Front. Med. 2022, 16(3): 358−377
https://doi.org/10.1007/s11684-021-0903-0

REVIEW

Microorganism-derived biological macromolecules for tissue engineering

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Abstract **According to literature, certain microorganism productions mediate biological effects. However, their beneficial characteristics remain unclear. Nowadays, scientists concentrate on obtaining natural materials from live creatures as new sources to produce innovative smart biomaterials for increasing tissue reconstruction in tissue engineering and regenerative medicine. The present review aims to introduce microorganism-derived biological macromolecules, such as pullulan, alginate, dextran, curdlan, and hyaluronic acid, and their available sources for tissue engineering. Growing evidence indicates that these materials can be used as biological material in scaffolds to enhance regeneration in damaged tissues and contribute to cosmetic and dermatological applications. These natural-based materials are attractive in pharmaceutical, regenerative medicine, and biomedical applications. This study provides a detailed overview of natural-based biomaterials, their chemical and physical properties, and new directions for future research and therapeutic applications.**

Keywords biological macromolecules; regenerative medicine; tissue engineering; exopolysaccharide; carbohydrate

Introduction

Biological macromolecules are high-molecular weight natural polymers discharged by microorganisms into their environment. These biomaterials are called exopolysaccharides (EPSs) [1], which can be homopolymeric or heteropolymeric components, and they contribute to the materials' structural diversity and large molecular weights (from 10 kDa to 1000 kDa) [2]. Polysaccharides produced by microbes may be categorized as follows, with the aid of their natural and biological functions: extracellular bacterial polysaccharides (e.g., sphingan, xanthan, and alginate), intracellular storage polysaccharides (e.g., glycogen), and capsular polysaccharides (CPSs) that are strongly related to the cell membrane, such as K30 O-antigen [3]. These polymers can play a defensive role and attach to both natural and inert surfaces, depending on their structural characteristics. During the last decade, many new microbial EPSs were discovered. In 1972, Sutherland proposed EPSs to represent polysaccharides found in the medium outside microbial cells, generally collaborating within the formation of microbial aggregates [4]. In addition, they are secreted through microorganisms gathered outside the cells and can remain in the nearby environment [5,6].

Furthermore, EPSs are vital for biofilm pathogenicity and arrangement. The biofilm network incorporates nucleic acids, proteins, humic substances, and EPS. Bacterial EPS helps attach microbes to surfaces; each EPS is different and confers strength and structure to the mature biofilm [7]. The inherent biocompatibility and apparent nontoxic nature of some of these bacterial EPSs contribute to different medical applications as scaffolds in wound dressing and tissue engineering [8,9]. Additionally,

Received: May 26, 2021; accepted: September 23, 2021

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bacterial EPSs prove biological activities, such as antitumor and immunomodulatory properties, which considerably vary with the degree of branching, molecular mass, conformation, and chemical modification [10,11]. Thus, they can become more attractive than polysaccharides from microalgae and plants. Some biopolymers are gradually degraded *in vivo*, making them appropriate for substituting tissue and releasing controlled drugs [12,13].

EPSs are extensively utilized in pharmacological, nutraceutical, and cosmeceutical areas, nutriment, insecticides, and herbicides. However, they are used for anticoagulants, drug delivery, wound dressing, antithrombotic, immunomodulation, anticancer, and bioflocculants [14].

The review sought to obtain a helpful insight into EPSs' chemical composition and structure, physical properties, extraction methods, and current commercial clinical applications, which are officially confirmed by accepted documents related to medical authorities based on their promising potential uses focused on various studies.

EPS biosynthesis pathways

The overall pathways of the EPS biosynthesis, including the Wzx/Wzy-dependent pathway, adenosine triphosphate (ATP) binding cassette (ABC) transporter-dependent pathway, and synthase-dependent pathway, and the extracellular synthesis produced by using only sucrose protein are considered four common mechanisms currently recognized for synthesizing carbohydrate polymers (Fig. 1).

Wzx/Wzy-dependent pathway

In this path, individual repeating components are first built from monomers attached to each undecaprenol diphosphate anchor (Und-P) at the cytosolic side of the membrane. This anchor is constructed by very specific glycosyltransferases (GTs) and moved to the periplasmic area by a Wzx protein (known as flippase) after its completion. Subsequently, polymerizing the repeated unit is catalyzed by the Wzy protein before being exported to the cell membrane [15,16]. Based on the results, extra protein (s) is allocated to the polysaccharide co-polymerase (PCP) and the external membrane polysaccharide export (OPX; formerly OMA) to transport the polymerized components from the periplasm space to the cell membrane requirements [16,17].

Polysaccharides synthesized by the Wzx/Wzy pathway are heteropolymers, and up to four or five kinds of sugar within their chemical structure are considered standard. Xanthan, succinoglycan, and different sphingans are

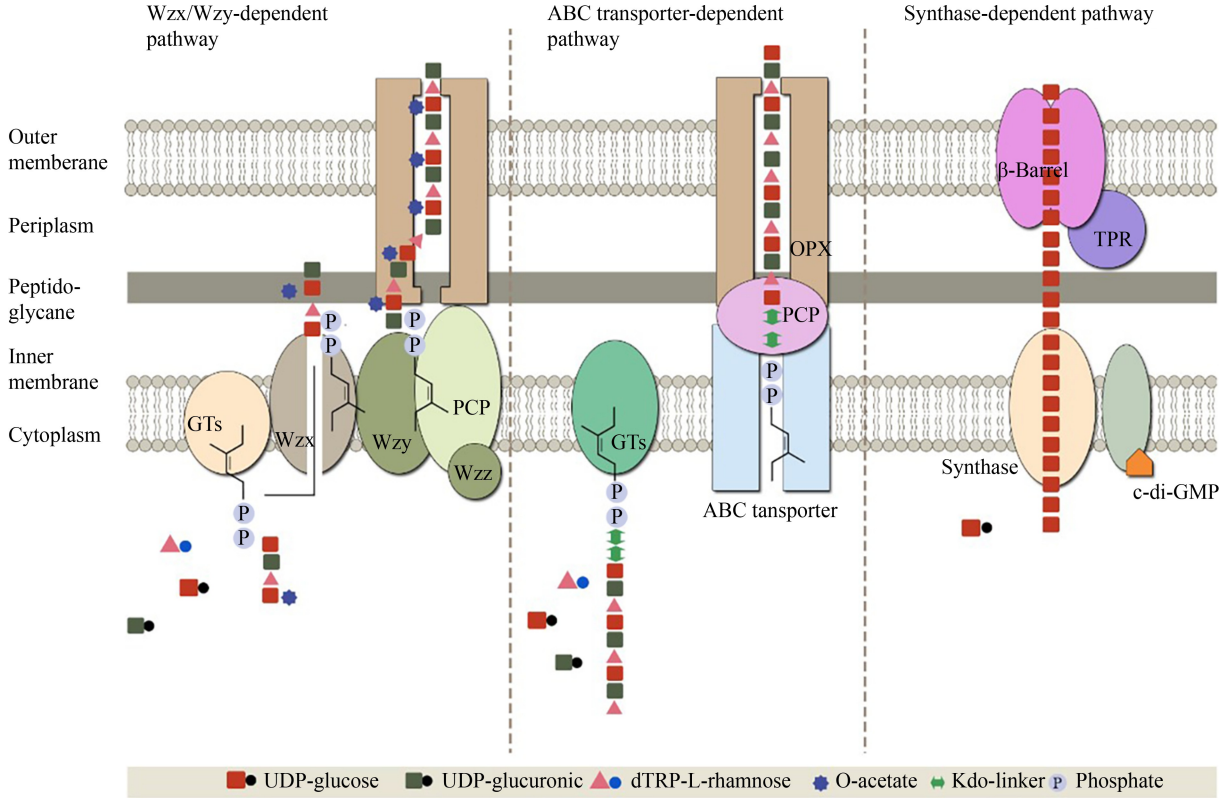


Fig. 1 Three different pathways of EPS biosynthesis. (1) Wzx/Wzy-dependent pathway, (2) ABC transporter–dependent pathway, (3) synthase-dependent pathway. Reproduced with permission from Ref. [3].

examples of polysaccharides synthesized through this pathway [18,19].

ABC transporter-dependent pathway

The ABC transporter-dependent pathway is considered the second biosynthesis method of bacterial EPS polymers [20]. CPSs are polymers synthesized through the ABC pathway attached to the cell surface. As shown in Fig. 1, the GT action in the cytoplasmic side of the internal membrane is required for the ABC transporter-dependent pathway. Polysaccharides synthesized by this pathway can be classified as homopolymers, when only a single GT-containing operon is included, or as heteropolymers, when multiple GT is utilized for the assembly process [20]. The tripartite efflux pump, including the inner membrane-spanning region of ABC transporters, and the periplasmatic proteins of the PCP and OPX family are responsible for translocating the molecules to the cell surface [2,3]. A poly-2-keto-3-deoxyoctulosonic acid (Kdo) linker and phosphatidylglycerol remain are attached to the polymer chain and synthesized by the ABC transporter-dependent pathway. This linker is not detached from the Wzx/Wzy pathway product [14,21].

Synthase-based biosynthesis

The synthase-based pathway is considered the third pathway of EPS biosynthesis. In this pathway, a single synthase protein conducts translocation and polymerization processes (Fig. 1) [22].

Extracellular biosynthesis by sucrase enzymes

Dextran and levan are the two prominent examples of polysaccharides synthesized by dextransucrase enzymes (Table 1) [3]. Fructans (levan) or glucans (dextran) are produced. The monosaccharide unit is transferred to a primer molecule. These polymers may be linear or branched at distinct levels [23].

Extraction of EPSs

The interactions maintaining the EPS components together in the matrix should not be disturbed. This approach is considered the most important issue in the extraction method. The extraction strategy should be chosen given the technique's efficiency in extracting EPSs in high yield. In this regard, several physical and chemical approaches or their combinations are used to extract EPSs. Several physical methods have attempted to evaluate extraction yield, among which centrifugation, sonication, and heating are regarded as the three physical extraction methods studied by Comte *et al*. [24]. Compared with other physical methods, such as centrifugation and sonication, heating results in excessive extraction of protein and polysaccharides content.

Various methods are used for cation exchange resin (CER), such as ethylenediaminetetraacetic acid, CER, and NaOH methods [25,26]. The CER method is considered the most widely used EPS chemical extraction strategy because the resin used for extracting EPSs can be evacuated and recycled without any difficulty. Chemical extraction, together with physical methods, seems to be highly effective. Liu and Fang [25] reported that as combined EPS extraction methods, formaldehyde and ultrasonication are more effective in extracting high proteins, humic acid, carbohydrates, and DNA content than those obtained only from formaldehyde. In another study, Comte *et al*. [24] extracted carbohydrates, high proteins, and DNA content by integrating ultrasonication and CER methods and compared them with those obtained separately [27].

Physical properties of EPSs

The effect of chemical composition, molecular mass, and structure on the physical properties of EPSs can contribute to the definition of their final confirmation. Polysaccharides can form different structures, such as primary (strands), secondary (double or triple helices), or tertiary (a highly complex network). In general, the molecular mass of polysaccharides is approximately 0.5×10^6 Da to 2.0×10^6 Da. Polysaccharides are very long molecules. Some criteria of physical properties, such as hydrophilicity/hydrophobicity, biodegradability, and adsorption characteristics of EPS, are discussed below [28].

EPS has several functional groups; some of them are charged (e.g., hydroxyl, carboxyl, phosphoric, phenolic, and sulfhydryl groups), whereas some are apolar groups (e.g., aromatics, hydrophobic regions in carbohydrates, and aliphatics in proteins) [28]. Molecules represent that EPS is amphoteric regarding the existence of hydrophobic and hydrophilic groups in EPS. The relative proportion of these two groups is identified with the composition and structure of EPS [29].

The connections (1,2)- and (1,6)- in the structure provide the strands more water solubility and flexibility than the connection (1,3)- or (1,4)- in the structure does [30]. The presence of side chains or organic and inorganic components in the EPSs affects their second structure [31]. The charged peripheral macromolecules are the most satisfactory confirmation in the tertiary structure, making highly dense networks or jellification by interacting with water and ions, as seen in alginate and xanthan [6]. These tertiary structure models need salts and are found in low temperatures.

Several enzymes degrade EPS, given that carbohydrates and proteins are regarded as the main components of

Table 1 Overview of the important bacterial EPSs concerning monomer composition, biosynthesis pathway, and applications

EPS	Components	Biosynthesis pathway	Properties	Applications
Alginate	GulA, ManA	Synthase dependent	Gelling capacity, film-forming ability	Food, feed, medicine, research
Cellulose	Glc	Synthase dependent	Not soluble in most solvents and high tensile strength	Food, medicine, acoustics
Colanic acid	Glc, Fuc, GlcA, Gal	Wzx/Wzy dependent	Gelling capacity	N.a.
Curdlan	Glc	Synthase dependent	Gel-forming ability, water insolubility, edible and nontoxic, has biological activity	Food, cosmetics, medicine, construction chemistry
Diutan	Glc, Rha, GlcA,	Wzx/Wzy dependent	High-molecular weight gum	Construction chemistry
Gellan	Glc, Rha, GlcA	Wzx/Wzy dependent	High viscosity and forms thermoreversible gels	Construction chemistry, food, feed
Hyaluronic acid	GlcA, GlcNAc	Synthase dependent	Gelling capacity, nontoxic	Medicine, cosmetics
Succinoglycan	Glc, Gal	Wzx/Wzy dependent	High viscosity and acid stability	Oil industry, cosmetics
Welan	Glc, Rha, GlcA, Man	Wzx/Wzy dependent	Stable in a wide pH range, viscosity retention at high temperature	Construction chemistry
Xanthan	Glc, Man, GlcA	Wzx/Wzy dependent	High viscosity, stable over a wide temperature, pH, and salt concentration ranges	Food, feed, technical applications, oil drilling
Dextran	Glc	Extracellular, dextransucrase	Nonionic, good stability Newtonian, fluid behavior	Medicine, chromatography
Levan	Fru, Glc	Extracellular, levansucrase	Does not swell in water, has low intrinsic viscosity, strong adhesive	Food (prebiotic), feed, medicines, cosmetics, industry, glue

Glc, glucose; Rha, rhamnose; Fuc, fucose; Fru, fructose; Gal, galactose; Man, mannose; GlcA, glucuronic acid; ManA, mannuronic acid; GulA, guluronic acid; GlcNAc, N-acetyl-glucosamine; Pyr, pyruvate; Ace, acetate; Gly, glycerate; Suc, succinate; N.a., not announced.

these polymers. Park and Novak [32] reported that different methods of extracting EPSs affect their biodegradability. For example, the EPS extracted via the CER strategy is oxygen-consuming biodegradable, and even the EPS extracted via sulfide approach is anaerobic biodegradable.

EPSs have different functional groups, such as carboxyl, hydroxyl, sulfhydryl, phenolic, and phosphoric groups; thus, they can bond with heavy metals [33,34]. Furthermore, protein, carbohydrates, and nucleic acids in EPSs can bond with heavy metals [35,36]. These intermolecular interactions between the components of EPSs and divalent cations, including Ca^{2+} and Mg^{2+}, can maintain the EPS structure [37].

Chemical properties of EPSs

Thus far, various types of EPSs with different structures (Fig. 2 and Table 2) and chemical properties have been characterized. The physicochemical properties of the EPSs can influence their behavior and function in the environment. The most applicable EPSs in medicine are discussed as follows.

Dextran

Dextran is an extracellular bacterial polymer made of D-glucopyranose, which displays at least 50% α-(1→6) linkage in the main chain and various branched linkages (α-(1→2), α-(1→3), α-(1→4)) [43]. Dextran can also be isolated from different strains, and the most famous among these strains are *Leuconostoc* strains [43,44]. For instance, the isolated dextran from *L. mesenteroides* B512F has α-(1→6) linkages and α-(1→3) branch linkages in its structure, whereas the extracted dextran from *L. mesenteroides* 1299 is insoluble and has linkage in α-(1→2) and α-(1→3) [45].

Curdlan

Agrobacterium biovar is considered the primary source of curdlan. Other types of polysaccharides, such as succinoglycan and a β-D-glucan, are found in these microorganisms [46,47]. Curdlan is linear and has (1→3)-β-glucan linkage with interchain (1→6) linkages [48].

Alginates

Alginate, the most abundant multifunctional polymer, is usually extracted from the cell walls of a marine organism (*Macrocystis pyrifera*, *Laminaria*, *Ascophyllum nodosum*) with unique physicochemical properties. In addition, it is extracted from the several bacteria strains, such as *Azotobacter* and *Pseudomonas*. These strains attract much attention not only as available resources but also as new efficient tools with high potential in biomedical and pharmaceutical areas, such as using alginate as an adjuvant of antibiotics and a substrate for 3D cell culture and antiviral drugs. The recent development in alginate chemistry is quite remarkable.

Alginate, including 1,4-linked β-D-mannuronic acid (M) and 1,4 α-L-guluronic acid (G) residues, is well known. Alginate can be degraded in our body, intensely

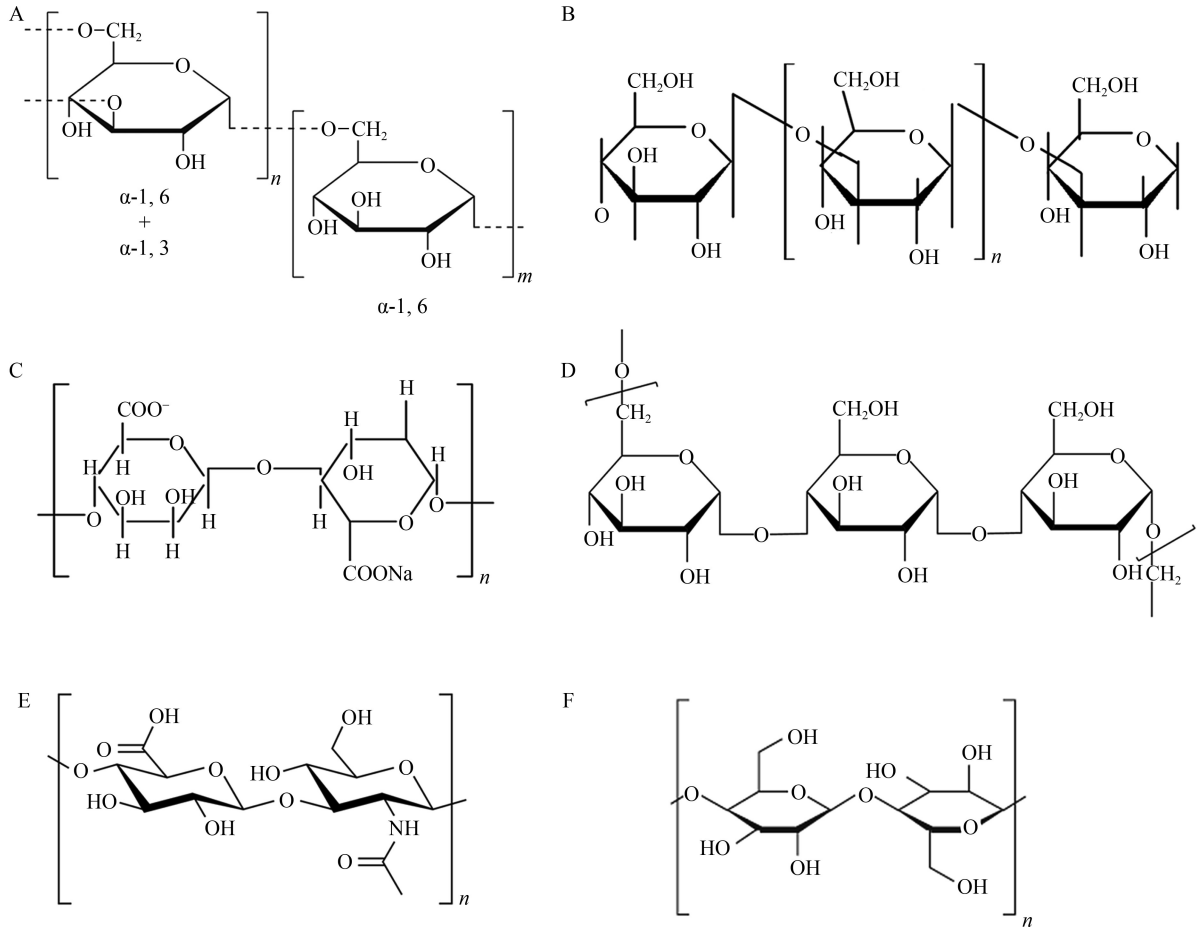


Fig. 2 Chemical structures of certain EPSs. (A) Dextran, (B) curdlan, (C) alginate, (D) pullulan, (E) hyaluronic acid, (F) cellulose.

depending on pH and its molecular weight. This natural polymer may provoke the immune response following degradation. Alginate is available in different molecular weights from 33 000 g/mol to 400 000 g/mol and chemical compositions. Alginate esters commonly form viscous and water-soluble alginate; in particular, alginate acid is a water-soluble type. The polymeric chain in the alginate structure is intensely physically cross-linked via divalent ions (Ca^{2+}).

Furthermore, alginate has free carboxyl groups, making it a suitable mucoadhesive material because of the hydrogen formation and electrostatic bonding within mucin. This property is useful for delivering drugs to the gastrointestinal mucosa. The sodium alginate properties are summarized in Table 3.

Pullulan

Pullulan is an EPS produced by *Pullularia pullulans* (or *Aureobasidium pullulans*) [49]. Commercially available pullulans of different molecular weights ranging from 1000 Da to 2 000 000 Da are made from *A. pullulans* under special culture methods. This polymer is biodegradable, biocompatible, and safe immunologically, and has tumorigenicity [3–6]. A transparent and viscous solution can be produced by dissolving pullulan in water to form a film. These films are heat stable and have anti-static and elastic characteristics [50–52].

Hyaluronic acid

The uronic acid and amino sugar in the disaccharide are d-glucuronic acid and d-N-acetyl-glucosamine, joined together through alternating β-1,4 and β-1,3 glycosidic bonds. Both sugars are spatially linked to glucose that allows all of its bulky groups (hydroxyls, carboxylate moiety, and anomeric carbon on the adjacent sugar) in the β configuration to be placed in sterically favorable equatorial positions. At the same time, all of the small hydrogen atoms occupy slightly sterically favorable axial positions [53]. Thus, the disaccharide structure is very stable energetically. Hyaluronan synthase enzymes are responsible for synthesizing large and linear polymers of the repeating disaccharide structure of hyaluronan by adding glucuronic acid and N-acetylglucosamine to the growing chain utilizing their activated nucleotide sugars (uridine diphosphate (UDP)-glucuronic acid and UDP-N-

Table 2 Microorganism-derived EPSs as functional biopolymers

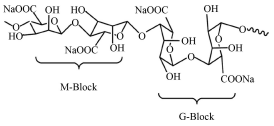
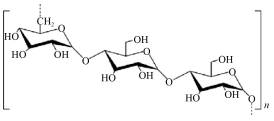
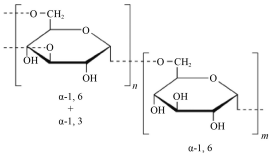
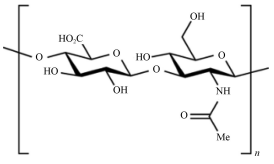
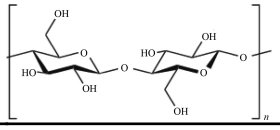
Polymer	Producing microorganism	Chemical structure	Reference
Microbial alginate	*Pseudomonas* and *Azotobacter*		[38]
Pullulan	Fungus *Aureobasidium*, Fungus *Tremella mesenterica*, *Rhodotorula bacarum*, Hypovirulent strains of *Cryphonectria parasitica*, and *C. parasitica*		[39]
Dextran	*Leuconostoc*, *Weissella*, *Lactobacillus*, *Streptococcus*, and *Leuconostoc mesenteroides*		[40]
Hyaluronic acid	*Streptococcus pyogenes*, *Streptococcus uberis*, *Pasteurella multocida*, and *Cryptococcus neoformans*		[41]
Bacterial cellulose	Gram-negative bacteria species, such as *Acetobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, and *Salmonella*, *Alcaligenes*, and Gram-positive bacteria species, such as *Sarcina ventriculi*		[42]

Table 3 Sodium alginate properties suggested by the European Pharmacopeia (Eur. Ph.) and the United States Pharmacopeia (USP) [131]

Parameter	Eur. Ph. 8.0	USP 32-NF 27
The appearance of the solid product	White or pale yellowish-brown powder	n.d.
Content	n.d.	90.8%–106.0% of the dried basis
Packaging and storage	n.d.	Preserved in tight containers
Solubility	Slowly soluble in water, practically insoluble in 96% ethanol	n.d.
Appearance of solution	Not more opalescent than reference formazin suspension in water and not more intensely colored than intensity 6 of the range of reference solutions of the most appropriate color	n.d.
Heavy metals	⩽20 ppm	⩽ 0.004%
Chlorides	⩽1.0%	n.d.
Calcium	⩽1.5%	n.d.
Arsenic	n.d.	⩽ 1.5 ppm
Loss on drying	⩽15.0%	⩽ 15.0%
Total ash	n.d.	18.0%–27.0%
Sulfated ash	30.0%–36.0%	n.d.
Microbial limits	TAMC: ⩽1000 cfu/g TYMC: ⩽100 cfu/g	⩽ 200 cfu/g
Absence of specified microorganisms	*Salmonella* sp., *Escherichia coli*	*Salmonella* sp., *E. coli*

n.d., not determined; TAMC, total aerobic microbial count; TYMC, total yeast/molds count.

acetylglucosamine) as substrates [54]. The mediocre length of a disaccharide is ~1 nm. Thus, a hyaluronan molecule of 10 000 repeats can be extended up to 10 μm when stretched from end to end, and a length becomes equal to human erythrocyte diameter [55].

Cellulose

Cellulose synthesis by the genus *Acetobacter* is a complicated process, which involves (1) the polymerization of one glucose residue into linear β-1,4-glucan chains, (2) the extracellular secretion of these linear

chains, and (3) the assembly and crystallization of the glucan chains into hierarchically composed ribbons [56]. These methods form a 3D jelly-like construct on the surface of a liquid medium [57].

Bacteria-derived phages

Bacteria-derived phages are located in the cytoplasm of bacteria and contain rodlike shapes [58]. Bacteriophages are made up of circular single-stranded DNA encased in roughly 2700 copies of the primary coat protein. Bacteriophages are a varied group of viruses that require bacterial hosts to reproduce. Most have a simple structure, consisting solely of a protein coat (known as the capsid) and encapsulated nucleic acid, with no outer lipid layer. The capsid is made up of some distinct structural proteins that are organized in a geometric design. The presentation of peptides and proteins on viral particles serves as a platform for developing novel smart delivery systems. Filamentous phages and lytic phages are two types of phages discovered. The filamentous phage, which includes the subspecies M13, f1, and fd, seems to be the most used platform for phage display. The genome of filamentous phages is tiny and easy to manipulate genetically. Filamentous phage is an effective nanoscale scaffold for chemical conjugation. In one approach, two fluorophores, one pH sensitive and one pH insensitive, are conjugated to wt-capsid amine groups to create a biosensor for intracellular pH imaging [59].

EPS-based biomaterials: a game-changer for future medicine

Dextran

Dextran hydrogels can be widely used in different biomedical fields, such as contact lenses, cell encapsulation, drug delivery, and tissue engineering [60]. Given the immense commercial implications of functional dextrans, the synthesis and characterization of dextran from a unique strain of *Pediococcus pentosaceus* have been discussed. The structural characteristics of dextran were investigated using Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), and rheological testing. The cytotoxicity of dextran was examined using human cervical cancer (HeLa) cell line to assess its possible biomedical applications [61].

Curdlan

Curdlan indicates its potential in biomedical and pharmaceutical applications, along with its physicochemical properties. It was also utilized as a release suppository for gel encapsulation related to prednisolone, indomethacin, or salbutamol sulfate. Furthermore, compared with other rectal suppository systems that release the drug through dissolving or melting, curdlan drug-impregnated gels made by different heat treatments stayed intact in the rectum, allowing the diffusion of localized drugs and their delivery to the lower rectum [62]. Thus, these curdlan drug–impregnated gels prevent the drug from migrating into the colon; this scenario occurs with other suppository types, avoiding drug uptake into the portal vein and subsequently clearing first-pass in the liver [62]. Furthermore, the kinetics related to zero-order release may be preferred for enabling once-daily administration in the delivery systems of oral drugs. *In vitro* drug release related to curdlan tablets made from spray-dried curdlan/theophylline particles is diffusion controlled, and the rate of drug release is constant over the first 8 h [63]. Curdlan is related to the class of biological response modifiers, which increase or restore normal immune defenses, such as anti-inflammatory, anti-infective, antitumor, and anticoagulant activities [64]. The efficiency of curdlan derivatives relies on molecular weight, chemical structure, and adjustable conformation.

Alginate

Alginate can play a crucial role in controlled-release drug products. Thus, its conventional role in pharmaceutics includes thickening, gel-forming, and stabilizing agents. At present, alginate is most frequently used in oral dosage forms in pharmaceutical applications, but alginate hydrogels as depots for localized drug delivery in the tissue are increasing [65].

Pullulan

In recent years, pullulan has been evaluated for its biomedical use in different aspects, such as gene delivery and targeted drug, wound healing, tissue engineering, and even diagnostic imaging by utilizing quantum dots. It is also considered highly water soluble; however, hydrophobized pullulan is mainly used as drug delivery vehicles for drug delivery. These hydrophobized pullulan molecules can produce colloidally stable nanoparticles upon self-aggregation in water with monodispersity. Pullulan, related to its film-forming features, can entrap biological molecules; these molecules are consistent with increased shelf life because of the properties related to its excellent oxygen barrier [66].

Hyaluronic acid

Hyaluronic acid (HA) is available in the majority of biological fluids and tissues. In clinical medicine, it is used as a diagnostic marker for many diseases, such as cancer, rheumatoid arthritis, and liver pathologies.

Moreover, it is used in supplementing impaired synovial fluid in arthritic patients by using intra-articular injections. It is also used in certain ophthalmological and ontological surgeries, along with cosmetic regeneration and reconstruction of soft tissue [54].

Colanic acid

Based on previous studies, colanic acid synthesis is upregulated in biofilms [67,68]. However, colanic acid is not synthesized in planktonic cells under situations related to normal laboratory growth. Some studies indicated that CPSs function well in pathogenicity [69–71]. In addition, the expression of colanic acid is necessary for creating typical *E. coli* biofilm architecture. However, some studies demonstrated that the expression of colanic acid is not linked to the events related to initial adhesion. The present study emphasized the initial stages of adhesion for 30 min to different surfaces by using uropathogenic *E. coli* strains. An earlier investigation assessed the role of colanic acid in the adhesion of *E. coli* K-12 strains to polyvinyl chloride after increasing the incubation times to 10 h with the surface [67].

EPS-based biomaterials for tissue engineering

In recent years, hydrogels have been evaluated for their use as scaffolds to support and improve tissue regeneration in tissue engineering, as shown in Table 4 [72–74]. Furthermore, hydrogels for soft tissues have attracted much attention because of their designing capacity to match the mechanical features and water content related to these tissues. However, these physical properties are not enough for hydrogels to meet their role in improving tissue growth. Regarding scaffolding, utility, porosity, interconnectivity, and pore size can play a role in regenerating tissue, penetrating cells, and diffusing nutrient diffusion [75,76].

Dextran

Dextran has increasingly become a promising material in tissue engineering. It is a biocompatible, biodegradable, nonimmunogenic, and nonantigenic biopolymer that has been widely used in biomedical applications, such as drug delivery and tissue engineering scaffolds [77]. Scaffold materials composed of dextran are highly durable, improving manipulation and tissue regeneration application [78]. Furthermore, dextran-based hydrogel materials have been utilized as scaffolds in soft tissue and cutaneous tissue engineering. In chronic wounds, dextran can accelerate neovascularization, re-epithelization, and skin regeneration. Unnithan *et al*. performed an experimental demonstration of this effect. They fabricated uniform nanofibers based on polyurethane-dextran loaded with estradiol using the electrospinning method and successfully applied these nanofibers as a wound dressing material [79].

Formerly developed dextran hydrogels show minimal size porosity for cell infiltration to design highly porous scaffolds with a proper size for cellular attachment and penetration. Therefore, they used poly (ethylene glycol) (PEG) because of its liquid–liquid immiscibility property, and dextran was modified to methacrylate form. Some researchers found that the dextran-based constructs, which need interconnected macroporous geometry, are useful in clinical usages [80].

Dextran can be easily extracted from bacteria, and many different dextran-based scaffolds can be developed via the cross-linking process for tissue engineering applications [81]. Some dextran-based scaffolds with the desired porosity are fabricated using the salt-leaching technique. These scaffolds are helpful as a cell and biological molecule carrier (Fig. 3) [82].

The porosity measured by scanning electron microscope (SEM) is approximately 37%; however, it can be improved to more than 50% to increase cell homing if required [83]. The chemical characteristics of dextran-based scaffolds make adding other materials in the bulk of the scaffold possible. A nanoscale composite scaffold consists of polysaccharides, and hydroxyapatite is developed for bone regeneration [80].

In several investigations, dextran-based polysaccharides can grow and proliferate mesenchymal stem cells and endothelial progenitor cells *in vitro*. Marie and colleagues developed a pullulan–dextran–interfacial polyelectrolyte complexation (IPC) fiber composite scaffold incorporated with extracellular matrix protein to enhance its ability to stimulate adherent cell growth. The pullulan–dextran–IPC fiber composite scaffold incorporated with extracellular matrix protein surpasses a basic polysaccharide scaffold in terms of cell adhesion and proliferation [77] (Fig. 4).

Dextran-based scaffolds also are used for cardiac regeneration. Samhita Banerjee and his colleagues [84] identified the potential of a modified dextran-based matrix for cardiac cell encapsulation and its capacity to maintain cell contractile properties. They also reported that this construct can be a promising injectable material for generating bioartificial cardiac tissue [84].

Alginate

Hydrogels are considered cross-linked hydrophilic polymers, comprising a large volume of water without dissolution. In addition, they are regarded as exciting materials for special tissue engineering uses because of their ability to fill the defects related to irregularly shaped tissues, allow for minimally invasive procedures, such as arthroscopic surgeries, and facilitate the inclusion of cells or bioactive agents. Hydrogels can also be produced by

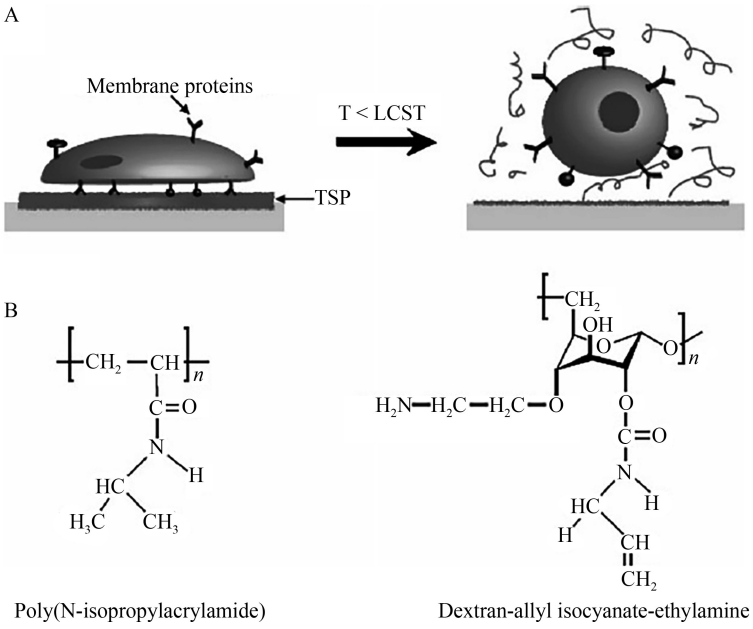


Fig. 3 Cells detach from the dextran-based thermoresponsive membrane without using any enzyme. Dextran-allyl isocyanate-ethylamine is a biodegradable, thermoresponsive polymer presented for detaching cells. Above LCST, dextran-allyl isocyanate-ethylamine is hydrophobic, and cells adhere to it. Below LCST, it becomes hydrophilic and nonadhesive to cells. LCST, lower critical solution temperature; T, temperature; TSP, thermosensitive polymer. Reproduced with permission from Ref. [129].

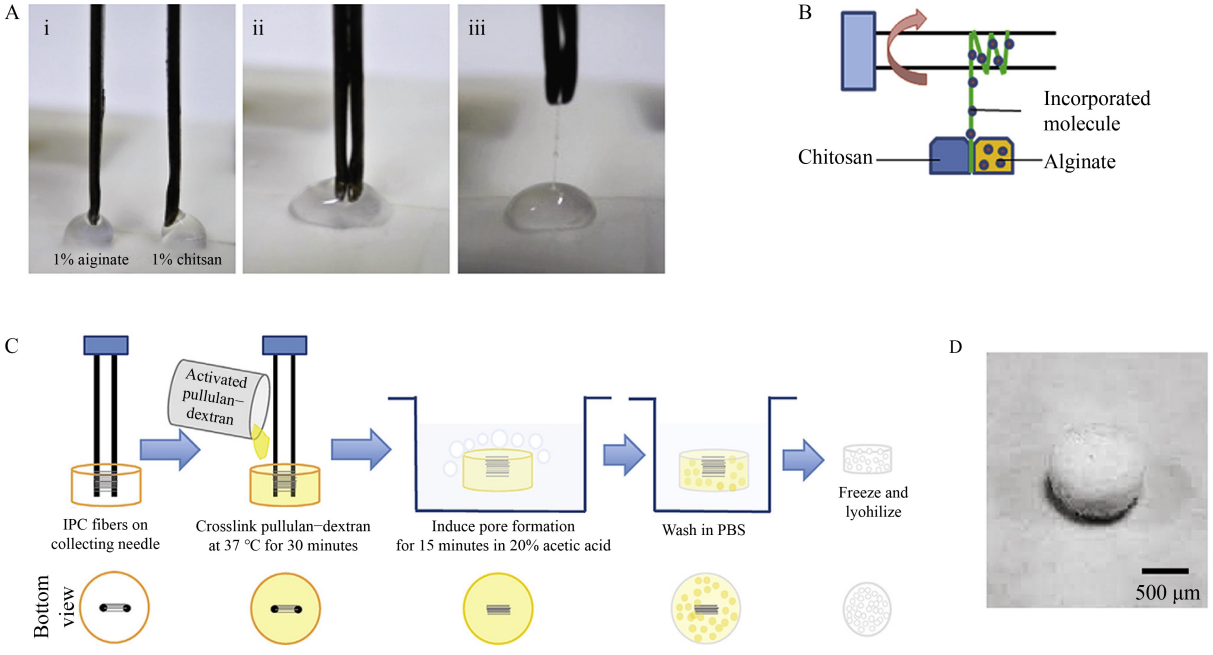


Fig. 4 IPC fibers are created and then incorporated into a polysaccharide scaffold. (A) IPC fibers are formed by pulling oppositely charged polyelectrolytes together. (B) Illustration of an IPC fiber gathered with a pair of collecting needles. (C) The IPC fibers are incorporated into the PD to produce a composite scaffold. (D) Lyophilized polysaccharide scaffold. Reproduced with permission from Ref. [77].

synthetic or natural polymers because the polymers are hydrophilic and can be cross-linked either physically or chemically to avoid dissolving the polymers. Poly (vinyl alcohol) (PVA), PEG, acrylic polymers, and their derivatives are considered the most researched synthetic polymers for tissue engineering. Hyaluronate, alginate, collagen, and their derivatives are naturally derived polymers (macromolecules), which are used most frequently as hydrogel scaffolds in tissue engineering [75].

Alginate has been used as a raw material for the food and drug industry, tissue engineering and regenerative

medicine, and several other applications [72]. For instance, Saman Naghieh *et al*. [85] used different alginate concentrations ranging from 0.5% to 3% to develop an alginate-based scaffold. Based on the results, alginate-based scaffolds can be developed using the indirect-bioprinting process, whereas the construct characteristics are altered by concentrating material. These items offer the primary tool for regulating the properties of alginate-based scaffolds designed using the indirect-bioprinting process. In addition, the cell compatibility of cell-loaded constructs is better than that of bulk gels. Additionally, compared with scaffolds developed with a high alginate concentration, scaffolds developed with a low alginate concentration demonstrate increased cell functionality (Figs. 5 and 6) [85,86].

Cellulose

Microbial cellulose is considered a natural alternative for many biomedical and tissue-engineered applications because of its unique nanostructure and properties. For instance, a microbial cellulose membrane has been successfully utilized as a small-diameter blood vessel replacement and a wound-repair tool for injured skin (Fig. 7). The nonwoven ribbons of microbial cellulose microfibrils are approximately similar to the structure related to native extracellular matrices. Thus, they can play a role as a scaffold for producing many tissue-engineered constructs. Microbial cellulose membranes can be also used in regenerative medicine and wound healing applications, such as periodontal treatments and guided tissue regeneration, because of their unique nanostructure.

Additionally, they can be used to replace the dura mater as a membrane surrounding brain tissue. Microbial cellulose can play the role of scaffold material for regenerating different tissues, representing that it can be considered an outstanding platform technology for biomedicine. Microbial cellulose is used to create many medical devices and consumer products when mass-produced successfully [87,88].

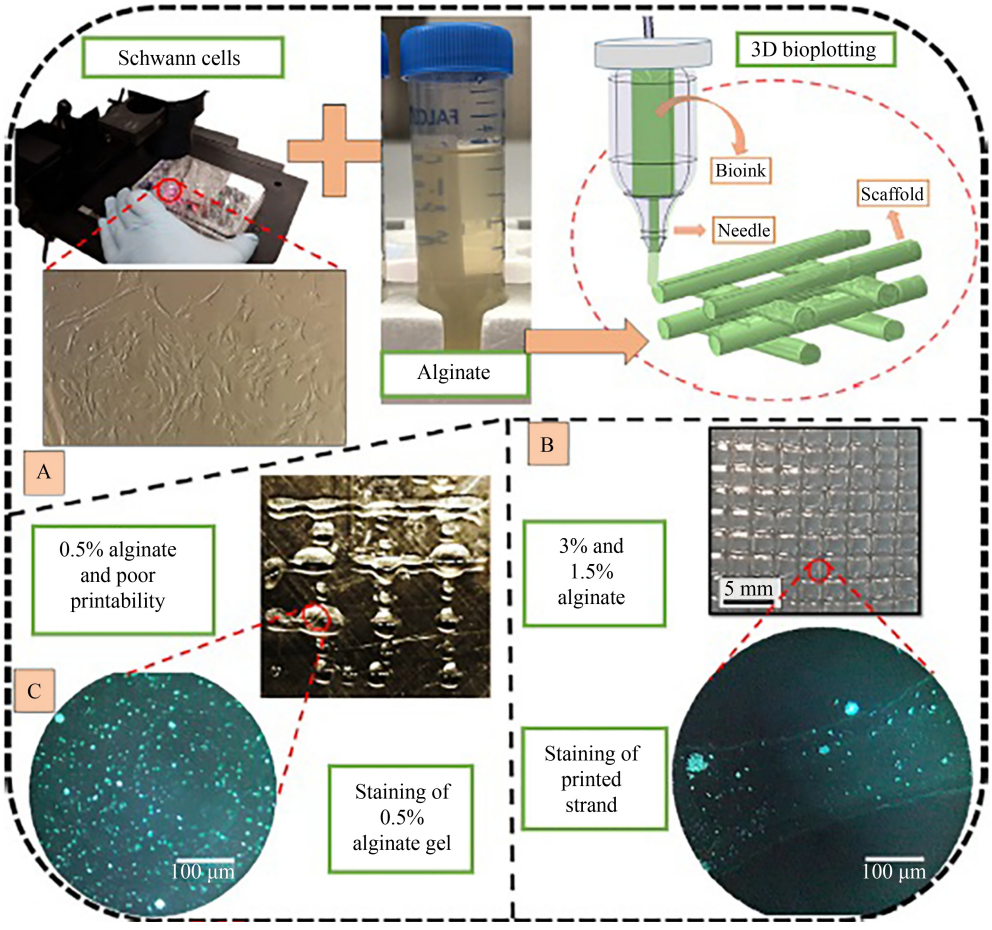


Fig. 5 3D bioplotting of alginate hydrogels. (A) Schwann cells are first mixed with alginate hydrogels and then bioplotted. (B) Cell-loaded alginate constructs and staining conclusion showing one strand. (C) Using a 100 µm needle for printing 0.5% alginate with poor printability and staining of the cell-loaded gel. Reproduced with permission from Ref. [85].

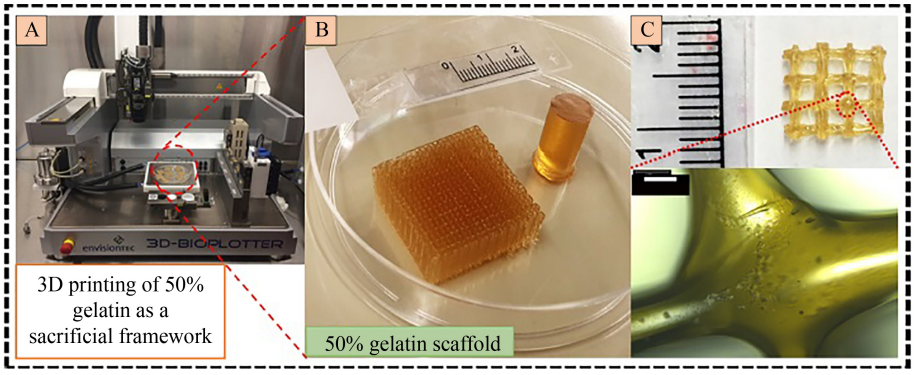


Fig. 6 Indirect biofabrication. (A) 3D bioplotter applied to the development of gelatin scaffold. (B) Gelatin construct and bulk gel models. (C) Gelatin scaffold and a close view of this sacrificial framework. Reproduced with permission from Ref. [85].

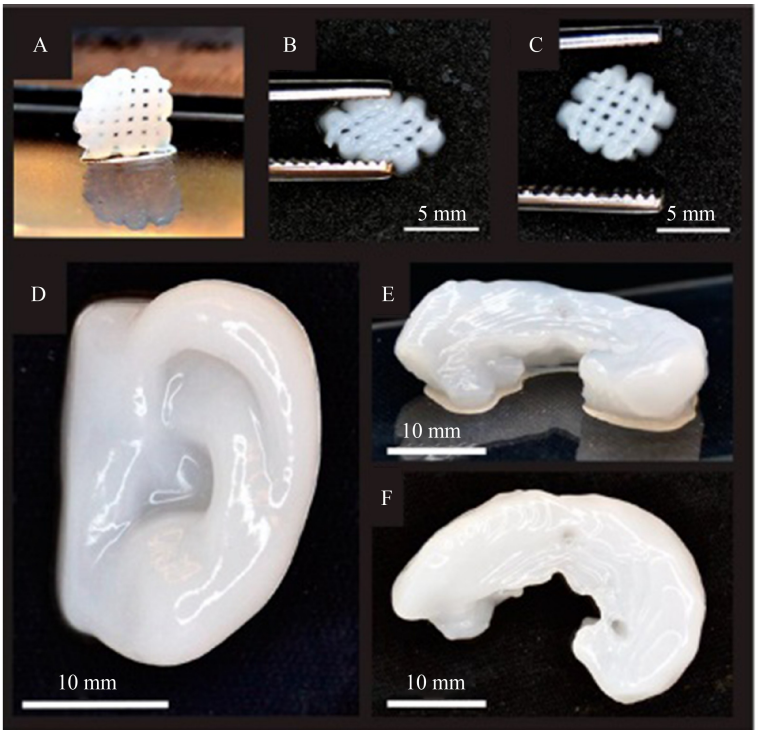


Fig. 7 (A) 3D printed model of nano cellulose–alginate scaffold. (B) The scaffold distorts under compression, but (C) returns to its original form after the pressure is completely removed. (D) Bioprinted human ear. (E and F) Sheep meniscus constructs. Reproduced with permission from Ref. [130].

Pullulan

Numerous applications of pullulan in biomedical engineering and pharmaceutical manufacturing have been reported in the literature [89]. Pullulan and its derivatives can be used to make scaffolds. Pullulan ester and/or pullulan ether are dissolved or dispersed evenly in water or a water–acetone combination to make designed scaffold forms. Pullulan can be formed into hydrogel form combined with synthetic or natural polymers. Iuliana Samoila and colleagues developed composite pullulan (HP) and PVA hydrogels with high hydration and good mechanical characteristics [90]. They claimed that the HP/PVA cryogel can best differentiate cells into adipocytes and is acceptable for biomedical use (Fig. 8).

Furthermore, the aqueous solution viscosity of pullulan is lower than that of any other high polymer used in cosmetics. Leung *et al*. presented biologically tolerable films, including digestible pullulan pills. These digestive pills containing antimicrobial agents, such as thymol, methyl salicylate, eucalyptol, and menthol, are effective killers of plaque-producing gums that cause mouth and dental diseases [91]. Feng Chen and coworkers synthesized injectable hydrogels by enzymatic cross-linking carboxymethyl pullulan-tyramine (CMP-TA) and chondroitin sulfate-tyramine (CS-TA) conjugates under defined

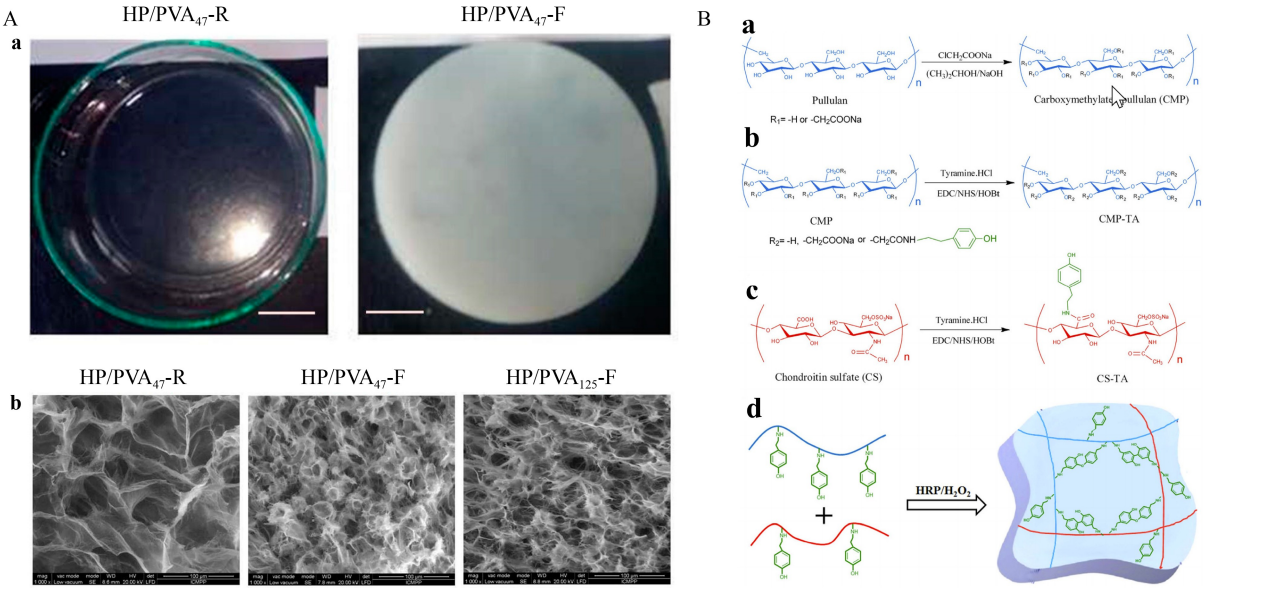


Fig. 8 (A)(a) Photomicrograph of the composite hydrogels based on HP/PVA produced using a freeze–thawing technique. (b) SEM microphotographs of the HP/PVA hydrogels [90]. (B)(a–c) Synthesis of carboxymethyl pullulan, CMP-TA, and CS-TA. (d) Hydrogel formation from CMP-TAs and CS-TAs via HRP-mediated cross-linking in the presence of H_2O_2 [92]. Reproduced with permission from Refs. [90, 92].

circumstances utilizing horseradish peroxidase (HRP) as a catalyst and hydrogen peroxidase (H_2O_2) as an oxidant [92].

Bacteriophages

The filamentous bacteriophage M13, biocompatible to biological systems, has recently been used to form scaffold substrates as a novel polymer. Given the unique structure of the filamentous bacteriophage M13, electrospinning has been suggested as a method of producing them as nanofiber scaffolds [59,93,94]. Yong Cheol Shin and colleagues create a nanofiber substrate for C2C12 myoblasts differentiation and myoblast development using RGD(Arg-Gly-Asp) peptides exhibiting M13 bacteriophages and poly(lactic-co-glycolic acid) (PLGA) hybrid nanostructures. They reported that the phage-modified hybrid scaffold is comparable with naïve tissue matrix and offers several characteristics, including porous structure, cell-adhesive properties, and hydrophilic surface, for engineered constructs [95]. Phage genetic engineering opens up new possibilities for creating functional nanomaterials in regenerative medicine and biomedical engineering. These functional materials have proved their versatility as biological platforms when combined with computational modeling techniques. Thus, phage-based constructs can serve as a viable model system for studying biological cues requiring a high density of functional peptides and accurate material tuning. A group of researchers created a genetically modified M13 phage with DGEA (Asp-Gly-Glu-Ala) peptide on the primary coat proteins shown in a nanofibrous scaffold. Then, scientists physiologically analyzed its influence on cultured cell morphologies [59]. Katarzyna Szot-Karpińska *et al*. developed a filamentous M13 bacteriophage with a point mutation in gene VII (pVII mutant-M13), which selectively binds to the carbon nanofibers (CNFs) [96] (Fig. 9).

Use of flagella for modulating stem cell differentiation

Flagella are iconic bacteria appendages, one of the earliest morphological characters recognized by microbiologists. Flagella deploy protein nanofibers from the bacterial cell body on their own [97]. Given their linear structured nanostructures and ability to be genetically manipulated to exhibit diverse peptide patterns, flagella may be utilized as units to create an extracellular matrix of the bone tissue. Flagella contains collagen-like peptides that may interface with Ca^{2+} ions to create bundles, resulting in extracellular matrix mineralization. The flagella-based matrix appears biocompatible and capable of managing bone marrow mesenchymal stem cell (BMSC) adhesion and proliferation. Furthermore, the flagella-based nanotopography and surface chemistry substantially promoted BMSC osteogenic differentiation. The bioengineered biomimetic nucleation and self-assembly provide a unique approach to bioinspired organized and hierarchical materialism [98].

Dong Li *et al*. showed that bioengineered and mineralized bacterial flagella substrates can efficiently differentiate BMSCs from osteoblast cells. This approach can support BMSC adhesion and proliferation, showing that BMSCs recognize the bioengineered surface peptide or protein motifs [99] (Fig. 10).

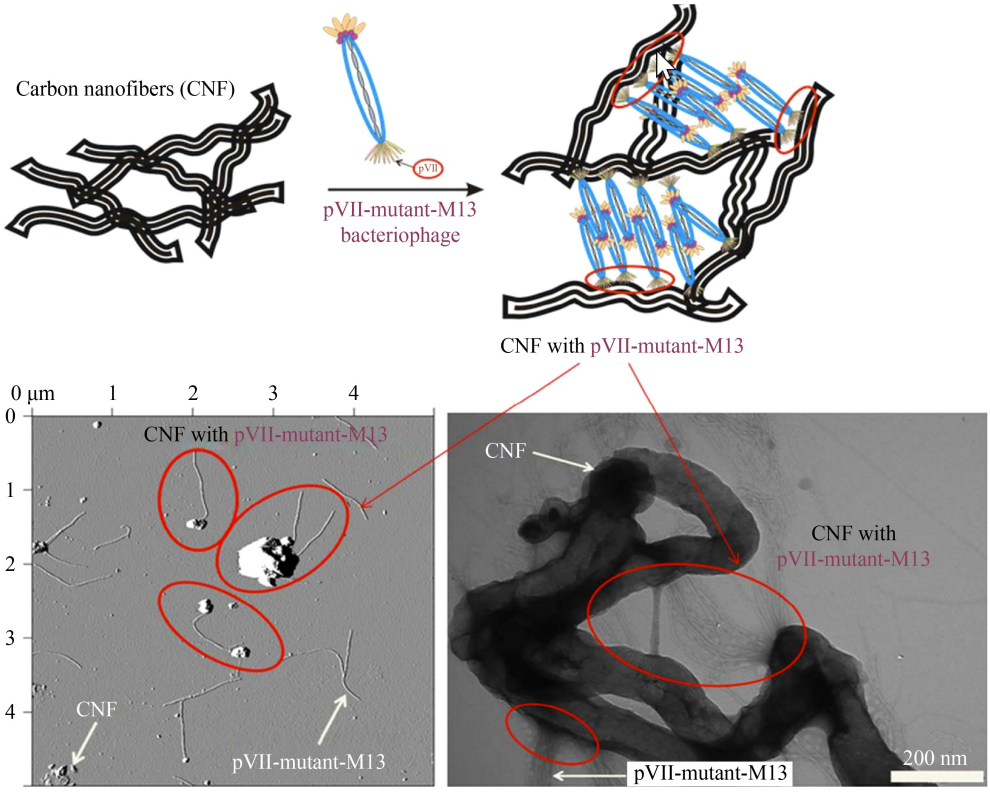


Fig. 9 M13 phage binds CNFs with a point mutation in the pVII protein (pVII mutant-M13). Reproduced with permission from Ref. [96] (Copyright © 2016, American Chemical Society).

Table 4 Some examples for using exopolysaccharides in the field of tissue engineering

EPS' type	Additional material	Scaffold type and fabrication technique	Application	Reference
Alginate	Polyurethane and cobalt	A hybrid cobalt-doped alginate/waterborne polyurethane 3D porous scaffold with nano-topology of a "coral reef-like" rough surface via two-step freeze–drying method	Nerve repair	[100]
	Chitosan	Using the lyophilization method, polypyrrole–alginate (PPy–Alg) mix is combined with chitosan to create polypyrrole–alginate (PPy–Alg) conducting scaffold	Bone tissue engineering	[101]
	Poly (3,4-ethylenedioxythiophene) (PEDOT)	Chemically cross-linked alginate networks are created in the PEDOT/Alg scaffold utilizing adipic acid hydrazide as the crosslinker, and PEDOT is generated *in situ* in the alginate matrix at the same time	A platform for controlling cell behavior	[102]
	Bovine serum albumin and hydroxyapatite nanowires	The freeze–dried hydrogel scaffold is immersed in an aqueous solution of $CaCl_2$ A dual-network bovine serum albumin/sodium alginate with hydroxyapatite nanowires composite (B-S-H) hydrogel scaffold	Cartilage tissue engineering	[103]
	Poly (caprolactone) and CNC nanoparticles	Poly (ε-caprolactone) (PCL)/CaAlg nanofibers are successfully produced using hybrid electrospinning	Wound healing	[104]
	Cardiac ECM and chitosan	Using freeze–drying method, cardiac tissue is prepared by decellularization technique, and the different concentrations of the solubilized ECM and chitosan/alginate are prepared and finally freeze-dried	Cardiac tissue engineering	[105]
Dextran	β-tricalcium phosphate (β-TCP)	A dextran nanocomposite hydrogel By dispersion of β-TCP in the aqueous solution and adding epichlorohydrin (ECH) 12 v/v% as a chemical cross-linking agent	Bone regeneration	[106]
	No	Electrospun dextran nanofibers cross-linked using boric acid	Wound dressing	[107]
	PVA and ciprofloxacin	Core-shell nanofibers are fabricated by emulsion electrospinning from PVA/dextran	As a drug delivery system	[108]
	Cellulose nanocrystal and gelatin	Extrusion-based 3D printing method	This hydrogel is suggested as a 3D bioink for application in tissue repair	[109]

(*continued*)

EPS' type	Additional material	Scaffold type and fabrication technique	Application	Reference
Pullulan	PVA	Aerogel composites are synthesized by impregnating nanofibrous pullulan-PVA scaffolds with hydrophobic silica aerogel	Tissue regeneration	[110]
	Gelatin	Electrospinning	Tissue regeneration	[111]
	Collagen	Hydrogel	Wound healing	[112]
	Polyethyleneglycoldiacrylate (PEGDA) and methacrylic anhydride	Hydrogel; photoinitiator is added to the solution to promote the (meth)acrylic units' polymerization to PulMA and PEGDA through a radical mechanism	Tissue adhesive scaffold with healing properties	[113]
	Poly (hydroxybutyrate-co-hydroxy valerate)/poly(ε-caprolactone)	A multifunctional 3D fibrous scaffold fabricated by a co-electrospinning system	As a drug delivery system for releasing cefuroxime axetil and also for bone regeneration	[114]
HA	Bacterial cellulose (BC)	Cross-linked BC/HA composites BC/HA composites are prepared by solution impregnation, and a chemical cross-linking is established in the BC/HA system by using 1,4-butanediol diglycidyl ether	Wound healing	[115]
	ε-polylysine (EPL) as a natural antimicrobial peptide	Electrospinning	Wound healing	[116]
	Hyperbranched PEG	An injectable hydrogel is reported by combining hyperbranched PEG-based multihydrazide macro-crosslinker and aldehyde-functionalized HA (HA-CHO), with gelatin added to increase the cross-linking density	Tissue regeneration	[117]
	γ-poly (glutamic) acid (γ-PGA) and glycidyl methacrylate as the photo-crosslinker	Digital light processing bio printed human chondrocyte-laden poly (γ-glutamic acid)/HA bio-ink	Cartilage tissue engineering	[118]
Bacterial cellulose	ECH as a cross-linking agent	The hydrogel is prepared by solving carboxymethyl-diethyl amino ethyl cellulose (CM-DEAEC) powder in deionized water and adding a cross-linking agent	Drug delivery	[119]
	Graphene	A novel scaffold for culturing neural stem cells (NSCs), three-dimensional bacterial cellulose−graphene foam, which is prepared via *in situ* bacterial cellulose interfacial polymerization on the skeleton surface of porous graphene foam	Treatment of the neurodegenerative diseases	[120]
	Citric acid as a cross-linking agent	Citric acid cross-linked carboxymethyl cellulose (C3CA) scaffolds are fabricated by a freeze–drying process	Bone tissue engineering application	[121]
	ε-polylysine (ε-PL), mussel-inspired polydopamine (PDA)	BC membranes are coated with PDA by a simple self-polymerization process, followed by treating with different contents of ε-PL	Wound dressing	[122]
	PVA	Composite hydrogel	Substitute for corneal stroma	[123]
Bacteriophages	Carbon	Wild M13 bacteriophage particles are used for CNF electrode modification	Surface modification	[124]
	Polycaprolactone and collagen	Electrospinning	Wound dressing with antibacterial hemostatic dual-function properties	[125]
	Chitosan and alginate	Microencapsulation procedure	Therapeutic phage by oral delivery	[126]
	Alginate and poly ε-caprolactone	A hybrid scaffold consisting of microsized core-sheath struts based on chemically conjugated M13 bacteriophage (phage)/alginate and PCL	Bone tissue regeneration	[127]

Conclusions and future perspectives

EPSs are biologically active components and specialized information carriers in cellular functions. Most of the extracted components have functional groups, including carboxyl, hydroxyl, sulfhydryl, phenolic, and phosphoric groups, which are useful for loading biologically active molecules. These capacities are most crucial for fabricating many devices or drugs for biomedical applications.

As emerging components in tissue regeneration, EPSs promote fundamental steps of tissue hemostasis, such as cell proliferation, migration, and differentiation. In addition, some studies indicated that these components can regulate cell–cell communication processes. Finally, the recent efforts in finding the use of EPSs as regenerative tools and understanding the basic biology of EPSs may signify a prospect for innovative therapeutic approaches.

Some concerns regarding utilizing polymers produced from microorganism-derived EPSs in medicine have been raised. For example, although pullulan has a wide range of practical uses, its cost is a major barrier to its adoption. The cost of pullulan is higher than that of other polysaccharides, such as dextran. Technological advancements or efficient manufacturing varieties, particularly those with low melanin synthesis, may enhance the economics of production, opening up new options for pullulan use [39]. Moreover, the expensive cost of HA synthesis, the necessity to chemically alter HA to extend its *in vivo* half-life, and the requirement to regulate the chemical,

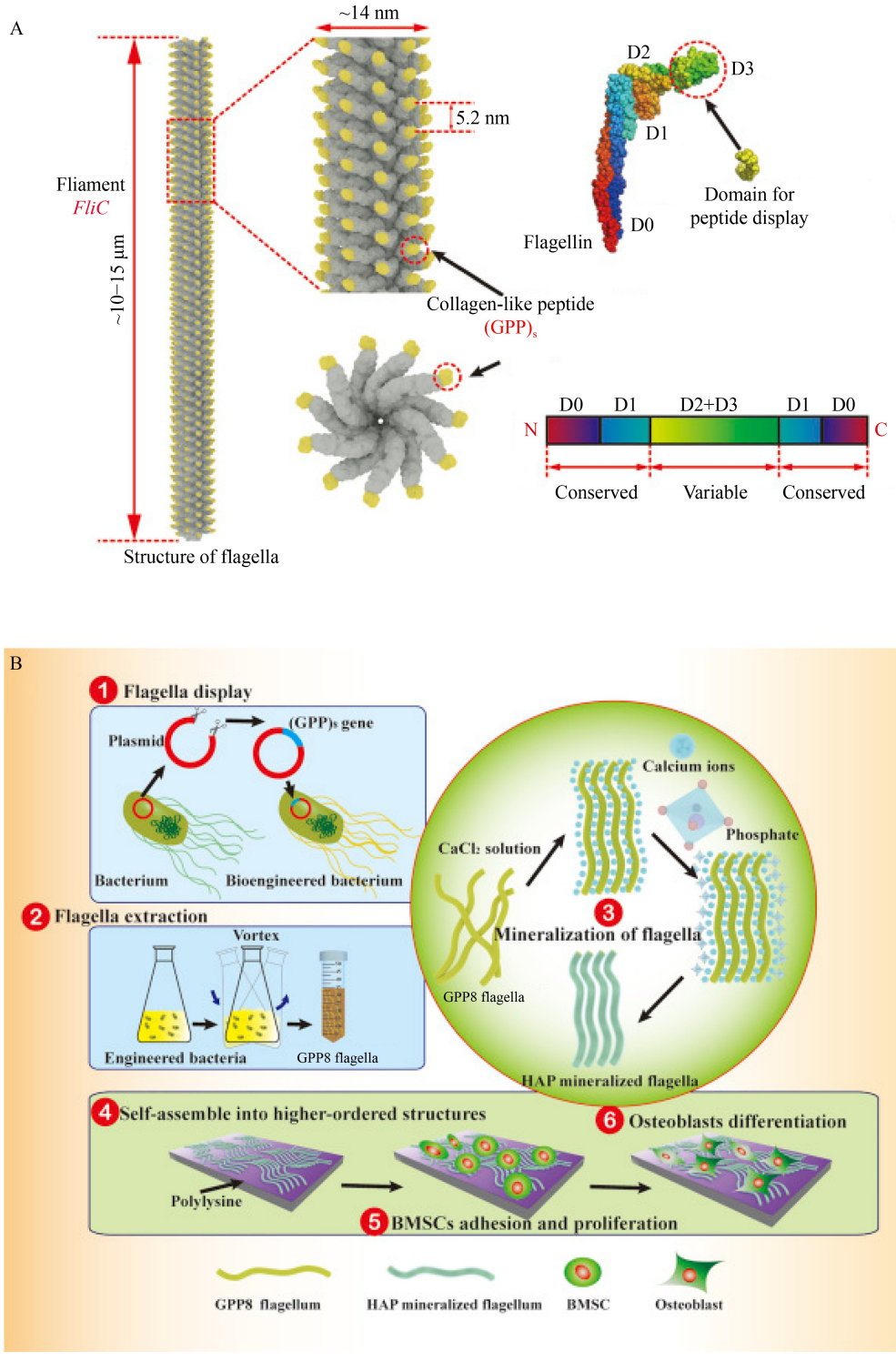


Fig. 10 Display of a collagen-like peptide $(GPP)_8$ on flagella, biomimetic assembly and mineralization of the resultant GPP8 flagella, and BMSCs' differentiation on the GPP8 flagella film. Reproduced with permission from Ref. [99].

physical, and biological characteristics of modified HA and final HA-based product to establish safety are all obstacles to the development of HA-based goods [128].

Additionally, researchers working with bacteriophages and phage-based polymers encounter obstacles, and they must hypothesize strategies to overcome them. Using viruses as building blocks for tissue engineering materials and platforms has excellent features. First, viruses can be chemically conjugated and genetically changed to present biological cues for cellular actions. Second, conjugated

molecules can improve cell–material interactions. Given the benefits of using virus scaffolds in biomedical applications, their use has many issues. For example, these materials lack the necessary mechanical properties for bone tissue synthesis and other applications. The biosafety of nanoparticles in the human body must be considered for future clinical applications.

Overall, the investigation of microorganisms that produce many EPSs and the chemical modification of such materials remains ongoing [78]. Although genetic and metabolic engineering and the exploration of inexpensive fermentation substrates for their production are recommended solutions to enhance the possibility of industrial-scale development and field application of these compounds, research on these subjects is ongoing.

Compliance with ethics guidelines

Naser Amini, Peiman Brouki Milan, Vahid Hosseinpour Sarmadi, Bahareh Derakhshanmehr, Ahmad Hivechi, Fateme Khodaei, Masoud Hamidi, Sara Ashraf, Ghazaleh Larijani, and Alireza Rezapour declare that they have no conflict of interests. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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