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DECELLULARIZED UMBILICAL CORD STROMA IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE: A SYSTEMATIC REVIEW

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Despite great progress in the field of biomaterials for tissue engineering and regenerative medicine, the high requirements placed on artificial matrices (matrices, carriers, scaffolds) are the reason for the ongoing search for natural or synthetic extracellular matrix mimetics. Among such materials, decellularized umbilical cord (UC) stroma appears to be very attractive – it has a high content of hyaluronic acid, cytokines, and growth factors, and there are no ethical restrictions for its production. Decellularized UC stroma has been found to promote cartilage, liver tissue and nerve tissue repair, as well as wound healing. The review critically analyzes and summarizes published data on the ability of decellularized UC stroma to maintain the necessary conditions for adhesion, migration, differentiation and functional activity of adherent cells, thus stimulating the internal (physiological) regenerative potential of tissues. Literature was searched for in the following electronic databases: Medline/PubMed (www/ncbi.nlm.nih.gov/pubmed), Cochrane library (https://www.cochrane.org), and eLIBRARY/Russian Science Citation Index (https://www.elibrary.ru). Inclusion criteria were the presence of biomaterials obtained from decellularized human UC stroma. Exclusion criteria for papers included research objects as decellularized umbilical cord vessels (veins and arteries) and umbilical cord cell cultures. Twenty-five original articles in English and Russian were selected for analysis of the products obtained, their applications, decellularization methods and research results. The review also discusses the prospects for decellularized umbilical cord in medicine.

Keywords: umbilical cord stroma, decellularization, extracellular matrix, regenerative medicine, tissue engineering.

The concept of tissue engineering is to create functionally active cell-engineered constructs (CECs) to stimulate physiological regeneration of damaged tissues or tissue-engineered constructs (TECs)/tissue equivalents formed *in vitro* or *in vivo*, intended for temporary/permanent replacement of irreversibly damaged organs and tissues [1–3].

The main components of CECs/TECs are: cells and a cell carrier (synonyms: scaffold, framework, matrix or artificial matrix) whose function is to deliver and retain cells in the implantation site [4]. The most interesting are extracellular matrix (ECM) mimetics that mimic the natural ECM in composition and can maintain the viability and functional activity of cells for a long time, creating the necessary microenvironment for them [1, 5, 6].

When creating CECs/TECs *in vitro*, reproduction of not only biochemical but also biomechanical stimuli, which ensure the vital activity of cells in the body, is an important condition for achieving a high degree of resemblance to the natural tissue [7]. To simulate *in vitro* biomechanical forces, such as compression, tension, shear force, and hydrostatic pressure, special devices – bioreactors – are used, and for each organ the complex of such influences is individual [6–8].

An alternative way is the formation of CECs/TECs in the body by implanting a cell-free artificial matrix into the body. The task is to ensure migration of the recipient's own cells to it and stimulate their proliferation with subsequent replacement of the damage by a functionally active tissue [9]. Cell-free products created by decellularization from the biomaterial of animal or human organs and tissues have shown high bioactivity. In addition, such a product can be manufactured in advance and applied without prior preparation. This potentially accelerates its introduction into clinical practice and is of particular importance for military medicine [10–11].

Among them, decellularized umbilical cord stroma (Wharton's jelly, WJ) seems promising [12]. The WJ is a connective tissue that forms the bulk of the umbilical cord in humans and other mammals. The ECM obtained from WJ-derived mesenchymal stem cells (MSCs) contains structural components (collagens types I, II, III, IV, V, VI, XII, XIV, fibronectin, fibrillin and high-molecular-weight hyaluronic acid and sulfated glycosaminoglycans

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(GAGs) such as chondroitin sulfates, heparan sulfate, dermatan sulfate) and numerous growth factors such as insulin-like growth factor (IGF-1) and IGF-binding proteins (IGFBPs) 1, 2, 3, 4 and 6, transforming growth factor alpha (TGF-α) and platelet-derived growth factor (PDGF), fibroblast growth factors (αFGF, βFGF), epidermal growth factors (EGFs), various isoforms of transforming growth factor beta (TGF-β1, 2, 3), vascular endothelial growth factor (VEGF), cytokines (with predominance of anti-inflammatory), matrix metalloproteinases (MMPs) and matrix metalloproteinase inhibitors (TIMPs). Expression of several immunomodulatory cytokines, such as RANTES (regulates activation, normal T-cell expression and secretion), interleukin receptor 6 (IL-6R), interleukin 16 (IL-16) and interferon gamma (IFN-γ), and proinflammatory cytokines, such as macrophage colony stimulating factor (MCSF), macrophage stimulating protein 1-alpha (MIP1a) were also found; tumor necrosis factor 1α and 1β receptor superfamily (TNF-RI and TNF-RII), interleukin 1 receptor antagonist (IL1RA), and wound healing-related cytokines including intercellular adhesion molecule 1 (ICAM-1), granulocyte stimulating factor (G-CSF) [13–17]. In addition, ECM derived from WJ-derived MSCs supposedly has immunomodulatory and some bacteriostatic effects [16, 17]. It has been described that decellularized WJ promotes cartilage repair [17], liver tissue [19], nerve tissue [20] and wound healing [21–23].

However, to the best of the authors' knowledge, no review has so far attempted to critically evaluate and summarize the evidence regarding the regenerative potential of decellularized UC stroma.

REVIEWED DATABASES AND SEARCH RESULTS

The literature search was conducted in electronic databases Medline/PubMed (www/ncbi.nlm.nih.gov/pubmed), Cochrane library (https://www.cochrane.org), and eLIBRARY, Russian Science Citation Index (https://www.elibrary.ru).

The following terms were used as a search query in Medline/PubMed: (umbil*[title] AND decell*[title]) OR (whart*[title] AND decell*[title]) OR (umbil*[title] AND acell*[title]) OR (whart*[title] AND acell*[title]) OR (umbil*[title] AND extracel*[title] AND matr*[title]) OR (whart*[title] AND extracel*[title] AND matr*[title]). Date of last search: March 19, 2023.

The following terms were used as a search query in Cochrane library: (extracell* AND umbil*) OR (decell* AND umbil*). Date of last search: March 25, 2023.

The following terms were used as a search query in eLIBRARY: decell* umbil* (search query 1), extracell* matrix* umbil* (search query 2), decellularization and umbilical cord (search query 3), "extracellular matrix" and umbilical cord (search query 4). Date of last search: March 25, 2023.

Inclusion criteria were: the presence of materials obtained from decellularized human UC stroma in the study. Full-text original articles in English and Russian were used in literature analysis. Exclusion criteria for articles were the use of decellularized vessels (veins and arteries) of the umbilical cord, as well as the study of umbilical cord cell cultures without using its ECM. In addition, conference proceedings, reviews, and preprints of articles were not included in the study.

The literature search process is shown in Fig. 1.

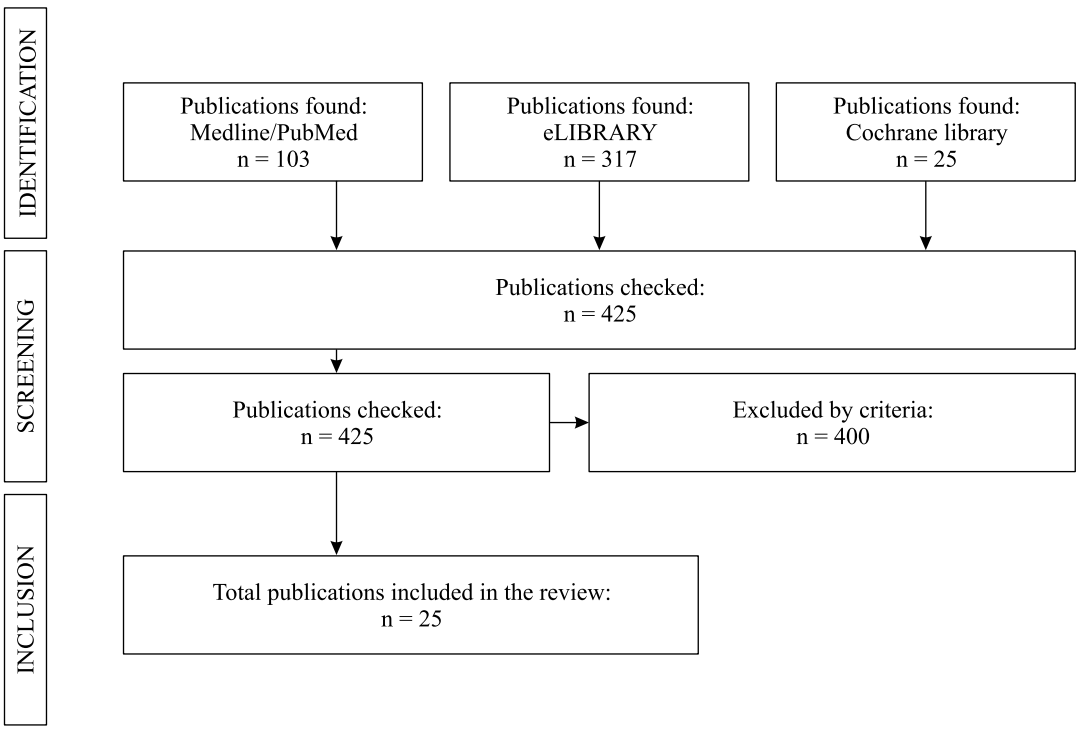

Fig. 1. Flow diagram of the literature search employed for this review

The initial search resulted in 425 publications. First of all, the results of the search for publications in each of the selected databases manually excluded articles on decellularization of umbilical cord vessels, the use of umbilical cord mesenchymal cells and their vesicles. Next, literature reviews and 2 clinical studies with no description of UC-derived products were excluded (decellularization was not confirmed). At the last stage, 4 repetitions were excluded: 3 publications were duplicated in the PubMed and eLIBRARY databases [17, 24, 25], one publication was reflected twice as a result of a PubMed search (publication [26] and its correction [27]). In 3 publications, the authors used only centrifugation as WJ processing. No quantitative control of genetic material content was presented. This was the reason for their exclusion from consideration in the review [10, 28, 29]. The results of another study were also decided not to be considered – the product obtained by the researchers was a fraction of the supernatant obtained after exposure of WJ to trypsin [30]. So, 25 articles were included in the study [11, 12, 16–21, 24–26, 31–44]. Eight described only *in vitro* studies [18, 19, 24, 25, 31–33, 37], four described only *in vivo* (animal) studies [34, 41, 43, 44], and 10 included both types of preclinical trials [11, 16, 17, 20, 21, 26, 35, 36, 38, 42]. No clinical studies meeting the inclusion criteria were found.

MAIN PARAMETERS AND RESEARCH RESULTS OF INCLUDED PUBLICATIONS

Results of the published works demonstrate that decellularized WJ (dWJ) refers to a biocompatible material capable of stimulating cell proliferation and positively influencing the regeneration processes of damaged organs and tissues (Table).

Gupta et al. when creating a matrix for vascular tissue engineering from silk fibroin, dWJ was functionalized to improve the remodeling properties and immunomodulation of the recipient response to introduction of the polymeric material. Powder of lyophilized dWJ was mixed with silk fibroin (5 mg dWJ per 1 ml of silk solution) before matrix molding. After lyophilization, the prosthetic porous vessel was coated with a nanofiber layer. The solution for obtaining the material by electrospinning consisted of silk fibroin and polycaprolactone in hexafluoro-2-propanol. After crosslinking using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in 80% ethanol for 12 hours, the scaffolds were washed in sterile water on a shaker for 12 hours and stored at 4 °C until further use [11]. Basiri et al. added dWJ, obtained using a similar technique, to a silk fibroin hydrogel before gelation. The biomaterial thus obtained, exhibited mechanical properties similar to those of cartilage [31].

Li et al. used dWJ as a cell carrier supporting the phenotypes and differentiation potential of hematopoietic progenitor cells [24]. Kehtari et al. demonstrated the ability of such a matrix to provide a microenvironment that promotes hepatocyte differentiation of pluripotent cells by activating transcription factors [19]. Azarbarz et al. presented the results of dWJ conjugated with gelatin for a 3D cell culture system. The differentiation of MSCs into insulin-producing cells was shown, confirmed by increased expression of insulin-specific genes and increased insulin release in response to glucose stimulation [32].

WJ is a reservoir of peptide growth factors involved in chondrogenesis. Xiao et al. showed that human dWJ can be a good alternative biomaterial for cartilage tissue engineering [33]. The biomechanical properties of dWJ and its homogeneous porous structure were sufficient to support rabbit chondrocytes cultured on them. Production of GAGs, collagen types I and II, and aggrecan by the cells was demonstrated [33]. At the same time, in a study by Foltz et al., during tracheal defect replacement, no chondrocyte recruitment into biocompatible dWJ and chondrocyte-induced collagen production were found. At the same time, the structure and physical characteristics of the biomaterial provided the necessary maintenance of tracheal patency [34]. Penolazzi et al. showed the therapeutic potential of dWJ and its effect on the functioning of degenerated intervertebral disc cells. The authors suggested that dWJ implantation may be sufficient for the functional repair of degenerated intervertebral discs [25].

A study by Jadalannagari et al. noted the migration of WJ-derived MSCs into the dWJ thickness on day 2. At the same time, the authors observed cell proliferation when cultured on dWJ, which, however, was lower than when cultured on the culture plate. Using a flat bone defect model, it was shown that human dWJ promoted adhesion and penetration of viable osteocytes into the biomaterial [26]. Yuan et al. used decellularized UC stroma for tendon healing. After cell removal, dWJ retained a significant amount of GAGs and collagen, preserved microstructure and tensile strength. The three-dimensional porous structure of dWJ promoted tenocyte migration, attachment, and proliferation. In an *in vivo* study, the decellularized product promoted tendon regeneration [35].

A study by Mann et al. established the stimulating effect of cryopreserved human dWJ on restoration of the integrity of a rat spine after intrauterine repair of spina bifida. The resulting biomimetic ECM improved organized cell growth and reduced acute inflammation [36]. In another study, a tissue-engineered coating consisting of rat adipose tissue MSCs and human dWJ accelerated healing of a rat tail wound. Reduced tail volume, improved angiogenesis and lymphangiogenesis were recorded [21].

Some researchers emphasize the applicability of human umbilical cord products to stimulate regeneration of different tissues. Dubus et al. showed by mass spectrometry in prepared lyophilized dWJ the presence of structural and adhesive proteins involved in wound healing

Table

General characteristics and results of studies included in this review

Study (last name, year)	Application	Product description	Decellularization methods	*In vitro* studies		*In vivo* studies		Results
				Object	Methods	Object	Methods	
1	2	3	4	5	6	7	8	9
Basiri, 2019 [31]	Cartilage repair and regeneration	dWJ/silk fibroin hydrogel	WJ was mixed with deionized water for 16 hours, centrifuged at 4 °C, the supernatant was stored at –80 °C	Human endometrial stem cells	Cell viability	–	–	The product improved the viability of cells studied
Gupta, 2021 [11]	Vascular tissue engineering	Two-layered silk fibroin tubular scaffold seeded with freeze-dried dWJ	WJ 1–2 cm in 4 °C deionized water for 16 hours, >70 μm fragments were removed, centrifuged at 5000 rpm for 10 minutes, 4 °C, freeze-dried	Human endotheliocytes, macrophages	Product cytotoxicity	Subcutaneous implantation in rabbits (n = 6), rabbit jugular vein implants (n = 3)	Histological studies, gene expression analysis, Doppler ultrasound	The biocompatible product induced cell recruitment after a month of subcutaneous implantation with expression of anti-inflammatory genes. Vascular implant was permeable after 3 months
Li, 2019 [24]	Bone marrow transplantation	3 mm thick fragments	Umbilical cord vessels were removed, umbilical cord stroma was decellularized with SAA solutions, cryopreserved	CD34+ cells	Flow cytometry, gene expression analysis	–	–	The product promotes megakaryocytic differentiation and supports primitive hematopoietic cell phenotypes
Kehtari, 2018 [19]	Liver tissue engineering	Porous matrix	Umbilical cord in Tris-EDTA buffer solution at 4 °C for 16 hours, decellularized with 0.03% SDS for 24 hours with shaking, washed, placed in a hypertonic saline at 37 °C for 3 hours, washed, cross-linked with acetic acid, freeze-dried	Human pluripotent stem cells	Viability, differentiation into hepatocytes	–	–	The cell-free product is able to support differentiation into hepatocytes

Continuation Table

1	2	3	4	5	6	7	8	9
Azarbarz, 2022 [32]	Differentiation of MSCs into IPCs	Gelatin-conjugated freeze-dried dWJ	The umbilical cord was incubated in NaCl (1 M) for 1 week, decellularized with 0.25% trypsin-EDTA solution for 24 hours, 1% Triton X-100 for 5 days. 24 hours, washed with PBS, freeze-dried. dWJ was gelatin-conjugated for 1 hour. 37 °C	WJ-MSCs	WJ-MSCs were differentiated into IPCs. IPCs were stained with dithizone. C-peptide secretion and expression of insulin-related genes were examined	–	–	Secretion of insulin by cells is higher than in the controls. The presence of the product is associated with an increase in dithizone-positive cells and increased expression of PDX-1, GLUT-2 and INS genes in IPCs
Xiao, 2017 [33]	Cartilage tissue engineering	Cylinders 8 mm in diameter, 2 mm thick	Homogenized WJ was frozen in water (4–5 times), centrifuged at 3000 rpm for 30 minutes, 5000 rpm for 30 minutes, 7000 rpm for 30 minutes, supernatant was withdrawn, centrifuged 10,000 rpm for 30 minutes, precipitate was freeze-dried	Rabbit chondrocytes	Cell viability, gene expression analysis, production of glycosaminoglycans and collagen	–	–	The biocompatible, bioactive cell-free product promoted chondrocyte activity *in vitro*
Foltz, 2022 [34]	Tracheal cartilage regeneration	dWJ	Amniotic membrane was removed, washed with PBS, decellularized with 0.01% SDS and sodium deoxycholate, centrifuged at 100 rpm, washed with 37 °C PBS for 24 hours, stored at 4 °C	–	–	Rabbit tracheal defect 10 × 20 mm, (n = 10)	Histological studies, determination of the relative content of collagen types 1 and 3, aggrecan	No product rejection reactions were detected. No induction of collagen production and chondrocyte recruitment was found. No significant differences in the content of collagen types 1 and 3 and aggrecan were observed at day 30

Continuation Table

1	2	3	4	5	6	7	8	9
Penolazzi, 2020 [25]	Intervertebral disc regeneration	dWJ	Umbilical cord was placed in 4 °C deionized water for 24 hours, decellularized with 4% sodium deoxycholate for 4 hours, treated with DNase for 3 hours (three times)	MSCs, human degenerated intervertebral disc cells	Cell viability and proliferation, gene expression analysis	–	–	Support of viability of the cells of the degenerated intervertebral disc and expression of critical homeostasis regulators
Jadalannagari, 2017 [26]	Tissue engineering	dWJ	Decellularized with 0.005% Triton X-100, SDS, sodium succinate, treated with DNase for 16 hours, SAAs removed	WJ-MSCs, HUVEC	Product cytotoxicity, PCR	Full-thickness parietal bone defect (5.0 mm in diameter) in mice (n = 4)	Histological studies, live-cell imaging	A biocompatible product that stimulates osteocyte migration after 24 hours and after 2 weeks *in vivo*
Yuan, 2022 [35]	Tendon regeneration	Freeze-dried dWJ, 20 × 10 × 2 mm	Decellularized with 1% SDS for 24 hours, 1% Triton X-100 for 24 hours, treated with nuclease for 12 hours, washed, freeze-dried	Rabbit tenocytes	Product cytotoxicity, proliferation, cell migration	Rabbit tendon defect (n = 54)	Histological studies, gene expression analysis	Biocompatibility with cells at day 7, stimulation of tendon maturation at week 12 after surgery
Mann, 2020 [36]	Regeneration after spina bifida repair	dWJ patch	–	Primary meningeal cells, human neonatal keratinocytes	Cell proliferation	Retinoid-induced spina bifida in fetal rats	Histological studies	The use of the patch reduced acute inflammation and cell apoptosis, and increased cell proliferation
Lu, 2023 [21]	Wound healing	dWJ, 3 × 5–8 cm fragment	Decellularized with 0.1% SDS, washed with PBS and medium 199 for 48 hours, 100 rpm, 37 °C	Rat adipose tissue MSCs	Cell viability and proliferation	Rats (n = 15)	Histological studies, tail volume measurements	The product stimulated cell proliferation at day 14 and accelerated the healing of rat tail wounds with reduced tail volume by week 5 of follow-up

Continuation Table

1	2	3	4	5	6	7	8	9
Dubus, 2022 [16, 17]	Regenerative medicine	Freeze-dried dWJ	Vessels and amniotic membrane were removed, decellularized with 1% Triton X-100 for 1 hour, treated with DNase for 24 hours, 37 °C with stirring, washed, freeze-dried	WJ-MSCs, human fibroblasts, osteocytes, neutrophils, and monocytes	Cytotoxicity, proliferation, flow cytometry, cytokine production	Subcutaneous implantation in rats (n = 4), calvarial bone regeneration (n = 2)	Histological studies	The biocompatible product did not activate neutrophil metabolic activity, it promoted anti-inflammatory macrophage polarization *in vitro*, and did not improve bone regeneration
Converse, 2017 [37]	Tissue engineering	dWJ fragments	Umbilical cord was placed in a hypertonic saline for 1 hour, decellularized with Triton X-100 for 1 hour, placed in hypertonic saline for 1 hour, deionized water for 1 hour, treated with enzyme for 12–16 hours, treated with N-lauroylsarcosine for 2 hours, with ethanol for 10 minutes, residual detergents were removed, cryopreserved	Bone marrow MSCs, umbilical cord hematopoietic and progenitor cells, human leukemia cell lines HL-60, Kasumi I, MV 411	Colonization of dWJ fragments by cells	–	–	Recellularized product
Koci, 2017 [20]	Nerve tissue repair and regeneration	dWJ-based hydrogel	Treated with 0.02% trypsin/0.05% EDTA solution, 0.1% peracetic acid, 4% ethanol, freeze-dried, solubilized with pepsin, and neutralized	Human bone marrow MSCs	Cell proliferation and migration	Focal cerebral ischemia in rats (n = 4)	Histological studies	*In vitro* cytocompatibility, active colonization of hydrogel by recipient cells was observed *in vivo* (anti-inflammatory macrophages were the predominant cell population)

End of Table

1	2	3	4	5	6	7	8	9
Ramzan, 2022 [18]	Cartilage tissue engineering	dWJ-based hydrogel	Umbilical cord was subjected to osmotic shock, treated with 0.05% trypsin for 2 hours at 37 °C, 1% Triton X-100 for 24 hours with stirring, washed, freeze-dried, crushed, solubilized with pepsin for 48 hours with stirring, neutralized, subjected to gelation	WJ-MSCs	Cell proliferation and viability, gene expression analysis	–	–	On day 7, the dWJ-based hydrogel provided scaffold support for cell proliferation and differentiation in the chondrogenic direction on day 28 of cultivation
Výborný, 2019 [38]	Nerve repair	Genipin-cross-linked dWJ-based hydrogel	Umbilical cord was treated with 0.02% trypsin/0.05% EDTA, 0.1% peracetic acid, 4% ethanol, freeze-dried, solubilized with pepsin, neutralized, cross-linked with genipin	WJ-MSCs, human fetal neural stem cells	Cell viability, proliferation and differentiation	Photochemical lesion of the cortex	Histological studies	Genipin-cross-linked dWJ-based hydrogel, retained *in situ* for up to 2 weeks without an adverse tissue response or inflammation
Kalyuzhnaya, 2019; Bolgarchuk 2020; Chebotarev 2020; Kondratenko 2021 [12, 39–44]	Regenerative medicine	dWJ and dWJ hydrogel lyophilisates, hydrogel wet form	Vessel was removed, homogenates were decellularized with SDS, washed, freeze-dried, solubilized with pepsin, and a liquid form was obtained. Liquid form was freeze-dried	Human, rat, mouse, porcine, and guinea pig fibroblasts	Viability	Full-thickness skin wounds in mice and pigs, cartilage defect in rabbits	Histological studies	Biocompatible products promote granulation tissue formation and epithelialization and hyaline cartilage healing

Note: SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; HUVEC, human umbilical vein endothelial cells; WJ, Wharton's Jelly; dWJ, decellularized Wharton's jelly; IPCs, insulin-producing cells; MSCs, mesenchymal stem cells; SAAs, surface active agents; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid.

process, such as collagen, fibronectin, tenascin, lumican, periostin, types I and II keratin, fibulin and fibrinogen beta chain [16, 17]. High levels of growth factors were detected in the culture medium after incubation in dWJ. A similar effect was not detected for native umbilical cord. At the same time, the release of bioactive molecules from the cell-free product did not lead to phagocyte activation and accumulation of reactive oxygen species. WJ-derived stromal cells and human fibroblasts that were cultured in dWJ maintained high viability. Subcutaneous implantation in rats showed no inflammatory reaction and formation of a connective tissue capsule or reaction to the foreign body with the presence of multinucleated giant cells. The implanted subcutaneous human UC product was completely remodeled within three weeks. Mouse macrophages produced significantly more anti-inflammatory mediators after 72 hours of contact with dWJ compared to the control. The action of native human UC on mouse macrophages under similar conditions resulted in dominant secretion of proinflammatory mediators by them [16, 17]. The same works established the antibacterial activity of dWJ, manifested by the presence of a microbial growth inhibition zone and reduction of bacterial adhesion. Converse et al. described in detail the procedures for making TECs from dWJ in the form of plates followed by recellularization [37].

The fabrication and properties of solubilized forms of dWJ are described in 4 publications. Koci et al. solubilized cell-free powdered WJ for hydrogel preparation with hydrochloric acid pepsin. The resulting product form contained higher amounts of sulfated GAGs compared with the product prepared from pig bladder, spinal cord, and brain using similar techniques. The dWJ products exhibited a short gel time, indicating rapid self-assembly of the structural molecules. They supported cell growth, proliferation and migration *in vitro*. And 24 hours after introducing the injected form of dWJ into the focal ischemic lesion in the motor area of the rat cerebral cortex, a compact gel structure populated by a dense layer of endogenous cells was formed within the lesion site. Macrophages were the predominant cell type present within the defect, with the M2 macrophage phenotype (CD206) accounting for 77.1 ± 6.5% of all macrophages contained in the gel [20].

Ramzan et al. obtained the hydrogel form by enzymatic digestion using pepsin in hydrochloric acid followed by gelation. The dWJ-derived gel thus obtained, functioned as a three-dimensional matrix that provided the necessary microenvironment for adhesion, migration, proliferation and differentiation of MSCs into the chondrogenic lineage *in vitro* [18].

Vyborny et al. used a solubilized form of chemically cross-linked dWJ hydrogel to repair rat cortical lesions. In *in vitro* experiments, the resulting product was shown to have no cytotoxic properties, and *in vivo* experiments showed *in situ* gelation without adverse inflammatory reactions [38].

A group of researchers from Kirov Military Medical Academy, headed by I.K. Kalyuzhnaya, patented dWJ products/products in lyophilized and solubilized hydrogel (which can be lyophilized) forms (Fig. 2). The decellularization procedure in this case can be performed using sodium dodecyl sulfate or 0.1 N sodium hydroxide [12, 39–44]. Biocompatible products have shown bioactivity regarding the healing of articular cartilage and skin defects [41–44].

Thus, the publications considered above show that dWJ-derived biocompatible and bioactive products can be used in various areas of regenerative medicine and cell technologies, such as a source of bioactive molecules in human cell culturing, in cellular immunotherapy, wound healing, hematopoietic cell transplantation, MSC differentiation in insulin-producing cells, repair of cartilage, intervertebral discs, tendons and nerve tissue, tissue engineering of vessels, liver and cartilage, and regeneration after spina bifida repair.

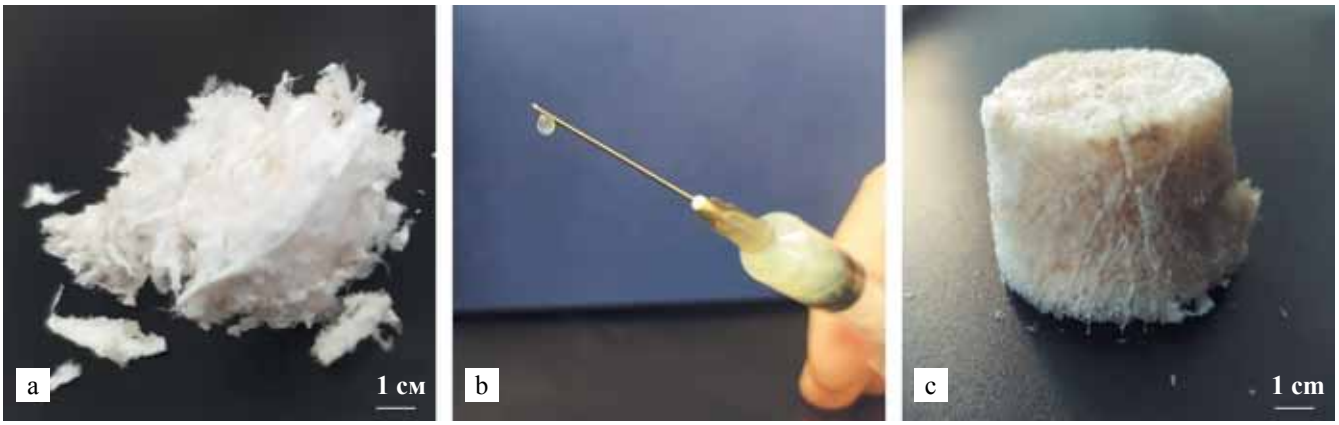

Fig. 2. View of various forms of decellularized human Wharton's jelly (dWJ). a, lyophilized dWJ [42]; b, solubilized form of dWJ; c, lyophilized solubilized form of dWJ [45]

PROSPECTS AND POSSIBLE APPLICATIONS OF DECELLULARIZED UMBILICAL CORD STROMA IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE

The search for biomaterials for tissue engineering and regenerative medicine focuses mainly on the development of biomimetics that are capable of inducing specific cellular responses and formation of tissue equivalents. For optimal reproduction of the natural cell niche, an ideal biomimetic should be biocompatible, i.e., it should easily integrate into the surrounding tissue and form a single whole with it, while its degradation products should also have high biocompatibility. The biomimetic should support cell adhesion, proliferation, differentiation and secretion of its own ECM. The rate of matrix degradation should correspond to the regeneration rate of the recipient tissue. To integrate into the body, the biomaterial must have the ability to neovascularize and innervate [46]. In some cases, the biomaterial must withstand the mechanical stress characteristic of the area of its implantation, i.e., specific mechanical strength and plasticity are required [47]. In addition, sterilization and storage should not change the properties of the matrices, and use of the biomaterial should be as convenient as possible. Decellularized tissues have the most accurate reproduction of the cell niche, due to preservation of specific composition and morphology. Due to this, decellularization is considered to be one of the most promising methods of creating ECM mimetics [1].

The nonimmunogenicity of the decellularized product, as one of the main parameters of biocompatibility, is mainly due to the absence of genetic material of the original tissue [48]. The use of various decellularization methods and protocols provides reliable purification against the donor's genetic material, but, at the same time, can lead to loss of bioactive components and change the structure of ECM proteins. Effective removal of cells while preserving the microstructure and composition of ECM makes the finished cell-free product able to stimulate regeneration, as well as maintain cell activity [48]. Note that the publications studied do not compare the effectiveness of different UC stromal decellularization protocols. For example, optimization of the decellularization protocol improved the functional properties of cell-free blood vessels and articular cartilage and liver [49–51].

Allogeneic biomaterial for making cell-free products from it is often limitedly available and is not optimal for several reasons. The composition and structure of adult donor tissues are influenced by external and internal factors during the donor's life. Birth defects, diseases, age-related changes, the effects of stress, medications, and exposure to unhealthy work or environmental conditions significantly alter connective tissue components and architecture. Loss of ECM components along with increased cross-linking of collagen leads to deterioration of tissue biomechanical properties. Fibronectin levels change with age, impairing cell binding to ECM via integrin receptors [52]. Age-related changes in donor tissues inevitably worsen the properties of cell-free mimetics made from them and reduce their regenerative potential.

ECM properties inevitably deteriorate with age, which has prompted many researchers to neglect the risks of using xenogeneic biomaterials for tissue engineering. Worldwide, research is underway to create cell-free products from animal organs and tissues, provided that protocols are developed to ensure complete removal of cells, including genetic material [49–51, 53].

For the needs of tissue engineering and regenerative medicine, there are currently offers of commercial products based on decellularized ECM of allogeneic and xenogeneic origin [54, 55]. Nevertheless, human biomaterial for creating such products is preferable to available xenogeneic materials due to the potential danger of undesirable immune response when implanted into the recipient. Carbohydrate residue galactose α-1,3-galactose (α-Gal epitope), also called the major xenoantigen, is a component of membrane glycoproteins and glycolipids of cells of many mammals, except humans and some monkey species. Human blood contains a high titer of anti-α-Gal antibodies. There is an assumption that the wall of *Enterobacteriaceae* bacteria of the normal intestinal microflora contains galactose residues and stimulates the human immune system to produce these antibodies [56].

It is impossible to completely avoid the recipient's immune system response to the implantation of decellularized tissue. However, the type of the emerging immune response that occurs during implantation determines the possibility of a favorable healing outcome [57]. Sikari et al. and Huleichel et al. showed the connection between the products of biomaterials degradation and macrophage tissue phenotype, their expression of anti-inflammatory genes and protein production [58, 59].

Collagen cross-linking technologies promote product resistance to enzymatic degradation but reduce the moisture-absorbing properties of the material [60]. In addition, chemical cross-linking agents change the ultrastructure, composition and topology of the product surface [60]. However, the use of crosslinking agents seems to be necessary when there is a need for the artificial matrix to function in the body for the long-term. This points to the possibility of their use for decellularized UC stroma, given the rapid degradation (about 3 weeks) revealed in the study.

Interest in the use of extraembryonic tissues (including placenta, amniotic membrane and umbilical cord) as raw materials for use in regenerative medicine is due to their unique composition and properties [61]. The composition of embryonic and neonatal tissues is markedly different from that of adult tissues and has a greater

regenerative potential because it plays an important role in tissue morphogenesis [62]. In contrast to the adult, the mammalian foetus heals its wounds spontaneously by regeneration without associated scarring, provided that the injury has been inflicted at a sufficiently early stage of gestation, typically before the third trimester, after which there is a transition to the postnatal type of wound healing with a gentle elastic scar formation [63]. The unique feature of fetal phenotype tissues for wound healing without scarring may be associated with a higher content of antifibrotic isoform TGF-β3 in them, relative to TGF-β1 and TGF-β2 isoforms inherent in postnatal tissues [62].

While the clinical use of fetal membranes is well documented, the use of UC tissue is relatively new. The human UC is widely used for obtaining MSCs and endothelial cells, and can also serve as a source of hyaluronic acid. At the same time, WJ consists mainly of collagen (>500 mg/g tissue), proteoglycans and GAGs, such as hyaluronic acid and heparan sulfate, immobilized and embedded in the collagen network. The proportional ratio of collagens in WJ is: 47% (type I), 40% (type III), and 12% (type V). Hyaluronic acid constitutes up to 70% of GAG content in UC (approximately 4 mg/ml), which gives the tissue a special hydrophilicity [61, 64, 65]. Unique structural characteristics and the presence of growth factors make the UC an attractive source of biomaterial for the needs of regenerative medicine and tissue engineering.

It should be noted that among the publications found, to the surprise of the authors, no studies using xenogeneic UC were found. Given the property of low immunogenicity of provisory organs and the admissible difficulties in ensuring a constant supply of biomaterial when transferring the technology to production, the use of this type of biomaterial may be justified.

As shown by analysis of publications, decellularized human UC stroma can be successfully used to stimulate regenerative processes of different organs and tissues. Cell-free UC stroma can be used as an independent product; it can also be used to improve the biological properties of other materials. For ease of transport and storage, decellularized UC stroma is lyophilized. A variety of product forms can be made from WJ-derived ECM, including porous matrices, hydrogel compositions, tubular scaffolds, etc. It is possible to supplement compositions with amniotic material or with mineralized or demineralized bone ECM. Combinations of UC biomaterial with pharmaceutical carriers can be created. In some cases, injectable forms of WJ-derived ECM hydrogels with *in situ* polymerization abilities are considered more feasible because these materials can easily adjust to the surface topography and volume of the injured area, and osmotic forces contribute to the stretching of the hydrogel polymer network as it swells. For this purpose, the decellularized UC stroma is enzymatically solubilized.

In general, work in the field of obtaining biomaterial from decellularized UC began relatively recently: the first publication in the English-language literature appeared in 2016. In Russia, studies devoted to the described topic are carried out by only one group of scientists from Kirov Military Medical Academy in St. Petersburg [12, 39–44, 66]. This is probably the reason for the lack of clinical works that meet the review's inclusion criteria. At the same time, two clinical trials were initiated in Iran in 2022, devoted to the treatment of wrinkles using UC [67, 68]. However, there is no information on whether the UC used in these studies is decellularized.

CONCLUSION

Based on analysis of papers published in electronic databases, we can conclude that scientific research on the possibilities of dWJ application is a very promising area of regenerative medicine and tissue engineering. Researchers are using different methods to remove cells and create convenient forms for application of cell-free products from UC stroma. However, more conclusive evidence – additional studies, clinical and preclinical – is needed to finally determine the prognosis for the use of the technology in medicine.

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