line; G-SF-SO3-FGF2 – gelatin-sulfated silk composite with fibroblast growth factor, 2-sulfonic acid group; CFFs – colony-forming fibroblasts.

DESIGN OF INCLUDED STUDIES

The primary categorization of papers was based on the study design. Twelve studies were conducted *in vitro* [23–34], while two were conducted *in vivo* [35, 36]. Four studies were conducted in both *in vitro* and *in vivo* settings [37–40].

METHODS FOR BIOPRINTING AND POLYMER CROSSLINKING

The extrusion-based bioprinting technique was the most prevalent, with only two studies reporting the use of inkjet bioprinting [26, 35]. Various crosslinking techniques were employed, with only six studies [29, 33, 34, 35, 37, 38] reporting the absence of crosslinking agents. The following methods were utilized:

1. Crosslinking by chemical reagent: Ca2+ [27, 30, 31], CaCl2 [32, 36], 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [28], N-hydroxysuccinimide-1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC-NHS) [23, 25, 32, 40], nebulized sodium bicarbonate (NaHCO3) [26], 1,4-butanediol diglycidyl ether (BDDE) [31].

2. Crosslinking by physical exposure to: UV [24, 27, 39] or cooling [30, 32].

BIOINK BASE MATERIAL

The vast majority of the bioink base materials utilized were a combination of gelatin and collagen hydrogels. While gelatin hydrogel exhibited optimal rheological properties, it demonstrated zero viscosity at temperatures above 27 ± 1 °C [25], and all gelatin studies investigated the use of different crosslinking agents [25, 27, 30, 32, 36, 37, 39, 40]. In contrast, four studies reported the ability to print collagen hydrogel without the need for chemical crosslinking agents [25, 33, 35, 38]. Furthermore, the integration of alginate hydrogel with gelatin [30, 32, 36] or honey [34] has also been reported.

USE OF CELL CULTURES

In general, *in vitro* studies tend to utilize fibroblasts as a cellular component. Among the various types of fibroblasts, human dermal fibroblasts (HDFs) were employed most frequently [24–26, 28, 31, 32, 35, 38–40]. The T3T [23, 27, 29, 33, 34] and L929 [37, 39] mouse fibroblast lines were used in a similar number of studies.

The human epidermal keratinocytes (HEK)/human keratinocyte cell line (HaCaT) was used in four studies [26, 35, 38, 39]. Wharton's jelly-derived mesenchymal stromal stem cells (WJMSC) and amniotic epithelial cells (AECs) were used in one study [30], Vero epithelial cells were also described in one study [33].

CELL VIABILITY RATE AFTER BIOPRINTING

It is believed that high-tech materials based on natural-based polymers exhibit superior biological properties. Of the 16 *in vitro* studies that have been conducted, 13 reported high cell proliferation rates. However, three studies [23, 26, 39] did not demonstrate a significant change in proliferation rates, yet reported high cell viability. Seven studies reported good cell viability [24, 26, 28, 29, 33, 34, 37]. Five studies reported minimal cell viability, with values ranging from 85.07 to 98% [26, 29, 30, 33, 38]. One study reported the appearance of dead cells, indicating low cell viability [28].

Furthermore, 14 studies reported high cell growth rates, and only a decellularized small intestinal submucosa suspension (dSIS) [28] and silk sericin / methacrylate gelatin-based bioink (SS/GelMA) [39] did not promote cell growth. All *in vivo* results were consistent with *in vitro* studies, with the exception of SS/GelMA [39], which demonstrated good healing properties in wounds two weeks after their treatment.

ANIMALS USED FOR THE EXPERIMENT

A total of 159 animals were used in animal studies, with each study including between 8 and 40 animals. Four studies employed mice [35–38], two studies employed rats [39, 40], and one study employed pigs [35].

REVIEW OF INCLUDED STUDIES

The results of 18 *in vitro* cell culture and *in vivo* animal studies indicate that 3D bioprinted natural polymer constructs can promote complete closure of skin wounds. The majority of 3D bioprinted skin substitutes demonstrated the ability to promote cell proliferation, adhesion, and differentiation, and the majority of *in vitro* studies reported high cell viability. Furthermore, all animal studies demonstrated reduction in wound area in animals two weeks after surgery. Nevertheless, it is

important to acknowledge the technical challenges and practical limitations of assessing cell viability *in vitro* and wound dynamics in *in vivo* animal studies. These factors must be taken into account when considering the potential clinical applications of such technologies.

The primary objective of employing 3D bioprinting in the context of wound healing is to facilitate rapid treatment of directly damaged tissues *in situ*. In a study conducted by [35], bioprinting was performed using a combination of fibrinogen and thrombin bioink with type I collagen, comprising cells from the mouse embryonic fibroblast line (HDFs) and human embryonic kidney-derived cell line (HEK 293).

This approach was employed directly on a wound in the back region of mice and pigs. Marker dots were applied around the wound, after which it was scanned with a handheld scanner. Based on the wound scan data, an STL file was generated for the bioprinter, which included information on the planimetry of the points for the movement of the bioprinter nozzle. This is necessary for volumetric filling of the wound during the bioprinting process.

This approach, as demonstrated in the conducted experiment, resulted in significant acceleration of the wound healing process, with an estimated three-week reduction in healing time compared to other treatment methods. The immunohistochemical analysis revealed the presence of HDFs and HEK 293 cells in the dermis and epidermis of the wound at three to six weeks post-surgery, in addition to endogenous cells.

BASIC 3D BIOPRINTING METHOD

As previously stated, the predominant method employed in the reviewed works for 3D bioprinting was extrusion printing, with only two studies utilizing inkjet printing. This pattern is logical, given that extrusion printing is technically the simplest method and allows for the printing of viscous bioinks (30 mPa·s to 6 x 10^7 mPa·s) with high cell density [41, 42]. In comparison to other methods, extrusion printing is associated with several disadvantages. These include a relatively low resolution (2000–1000 μm), potential nozzle clogging, and reduced cell viability at high printing speed, due to increased pressure in the extruder, which can lead to cell membrane damage [41, 43, 44]. Given that optimal printing rates do not lead to cell damage and there is no need for high resolution, coupled with lower equipment costs, extrusion printing remains the method of choice for creating bioengineered constructs for the treatment of skin defects.

MATERIALS FOR BIOINKS

A variety of bioinks have been employed in the studies, including single-component bioinks and composite bioinks comprising multiple components. The materials utilized in the form of hydrogels possess necessary physicochemical properties for printing and exhibit a high degree of similarity to the natural extracellular matrix of the skin, thereby providing them with high biocompatibility [14].

Collagen. Collagen hydrogel has demonstrated the required biodegradation (about 30 days), high shape stability at 37 °C, and excellent micro- and macropore structure that promote cell attachment and proliferation [33]. However, direct 3D bioprinting of collagen is still limited due to physical properties of a collagen solution, which make it poorly suitable for printing, especially when cells or tissue spheroids are incorporated [29]. Notably, despite the limited printing capabilities of pure collagen, most studies have not utilized chemical crosslinking. Instead, various methods have been employed, including mixing with other materials (fibrinogen and thrombin [35], chitosan [23]), using fibrillar collagen [33], using low concentrations of collagen (2–4%) [29], and adjusting the density of bioink by dosing the amount of cell suspension injected [26].

In the same context, the gelation of matrix proteins, such as collagen, is typically initiated by controlling pH, temperature, or both. However, this approach is only valid for thin structures (less than 1 mm) due to the limitations of diffusion or heat transfer in thick structures (1 to 3 mm). Consequently, ungelatinized regions are observed in the printed structure. The utilization of elevated pH or temperature to achieve the aforementioned outcome is not always feasible, as it may result in significant cellular damage within the solution [26].

Gelatin. Gelatin is a denatured form of collagen protein [45]. At low temperatures, gelatin filaments form helical structures, which result in a gel-like form [46]. Gelatin retains the Arg-Gly-Asp sequence and, in contrast to its predecessor, is less immunogenic

and promotes cell adhesion, differentiation, and proliferation [47]. However, pure gelatin solutions exhibit poor mechanical strength and low viscosity at temperatures above 27 ± 1°C, which limits their use in 3D bioprinting.

To overcome this limitation, gelatin is frequently combined with other natural biomaterials, such as alginate [30, 32, 36] and silk fibroin (SF) [37], to enhance its formability. Moreover, gelatin methacrylate (GelMA) is a promising candidate for wound-healing bioinks due to its high heat sensitivity and photocrosslinking ability. GelMA is also known to have good biocompatibility and to promote intercellular interaction and cell migration. Furthermore, the favorable mechanical stability of GelMA after UV crosslinking has been exploited to provide high shape accuracy of composite bioinks, where cellulose nanofibrils [27] and silk sericin [24] were used as the second component.

Alginate. Alginate is a polysaccharide consisting of β-mannuronate and its C-5 epimer α-L-gluronate [48]. It is a popular hydrogel used in bioprinting due to its biocompatibility, the possibility of various crosslinking options, and the ease of use [49]. However, alginate has several limitations. Delayed crosslinking can reduce the shape accuracy of bioprinted constructs, while rapid crosslinking limits the interaction of cells with the material, reducing their further viability.

One study attempted to overcome these limitations by reducing the viscosity of alginate through the addition of honey. It was hypothesized that the inclusion of honey would allow to increase cell viability without altering the printability of the alginate. Even printing with simple alginate solutions was found to have poor shape accuracy. Researchers have attempted to increase the viscosity of alginate or extrude it with chemical crosslinking agents, such as calcium ions (Ca2+) [30].

Skin decellularized extracellular matrix (S-dECM). The extracellular matrix (ECM) is the non-cellular component of a tissue or an organ. It is a network of microenvironments that allows cells to perform their functions. Every tissue has a well-constructed ECM composed of several components that maintain the native structure and promote cell migration. Interestingly, ECM can be obtained with an appropriate protocol and used as a matrix for tissue regeneration [50].

In one of the studies reviewed, the authors successfully decellularized pig skin and generated printable dECM-based bioink from it. In an *in vitro* study, they found that compared to collagen-based bioink, 3D bioprinted skin equivalent using dECM-based bioink promoted dermal stabilization, improved epidermal organization, and provided physiologically important skin functions. Furthermore, dECM-based 3D skin encapsulated endothelial progenitor cells (EPCs) and atypical squamous cells (ASCs) demonstrated the capacity to promote neovascularization and re-epithelialization, which was evidenced by accelerated wound healing *in vivo* [38].

MAIN PARAMETERS OF BIOINKS AFFECTING CELL VIABILITY

The biocompatibility of bioprinting materials has been extensively studied, and a number of factors that may affect cell viability, adhesion, proliferation, migration, and differentiation have been identified. In general, cytotoxicity is the primary criterion to be evaluated when considering a potential material for medical use. The majority of the included studies employed a colorimetric test to assess the metabolic activity of cells (MTT assay), thereby ensuring that there was no cytotoxicity or inflammation caused by chemical interaction between the cell and the material. Notably, only silk sericin / BioVernyl GelMA induced acute inflammation on day 7, which disappeared at the end of the observation period [39].

An additional property of bioinks is the size of pores formed in printed structures during crosslinking of hydrogels or lyophilization of samples. Small pore sizes result in a lack of nutrition and oxygen supply to the cells, which in turn leads to slower cell migration and low viability. The effect of gelatin hydrogel pore size on cell behavior was studied. The study revealed that a pore size of 580 μm led to a 14% increase in the proliferation rate of HDFs after 14 days in comparison to 435-μm pores [25]. Furthermore, the use of natural bioinks offers a favorable intermolecular network. For instance, it is well established that fibrillar collagen possesses an optimal micro- and macropore structure, which has been demonstrated to facilitate robust cell attachment and proliferation, ultimately enhancing cell viability [33].

A crucial aspect of bioinks is the concentration of their primary structural component. This parameter has a profound impact on cell viability, as high concentrations lead to cellular compaction and, consequently, reduction in cell viability. The effect of varying the concentration of Viscoll brand collagen on cell viability was evaluated. The results demonstrated that reduction of the collagen concentration from 4 to 2% led to an increase in cell viability from 87.2 ± 2.1 to 97.2 ± 1.2% ($p < 0.05$) [29].

Another group of authors studied the effect of using a lower-molecular-weight collagen extract on the viability of the mouse embryonic fibroblast cell line (NIH 3T3). Their findings indicated that decreasing the concentration of the extract from 100 to 25% resulted in an increase in cell viability from 85.07 ± 6.73 to 111.31 ± 3.65% ($p < 0.05$) [33]. Another study sought to examine the impact of combining low concentrations of GelMA with cellulose nanofibrils (CNF) on cell proliferation. The results indicated that three days after culture, the number of cells on the CNF/GelMA composite bioink was approximately twice that observed on CNF bioink alone [27].

The density of the cell suspension is another critical factor. As previously described, the use of higher cell counts (greater than 1 million cells per ml) results in reduced cell viability. There is evidence for the use of an inkjet bioprinting system and a study on the effect of using different cell suspension densities and droplet sizes on cell viability. The study demonstrated that cell viability is proportional to cell suspension density and inversely proportional to the space between droplets for both keratinocytes and skin fibroblasts. At very low cell suspension density (0.5 million cells / ml) and large droplet spacing (400 nm), fibroblast viability was moderate (84%). This is likely due to the lack of intercellular communication at relatively low surface coverage. Similarly, at high cell suspension density (5 million cells / ml) and small droplet spacing during printing (400 μm), keratinocyte viability was equal (94%). The highest cell viability rates (98–99%) were achieved using cell suspension density of 1–2 million cells / ml and droplet spacing of 200 nm [26]. In addition, the thickness of the printed structure exerts a significant effect on cell adhesion. The percentage of cell attachment was found to be higher in 3-mm-thick samples than in 2-mm-thick samples. It was demonstrated that a thicker scaffold promoted cell adhesion [31].

Conversely, growth factors, crucial morphogenetic proteins that influence cell activity and guide tissue repair and regeneration, cannot be overlooked [51]. Published data indicate that the addition of 100 ng / ml of fibroblast growth factor (FGF2) to bioink significantly increases the proliferation rate (from ~40 to ~75%), improves the morphology of the construct (approaching the structure of native tissue), and accelerates the assembly of native collagen fibrils responsible for the formation of the ECM of the skin [40]).

THE STRUCTURE AND MECHANICAL PROPERTIES OF THE OBTAINED BIOENGINEERED CONSTRUCTS

Materials utilized for bioprinting should possess acceptable mechanical properties and should not collapse after printing. Additionally, they should have a high swelling coefficient to facilitate moisture and air exchange in the wound area, metabolism, and cell proliferation. According to published literature, human skin exhibits an average modulus of elasticity, with values ranging from 100 to 1,100 kPa [38]. The degree of swelling is inversely proportional to Young's modulus values. Nevertheless, an increase in the fiber spacing of decellularized small intestinal submucosa (dSIS) suspension from 500 to 700 μm has been observed to result in a notable increase in the degree of swelling from 69 to 79% and a simultaneous decrease in the Young's modulus from 26.6 ± 3.8 to 9.7 ± 3.1 kPa ($p < 0.05$) [24]. Similar outcomes were observed in studies that employed a crosslinked solution of cellulose nanofibrils (CNF) [31] and a crosslinked solution of alginate with gelatin [32].

It is essential that the bioinks retain their shape after leaving the tip of the printing nozzle. In general, proper hydrogel viscosity ensures high form accuracy and minimizes the possibility of structural failure after printing [36]. Another important parameter is sheer thinning. Bioinks must have a strictly defined thixotropic effect to avoid nozzle clogging during extrusion and to allow for structural consistency recovery after printing to be ready for the next layer [23, 30, 31]. For instance, collagen

requires approximately one minute to transit to a gel-like state and maintain a solid base for printing the subsequent layer [26]. Additionally, the stiffness of the printed scaffolds has been demonstrated to significantly influence cell proliferation. For instance, as CNF stiffness increased within the range of 3–8 kPa, cell proliferation was accelerated [31].

WOUND CARE IN ANIMAL MODELS

A high rate of wound healing is crucial to avoid prolonged treatment and the formation of hypertrophic scars. The success of using the new material as a wound treatment agent is primarily determined by its high biocompatibility and a lack of cytotoxicity *in vitro*. With further study, the material under consideration should stimulate wound healing and tissue re-epithelialization *in vivo.* The use of bioprinted constructs with the addition of human cell lines has been demonstrated to accelerate wound healing in animal models by approximately three weeks compared to other methods [35].

Implantation of skin constructs with the correct pore size and structure has been shown to significantly influence the nutrition supply and cell growth in the wound area [37]. Uniform and as early as possible application of the printed coating has been shown to maximally reduce the formation of scar tissue in the wound area. It has been demonstrated that the application of a scaffold of gelatin – sulfated silk composite containing fibroblast growth factor (G-SF-SO3-FGF2) to the back of wounded rats resulted in the wound surface becoming smoother after surgery. Furthermore, cross-sectional results indicated that the wound had completely closed, accompanied by the presence of more blood vessels [40]. Furthermore, the histologic examination of a cross-section of the SS/GelMA-treated wound seven days after surgery demonstrated the formation of new collagen accompanied by high fibroblast proliferation, which was comparable to that observed in healthy tissue. This was followed by complete wound closure at week 4 [39].

It is also important to note that the integration of a new tissue or organ into the surrounding tissue or cavity of a bio-object necessitates the presence of an appropriate vascular network. To address this challenge, researchers have employed a range of techniques, including the addition of growth factors that stimulate vascularization [40], the use of a network of interconnected pores with a diameter of 50 to 500 μm and micropores with a diameter of less than 10 μm [33], and the incorporation of skin decellularized extracellular matrix [38].

CONCLUSION

This review presents an analysis of scientific studies conducted *in vitro* on cell cultures and *in vivo* on animals. The aim was to ascertain the possibility of creating a skin substitute using 3D bioprinting. The review first confirms the significant advantages of using extrusion bioprinting with natural-based biopolymers for skin repair and regeneration. The majority of obtained images using this technology demonstrated an excellent ability to mimic the 3D structure of the native skin tissue microenvironment and promote cell adhesion, proliferation, and migration. *In vivo* visualization revealed that the use of a bioprinted construct with well-organized dermal and epidermal layers resulted in complete wound closure four weeks after surgery. Additionally, high significance of different properties of bioinks should be noted, as they greatly impact the acceleration of wound healing.

Despite the limited number of studies conducted, *in situ* bioprinting is one of the most promising advances in skin tissue engineering. It can be utilized by surgeons to efficiently and rapidly print complex organs. However, the main challenge lies in the difficulty of accurately constructing tissue parts. This requires integration of various fields of science, including not only medicine and biology, but also engineering, chemistry, and even IT. In addition, some new polymer crosslinking techniques, such as two-photon crosslinking and UV radiation directed at the nozzle tip, can help improve the speed and accuracy of printing with existing bioinks. Vascularization-prepared scaffolds are of particular interest because they retain their pre-vascularized microstructure after printing and, even when used without cells, are rapidly repopulated with autologous cells due to stimulation of the recipient's regenerative processes.

It is regrettable that the use of 3D bioprinting for wound healing is still being studied in animals. A meta-analysis of the available literature did not identify any randomized human clinical

trials. Another significant issue is that the time of observation and measurement, the cell lines used, the type and number of animals used, the severity and area of the wounds inflicted, and the method of application vary from study to study, contributing to high heterogeneity of results.

REFERENCES

1. Sen C.K. Human wounds and its burden: an updated compendium of estimates. *Adv. Wound Care.* 2019;8(2):39–48. DOI: 10.1089/wound.2019.0946.
2. Beldon P. Basic science of wound healing. *Surgery.* 2010;28(9):409–412. DOI: 10.1016/j.mpsur.2010.05.007
3. Dhivya S., Padma V.V., Santhini E. Wound dressings – a review. *BioMedicine.* 2015;5(4):22. DOI: 10.7603/s40681-015-0022-9.
4. Chouhan D., Dey N., Bhardwaj N., Mandal B.B. Emerging and innovative approaches for wound healing and skin regeneration: status and advances. *Biomaterials.* 2019;216:119267. DOI: 10.1016/j.biomaterials.2019.119267.
5. Ferry P.W. Melchels, Marco A.N. Domingos, Travis J. Klein, Jos Malda, Paulo J. Bartolo, Dietmar W. Hutmacher. Additive manufacturing of tissues and organs. *Prog. Polym. Sci.* 2012;37(8):1079–1104. DOI: 10.1016/j.progpolymsci.2011.11.007.
6. He P., Zhao J., Zhang J., Li B., Gou Z., Gou M., Li X. Bioprinting of skin constructs for wound healing. *Burn. Trauma.* 2018;6:5. DOI: 10.1186/s41038-017-0104-x.
7. Groll J., Burdick J.A., Cho D.W., Derby B. Gelinsky M., Heilshorn S.C., Jüngst T. et al. A definition of bioinks and their distinction from biomaterial inks. *Biofabrication.* 2019;11(1):013001. DOI: 10.1088/1758-5090/aaec52.
8. Cui H., Nowicki M., Fisher J.P., Zhang L.G. 3D bioprinting for organ regeneration. *Advanced Healthcare Materials.* 2017;6(1):1601118. DOI:10.1002/adhm.201601118.
9. Gopinathan J., Noh I. Recent trends in bioinks for 3D printing. *Biomater. Res.* 2018;22:1–15. DOI: 10.1186/s40824-018-0122-1.
10. Panwar A., Tan L.P. Current status of bioinks for micro-extrusion-based 3D bioprinting. *Molecules.* 2016;21(6):685. DOI: 10.3390/molecules21060685.
11. Xia Z., Jin S., Ye K. Tissue and organ 3D bioprinting. *SLAS Technol.* 2018;23(4):301–314. DOI: 10.1177/24726 30318760515.
12. Ng W.L., Wang S., Yeong W.Y., Naing M.W. Skin bioprinting: impending reality or fantasy? *Trends Biotechnol.* 2016;34(9):689–699. DOI: 10.1016/j.tibtech.2016.04.006.
13. Kumar A., Starly B. Large scale industrialized cell expansion: Producing the critical raw material for biofabrication processes. *Biofabrication.* 2015;7(4):44103. DOI: 10.1088/1758-5090/7/4/044103.
14. Griffith L.G., Swartz M.A. Capturing complex 3D tissue physiology in vitro. *Nature Reviews Molecular Cell Biology.* 2006;7(3):211–224. DOI: 10.1038/nrm1858.
15. Ahmed E.M. Hydrogel: preparation, characterization, and applications: A review. *J. Adv. Res*. 2015;6(2):105–121. DOI: 10.1016/j.jare.2013.07.006.
16. Yamamoto M., James D., Li H., Butler J., Rafii S., Rabbany S. Generation of stable co-cultures of vascular cells in a honeycomb alginate scaffold. *Tissue Engineering. Part A*. 2010;16(1):299–308. DOI: 10.1089/ten.TEA.2009.0010.
17. Thomas B.H., Craig Fryman J., Liu K., Mason J. Hydrophilic-hydrophobic hydrogels for cartilage replacement. *Journal of the Mechanical Behavior of Biomedical Materials.* 2009;2(6):588–595. DOI: 10.1016/j.jmbbm.2008.08.001.
18. Zhu J., Marchant R.E. Design properties of hydrogel tissue-engineering scaffolds. *Expert Review of Medical Devices*. 2011;8(5):607–626. DOI: 10.1586/erd.11.27.
19. Montero F.E., Rezende R.A., da Silva J.V., Sabino M.A. Development of a smart bioink for bioprinting applications. *Front. Mech. Eng.* 2019;5(56):1–12. DOI: 10.3389/fmech.2019.00056.
20. Valot L., Martinez J., Mehdi A., Subra G. Chemical insights into bioinks for 3D printing. *Chem. Soc. Rev.* 2019;48(15):4049–4086. DOI: 10.1039/C7CS00718C.
21. Kim J.E., Kim S.H., Jung Y. Current status of three-dimensional printing inks for soft tissue regeneration. *Tissue Eng. Regen. Med.* 2016;13(6):636–646. DOI: 10.1007/s13770-016-0125-8.
22. Liberati A., Altman D.G., Tetzlaff J., Mulrow C., Gøtzsche P.C., Ioannidis J.P. et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *J. Clin. Epidemiol.* 2009;62(10):e1–34. DOI: 10.1016/j.jclinepi.2009.06.006.
23. Heidenreich A.C., Pérez-Recalde M., González Wusener A., Hermida É.B. Collagen and chitosan blends for 3D bioprinting: A rheological and printability approach. *Polym. Test.* 2020;82:106297. DOI: 10.1016/j.polymertesting.2019.106297.
24. Xu W., Molino B.Z., Cheng F., Molino P.J., Yue Z., Su D. et al. On low-concentration inks formulated by nanocellulose assisted with gelatin methacrylate (GelMA) for 3D printing toward wound healing application. *ACS Appl. Mater. Interfaces.* 2019;11(9):8838–8848. DOI: 10.1021/acsami.8b21268.
25. Chen X., Yue Z., Winberg P.C., Dinoro J.N., Hayes P., Beirne S., Wallace G.G. Development of rhamnose-rich hydrogels based on sulfated xylorhamno-uronic acid toward wound healing applications. *Biomater. Sci.* 2019;7(8):3497–3509. DOI: 10.1039/C9BM00480G.
26. Shi L., Hu Y., Ullah M.W., Ullah I., Ou H., Zhang W. et al. Cryogenic free-form extrusion bioprinting of decellularized small intestinal submucosa for potential applications in skin tissue engineering. *Biofabrication.* 2019;11(3):035023. DOI: 10.1088/1758-5090/ab15a9.
27. Osidak E.O., Karalkin P.A., Osidak M.S., Parfenov V.A., Sivogrivov D.E., Pereira F.D.A.S. et al. Viscoll collagen solution as a novel bioink for direct 3D bioprinting. *J. Mater. Sci. Mater. Med.* 2019;30(3):31. DOI: 10.1007/s10856-019-6233-y.
28. Liu P., Shen H., Zhi Y., Si J., Shi J., Guo L. et al. 3D bioprinting and in vitro study of bilayered membranous construct with human cells-laden alginate/gelatin composite hydrogels. *Colloids Surfaces B Biointerfaces.* 2019;181:1026–1034. DOI: 10.1016/j.colsurfb.2019.06.069.

29. Huang L., Du X., Fan S., Yang G., Shao H., Li D. et al. Bacterial cellulose nanofibers promote stress and fidelity of 3D-printed silk based hydrogel scaffold with hierarchical pores. *Carbohydr. Polym.* 2019;221:146–156. DOI: 10.1016/j.carbpol.2019.05.080.
30. Albanna M., Binder K.W., Murphy S.V., Kim J., Qasem S.A., Zhao W. et al. *In situ* bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds. *Sci. Rep.* 2019;9(1):1–15. DOI: 10.1038/s41598-018-38366-w.
31. Xu C., Zhang Molino B., Wang X., Cheng F., Xu W., Molino P. et al. 3D printing of nanocellulose hydrogel scaffolds with tunable mechanical strength towards wound healing application. *J. Mater. Chem. B.* 2018;6(43):7066–7075. DOI: 10.1039/C8TB01757C.
32. Shi L., Xiong L., Hu Y., Li W., Chen Z.C., Liu K. et al. Three-dimensional printing alginate/gelatin scaffolds as dermal substitutes for skin tissue engineering. *Polym. Eng. Sci.* 2018;58(10):1782–1790. DOI: 10.1002/pen.24779.
33. Nocera A.D., Comín R., Salvatierra N.A., Cid M.P. Development of 3D printed fibrillar collagen scaffold for tissue engineering. *Biomed. Microdevices.* 2018;20(2):1–13. DOI: 10.1007/s10544-018-0270-z.
34. Kim B.S., Kwon Y.W., Kong J.S., Park G.T., Gao G., Han W. et al. 3D cell printing of *in vitro* stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: A step towards advanced skin tissue engineering. *Biomaterials.* 2018;168:38–53. DOI: 10.1016/j.biomaterials.2018.03.040.
35. Datta S., Sarkar R., Vyas V., Bhutoria S., Barui A., Chowdhury A.R. et al. Alginate-honey bioinks with improved cell responses for applications as bioprinted tissue engineered constructs. *J. Mater. Res.* 2018;33:2029–2039. DOI: 10.1557/jmr.2018.202.
36. Dong J.C., Sang J.P., Bon K.G., Young-Jin K., Seok C., Chun-Ho K. Effect of the pore size in a 3D bioprinted gelatin scaffold on fibroblast proliferation. *J. Ind. Eng. Chem.* 2018;67:388–395. DOI: 10.1016/j.jiec.2018.07.013.
37. Chen C.S., Zeng F., Xiao X., Wang Z., Li X.L., Tan R.W. et al. Three-dimensionally printed silk-sericin-based hydrogel scaffold: a promising visualized dressing material for real-time monitoring of wounds. *ACS Appl. Mater. Interfaces.* 2018;10(40):33879–33890. DOI: 10.1021/acsami.8b10072.
38. Xiong S., Zhang X., Lu P., Wu Y., Wang Q., Sun H. et al. A Gelatin-sulfonated silk composite scaffold based on 3D printing technology enhances skin regeneration by stimulating epidermal growth and dermal neovascularization. *Sci. Rep.* 2017;7(1):1–12. DOI: 10.1038/s41598-017-04149-y.
39. Liu J., Chi J., Wang K., Liu X., Gu F. Full-thickness wound healing using 3D bioprinted gelatin-alginate scaffolds in mice: A histopathological study. *Int. J. Clin. Exp. Pathol.* 2016;9:11197–11205.
40. Lee V., Singh G., Trasatti J.P., Bjornsson C., Xu X., Tran T.N. et al. Design and fabrication of human skin by three-dimensional bioprinting. *Tissue Eng. Part C Methods.* 2014;20(6):473–484. DOI: 10.1089/ten.tec.2013.0335.
41. Guillemot F., Mironov V., Nakamura M. Bioprinting is coming of age: report from the international conference on bio printing and bio fabrication in Bordeaux (3B'09). *Biofabrication.* 2010;2(1):010201. DOI: 10.1088/1758-5082/2/1/010201.
42. Peltola S.M., Melchels F.P., Grijpma D.W., Kellomäki M. A review of rapid prototyping techniques for tissue engineering purposes. *Ann. Med.* 2008;40(4):268–280. DOI: 10.1080/07853890701881788.
43. Malda J., Visser J., Melchels F.P., Jüngst T., Hennink W.E., Dhert W.J. et al. 25th anniversary article: Engineering hydrogels for biofabrication. *Advanced materials (Deerfield Beach, Fla.)*. 2013;25(36):5011–5028. DOI: 10.1002/adma.201302042.
44. Chang R., Nam J., Sun W. Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication-based direct cell writing. *Tissue Eng. Part A*. 2008;14(1):41–48. DOI: 10.1089/ten.a.2007.0004.
45. Gaspar-Pintiliescu A., Stefan L.M., Anton E.D., Berger D., Matei C., Negreanu-Pirjol T. et al. Physicochemical and biological properties of gelatin extracted from marine snail *Rapana venosa. Marine Drugs.* 2019;17(10):589. DOI: 10.3390/md17100589.
46. Chiou B., Avena-Bustillos R.D., Bechtel P.J., Jafri H., Narayan R., Imama S.H. et al. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. *European Polymer Journal*. 2008;44(11):3748–3753. DOI: 10.1016/j.eurpolymj.2008. 08.011.
47. Sakai S., Hirose K., Taguchi K., Ogushi Y., Kawakami K. An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. *Biomaterials.* 2009;30(20):3371–3377. DOI: 10.1016/j.biomaterials.2009.03.030.
48. Jung H., Pena-Francesch A., Saadat A., Sebastian A., Kim D.H., Hamilton R.F. et al. Molecular tandem repeat strategy for elucidating mechanical properties of high-strength proteins. *Proceedings of the National Academy of Sciences of the United States of America.* 2016;113(23):6478–6483. DOI: 10.1073/pnas.1521645113.
49. Ozbolat I.T., Hospodiuk M. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials.* 2016;76:321–343. DOI: 10.1016/j.biomaterials.2015.10.076.
50. Dzobo K., Motaung K.S.C.M., Adesida A. Recent trends in decellularized extracellular matrix bioinks for 3D printing: An updated review. *Int. J. Mol. Sci.* 2019;20(18):4628. DOI: 10.3390/ijms20184628.
51. Mitchell A.C., Briquez P.S., Hubbell J.A., Cochran J.R. Engineering growth factors for regenerative medicine applications. *Acta Biomater.* 2016;30:1–12. DOI: 10.1016/j.actbio.2015.11.007.

Authors' contribution

All authors participated in the conception and design of the study; primary search, analysis and interpretation of the data, critical revision of the manuscript for important intellectual content, and final approval of the article for publication.

Authors' information

Barsuk Ilya A. – Assistant, Research Department (Biomedical Research), Research Center, S.M. Kirov Military Medical Academy, Saint Petersburg, barsuk20220@gmail.com, http://orcid.org/0000-0002-3728-9966

Golovko Konstantin P. – Dr. Sci. (Med.), Associate Professor, Head of the Research Center, S.M. Kirov Military Medical Academy, Saint Petersburg, labws@mail.ru, http://orcid.org/0000-0002-1584-1748

Alexandrov Viktor N. – Dr. Sci. (Med.), Professor, Head of the Research Laboratory (Tissue Engineering), Research Department (Biomedical Research), S.M. Kirov Military Medical Academy, Saint Petersburg, vnaleks9@yandex.ru, http://orcid.org/0009-0001-9229-5293

Khasanov Artur R. – Assistant, Research Department (Biomedical Research), S.M. Kirov Military Medical Academy, Saint Petersburg, khasartrish@yandex.ru, http://orcid.org/0009-0003-0763-7194.

Edgeev Naran I. – Head of the Surgical Department, Branch No. 4 of Naval Clinical Hospital No. 1469, Murmansk region, Zaozersk, luxomjachok@mail.ru, http://orcid.org/ 0009-0006-4989-2523

Galiullin Rinat I. – Senior Resident, Surgical Department, Branch No. 4 of Naval Clinical Hospital No. 1469, Murmansk region, Zaozersk, rinat061989@list.ru, http://orcid.org/0009-0008-6079-956X

(✉) **Barsuk Ilya A.,** barsuk20220@gmail.com

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