The Use of Bacterial Polysaccharides in Bioprinting

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Abstract

Additive manufacturing or 3D printing has spearheaded a revolution in the biomedical sector allowing the rapid prototyping of medical devices. The recent advancements in bioprinting technology are enabling the development of potential new therapeutic options with respect to tissue engineering and regenerative medicines. Bacterial polysaccharides have been shown to be a central component of the inks used in a variety of bioprinting processes influencing their key features such as the mechanical and thermal properties, printability, biocompatibility, and biodegradability. However, the implantation of any foreign structure in the body comes with an increased risk of bacterial infection and immunogenicity. In recent years, this risk is being potentiated by the rise in nosocomial multidrug-resistant bacterial infections. Inks used in bioprinting are being augmented with antimicrobials to mitigate this risk. The applications of bacterial polysaccharide-based bioinks have the potential to act as a key battlefront in the war against antibiotic resistance. This paper reviews the range of bacterial polysaccharides used in bioprinting and discusses the potential of various bioactive polysaccharides to be integrated into these inks.

Keywords: Antimicrobial inks; Bacterial polysaccharides; Bioprinting; Tissue engineering; Biotherapeutics.

1. Introduction: Emergence of bioprinting technology

Additive manufacturing or 3D printing is a rapidly emerging field that is being integrated into a wide variety of areas such as tissue engineering, regenerative medicines, aerospace engineering, and even property construction (Loh et al., 2018; Shi et al., 2019; Zhang et al., 2019). The integration of bioscience and design has enabled the development of 3D biofabrication techniques that provide an assembly scaffold for tissue growth enhancement, and a means of incorporating cells and growth factors to encourage tissue generation (Derakhshanfar et al., 2018). The development of this bioprinting technology has facilitated treatments including wound dressings, bone repair, and the construction of responsive structures such as ear, liver, skin, neural tissues, and heart constructs (Aljohani et al., 2018b; Cornelissen et al., 2017). Several different 3D bioprinting technologies have been developed; the most popular include extrusion printing, droplet (inkjet) printing, laser-assisted printing, and stereolithography (Fig. 1). Extrusion-based printing (EBB) utilizes the mechanical or pneumatic dispensing of the bioink. Compared to other bioprinting technologies, EBB is able to generate the most structurally robust constructs. The viscosity of bioink is a key determining factor in this, as high resolution printing can be achieved with higher viscosity. Increasing the viscosity can also increase the risk of extrusion pressure and shear stress-induced cell mortality, however many functional hydrogels can be printed without increasing the shear stress and extrusion pressure to detrimental levels (Hölzl et al., 2016; Yi et al., 2017). EBB has the advantage of allowing the use of multiple print heads or precursor cartridges to extrude different bioinks increasing the capacity to print more complex human tissues (Kang et al., 2018; J. Li et al., 2016; Mandrycky et al., 2016). Droplet-based bioprinting (DBB) enables accurate ink deposition, with droplets generated by either thermal, piezoelectric, electrostatic, or drop techniques. The bioink droplet is generated by a

short electric pulse to the heating element, forming a bubble to exude the ink droplet. Similarly, a charge is applied to piezo crystals in piezoelectric inkjets, and the resulting vibration forces the ink droplet out. Though fast and low cost, using high-density inks can result in clogged print nozzles which affects the droplet size and precision deposition (Gudapati et al., 2016). This issue has largely been addressed by using acoustic ejectors such as a piezoelectric actuator (Murphy and Atala, 2014). DBB is still widely used to print replicating narrow complex biological structures; although factors such as heat, vibration, and physical stress can induce cell mortality (Yi et al., 2017). Droplet-based bioprinters are relatively cheap and contamination can be easier to manage compared to other bioprinters. The use of multiple print heads can facilitate the production of complex multi-cell constructs (Xu et al., 2013). Laser-assisted bioprinting (LAB) guides an individual cell with a laser pulse from a donor source to a given surface. As the pulse creates a bubble, it forces the cells to transfer. The near UV wavelengths provide the energy to enable nozzle-free, high-resolution precision printing of biological structures, and the use of more viscous bioinks (Trombetta et al., 2017). Stereolithography polymerizes photo-sensitive polymers using a digital mirror projector array for a uniform print. It is one of the most accurate of the solid freeform techniques, printing at a high resolution (100 μm) while maintaining high cell viability (Gou et al., 2014). Table 1 gives a comparative overview of different bioprinters in term of their cost, cell viability, printing speed, supported viscosities, resolution, quality of vertical structure, cell density, representative materials for bioinks, and the reported biomedical applications.. Bioprinting technologies are rapidly evolving yet; the search for suitable bioprinting materials remains a key limiting factor to the integration of these technologies to the biomedical sector.

Table 1. A comparison of inkjet, laser-assisted, extrusion, and stereolithography printers. The table has been modified from (Mandrycky et al., 2016) with permission from Elsevier.

Parameters	Inkjet	Laser-assisted	Extrusion	Stereolithography	Ref.
Cost	Low	High	Moderate	Low	(Orloff et al., 2014; Ozbolat et al., 2014)
Cell viability	>85%	> 95%	~90%	>85%	(Catros et al., 2011; Jovic et al., 2019)
Print speed	Fast (finite printing height)	Medium (limited availability of suitable biomaterials)	Slow (higher volume deposition and reduced overall fabrication time)	Fast	(Derakhshanfar et al., 2018; Murphy and Atala, 2014)
Supported viscosities	3.5 to 12 mPa/s	1 to 300 mPa/s	30 mPa/s to above 6×10^7 mPa/s	No limitation	(Luiz E Bertassoni et al., 2014; Luiz E. Bertassoni et al., 2014; Murphy and

Parameters	Inkjet	Laser-assisted	Extrusion	Stereolithography	Ref.
					Atala, 2014)
Resolution	High	High	Moderate	High	(Ozbolat and Yu, 2013; Z. Wang et al., 2015)
Quality of vertical structure	Poor	Fair	Good	Good	(Z. Wang et al., 2015)
Cell density	Low $<10^6$ cells/mL	Medium $< 10^8$ cells/mL	High (including multicellular spheroids)	Medium $<10^8$ cells/mL	(Aljohani et al., 2018b; Murphy and Atala, 2014)
Representative materials for bioinks	Alginate, PEGDMA, Collagen	Collagen, Matrigel	Alginate, GelMA, Collagen	GelMA, GelMA-PEGDA hybrid hydrogel	(Kolesky et al., 2014; Z. Wang et al., 2015; Yu et al., 2014)
Reported	Tissue engineering	Tissue engineering	Tissue engineering (blood vessel, bone,	Tissue engineering (blood vessel and	(Gou et al., 2014; Huang et

Parameters	Inkjet	Laser-assisted	Extrusion	Stereolithography	Ref.
applications	(blood vessel, bone, cartilage, and neuron)	(blood vessel, bone, skin, and adipose)	cartilage, neuron, muscle, tumor) Controlled release of biomacromolecules Organ-on-a-chip	cartilage) Organ-on-a-chip	al., 2014; Park et al., 2018)

One of the principle issues associated with the insertion of any foreign object such as a bioprinted scaffold into the human body is the increased capacity for bacteria to attach to that object and establish a biofilm. Bacteria growing in biofilms have been shown to be 10−1,000 fold more resistant to antibiotics than their planktonic counterparts (Römling and Balsalobre, 2012). Almost 80% of all hospitals-related bacterial infections involve biofilm formation (Pandin et al., 2017). A biofilm, by definition, is a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (Tshikantwa et al., 2018). The biofilm mode of growth offers protection from various environmental challenges such as the innate and the adaptive immune system as well as offering an increased tolerance to antimicrobial and disinfection agents. The annual cost for biofilm infections in the USA is estimated to be $94 billion, with more than half a million deaths (Römling et al., 2014). The association of bacterial biofilms with non-native implanted structures is one of the leading concerns when it comes to the transition of bioprinting technologies from the benchtop to the clinic, particularly as individuals requiring bioprinted devices or organs may often already have a diminished immune capacity (J. Yue et al., 2015). The ability to mitigate this risk by using bioinks or ink-substrates that have the capacity to prevent bacterial growth or biofilm formation has the potential to be a viable strategy to overcome the risk of infection with device implantation. Hydrogels have emerged as one of the most promising bases for bioprinted inks, and many of the hydrogels used in bioprinting today are composed of bacterial polymers (Gopinathan and Noh, 2018; McCarthy et al., 2019). In this review, we will explore different bacterial-based polysaccharides that can be used as raw materials in bioprinting and highlight the range of bacterial-derived polysaccharides exhibiting antibacterial or anti-biofilm activities that could be used to potentially decrease the likelihood of infection on bioprinted structures. We will

also explore the capacity for these polysaccharides to be impregnated with bioactive compounds to prevent bacterial adhesion and discuss the different areas of medicine that these bacterial polysaccharides can potentially impact. Identifying the right polysaccharide to utilize in a bioink can significantly influence the ultimate success of any fabricated structures using that particular substrate.

2. Bacterial polymers

Bacteria produce four primary classes of polymers: including polysaccharides, polyesters, polyamides, and inorganic polyanhydrides. Many of these polymers are secreted from the cell, with many forming the key matrix components of social structures such as biofilms. With respect to functionality, polysaccharides have demonstrated the highest capacity for integration into currently available printing technologies (Rehm, 2010) as these are stereoregular and can adopt an ordered conformation under given conditions. These polysaccharides can be divided into two groups based on the composition: homopolysaccharides composed of a single type of saccharide, and heteropolysaccharide consisting of multiple different saccharide species. Different sub-groups within these classifications are defined by their chemical nature and different bonds linking the monomers comprising the polymer. These bacterial polymers can be further classified based on functionality such as sorptive (Gupta and Diwan, 2017), nutritive (Flemming and Wingender, 2001), immunostimulatory (McCarthy et al., 2017), redox-active (S. W. Li et al., 2016), communicative (Irie et al., 2017, 2012), and architectural (Powell et al., 2018). These properties need to be considered with respect to downstream functionality particularly when assessing the suitability of a bacterial polysaccharide to be utilized as an ink constituent for bioprinting. The location of a specific polysaccharide may also impact the potential functionality as bacterial polysaccharides can be intracellular, stored in the cytoplasm such as glycogen and

bacterial starch or associated with the cell-surface such as peptidoglycan, lipopolysaccharides, lipooligosaccharides, teichoic acids, lipoteichoic acids, capsular polysaccharides (CPS) and exopolysaccharides (EPS) (Chapot-Chartier, 2014; Mistou et al., 2016; Tytgat and Lebeer, 2014). EPSs and CPSs differ in their degree of attachment to the cell surface: EPSs are loosely associated with the cell surface *via* electrostatic interactions and often form a slime layer, while the CPSs are tightly linked to the cell surface and form a capsule around the cell surface. EPSs serve as natural adhesive and protect the cells from environmental stresses such as extreme pH, temperature, action of antibiotics, and desiccation. EPSs also play an essential role in the host-pathogen interaction and biofilm formation (Limoli et al., 2015; Schmid, 2018; McCarthy et al., 2017). The location of a polysaccharide can also have a significant impact on its purification strategies and cost. For instance, the different methods used for recovery of EPS from the culture broth depend on the characteristics of the microorganisms, the EPS type, and desired purity. A simple drying of culture broth yields a crude product. In contrast, the recovery of high purity EPS requires extensive downstream processing that involves different steps, such as the removal of cells by centrifugation or filtration followed by recovery of polysaccharide from the cell-free supernatant, usually through precipitation. The contaminants are removed through additional purification procedures such as through re-precipitation, deproteinization (chemically or enzymatically), and membrane processes (Sugumaran and V, 2017). The favourable characteristics conferred by bacterial polysaccharides has led to several them becoming routine bioink components. The following sections describe various bacterial polysaccharides routinely used in bioprinting (Table 2).

2.1 Alginate

Alginates are one of the leading polymers used in bioprinting. These are unbranched polysaccharides produced by several algal genera such as *Laminaria, Macrocystis, Ascophyllum, Ecklonia, Lessonia, and Durvillaea*, and bacteria belonging to the *Azotobacter* and *Pseudomonas* genera (Lee and Mooney, 2012). In *Azotobacter*, alginate plays a key role in the formation of desiccation resistant cysts by being the principal component of the capsule-like layer that surrounds these cysts (López-Pliego et al., 2018). In *Pseudomonas* species, alginate is known to be a component of the extracellular matrix (ECM) that surrounds the bacteria in a biofilm. This is particularly relevant in the opportunistic pathogen, *Pseudomonas aeruginosa*, where alginate production has been shown to be a key pathogenicity determinant particularly in the infection of the lungs of cystic fibrosis patients (McCarthy et al., 2014; Ramsey and Wozniak, 2005). The structure of alginate consists of two uronic acid residues, including β-*D*-mannuronic acid (M) and its C5 epimer α-*L*-guluronic acid (G), linked *via* 1,4-glycosidic bonds. The combination and length of these M and G residues vary considerably in nature and can significantly impact the physiochemical properties of alginate, with more G residues are associated with a more rigid polymer (Moradali et al., 2018). Algal-derived alginates have traditionally been used in the biomedical and pharmaceutical sectors for a variety of different purposes including acting as thickeners and stabilizers. This is largely due to the low toxicity and immunogenicity and high-level tractability. These features have put alginates at the forefront of various applications including drug delivery, cell encapsulation, stem cell culture, and tissue engineering scaffolds. Calcium alginate microspheres have been developed as controlled delivery and release systems (Dounighi et al., 2017; Maestrelli et al., 2017; Remminghorst and Rehm, 2006). For example, islets have been encapsulated in poly-L-ornithine (PLO)-coated alginate microbeads (Khanna et al., 2012), methacrylated glycol chitosan-coated alginate capsules (Hillberg et al., 2015), and in a

scalable and conveniently retractable device TRAFFIC (thread reinforced alginate fibre for islets encapsulation) (An et al., 2017). The majority of the bioprinting strategies using alginates thus far use algal-derived alginates which are printable at 2–4% (w/v) and are structurally-stable and solidify rapidly upon contact with a calcium-based crosslinker ($CaCl_2$, $CaSO_4$) and maintain their 3D shape (Aljohani et al., 2018a; Zhang et al., 2019). These structures have been used to generate a range of synthetic tissue constructs comprised of amniotic fluid-derived stem cells, smooth muscle cells, and biliary epithelial cells (Freeman and Kelly, 2017; Hospodiuk et al., 2017; Xu et al., 2013). The engineering of alginate to improve its capacity for utilization in bioprinting is an area of significant research focus (Jia et al., 2014).

Algal alginates encounter several limitations hindering their use in bioinks, such as a lack of homogeneity in G/M residues and fluctuations in molecular weight in accordance with variable environmental conditions (Peteiro, 2018). These have downstream consequences on the capacity of algal-derived alginates to fulfil the specific needs necessary for their further successful uptake by the biomedical sector. Some of these limitations can be overcome by using bacterial-derived alginates, particularly if high-value applications are identified, that can help mitigate the increased cost associated with the bacterial alginate production. The basic viscoelastic properties of bacterial alginates differ from those of algal origin, with bacterial alginates displaying more capacity for modification such as *O*-acetylation, a higher level of monodispersity, and a higher molecular mass (Donati and Paoletti, 2009). The genetic tractability and functional characterization of the alginate biosynthetic pathways in both *Azotobacter* and *Pseudomonas* offer much greater capacity to refine and maximize the amounts of native alginates produced by each. These biosynthetic pathways are largely uncharacterized in algae (Moradali et al., 2018). Both bacterial genera also encode a wide variety of enzymes that

can modify the native alginates such as acetylases that can be used to alter the degree of *O*-acetylation and hence viscosity. These represent tools that can be harnessed to tailor bacterial alginates to specific biomedical needs in a fashion that is not feasible with algal alginates. A greater understanding of the genetic regulatory mechanisms that control the alginate biosynthetic pathways in these bacteria means that they can also be modified to maximize production (Hay et al., 2013).

Alginate has become a popular component of inks used for bioprinting due to its relative inertness, and while a lack of bioactivity is advantageous, it does count against alginate when compared to other bacterial polysaccharides that display dual functionality. It does not support cell adhesion due to its highly hydrate anionic surface and lack of cell binding receptors (Glicklis et al., 2000). To promote cell adhesion for cell culturing and tissue engineering applications, both alginate and alginate-based materials are usually chemically modified by introducing cell adhesive peptides such as Arg-Gly-Asp (RGD) (Llacua et al., 2018), Asp–Gly–Glu–Ala (DGEA) (Alsberg et al., 2001), and Tyr-Ile-Gly-Ser-Arg (YIGSR) (Dhoot et al., 2004), as side chains. RGD is extensively used model adhesion ligand that has complementary integrin receptors (e.g., αvβ3, α5β1) on various cell types (Koo et al., 2002; Llacua et al., 2018). It is chemically coupled to the alginate backbone using water-soluble carbodiimide chemistry (Lee et al., 2008). Alginate modification with YIGSR peptides *via* carbodiimide promoted the adhesion of neural cells (Dhoot et al., 2004). These modified alginate-based materials are widely used in 2D and 3D cell culture and as scaffolds in tissue engineering applications. The relative inertness and non-toxicity of alginate have been extensively evaluated *in vitro* and *in vivo*, it might still be immunogenic. For instance, alginates with high M content are immunogenic and approximately 10 times more potent to induce cytokine production as compared to the alginates with high G content (Otterlei

et al., 1991); however, a study has also reported no immunogenic response by alginate implants (Zimmermann et al., 1992). The immunogenicity of alginates could be due to the impurities present in it, in the form of heavy metals, endotoxins, proteins, and polyphenolic compounds, when obtained from different natural sources (Lee and Mooney, 2012), as studies have reported no immunogenic response in animals to a highly purified alginate obtained through a multi-step extraction procedure (Lee and Lee, 2009). Further, alginate-based inks can be impregnated with compounds that confer bioactivity and functionality. Indeed, several examples have been described where alginate-based inks or microbeads have been loaded with antimicrobials and shown to target *Helicobacter pylori* infection in the stomach (Adebisi et al., 2015; Gattani et al., 2010). This narrow spectrum delivery window has been shown to successfully prevent the pathogen colonization (Alboofetileh et al., 2014; Hay et al., 2013; Osmokrovic et al., 2018; Russo et al., 2008). The functionality of alginate as ink for bioprinting is continuously developing with different crosslinking agents or polymer combinations being identified to tailor the properties of these inks to a given purpose (Madzovska-Malagurski et al., 2016). The capacity for alginate-based inks to be used as a vector for the targeted delivery of antimicrobials or to act as antibiofilm coatings is rapidly developing and these inks may represent a key tool in the efforts to prevent and treat antibiotic-resistant infections.

2.2 Bacterial cellulose

Another common bacterial polymer used in bioprinting is bacterial cellulose (BC). BC is a natural polymer produced by several bacterial genera, such as *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Azotobacter, Sarcina ventriculi, Salmonella, Escherichia, and Rhizobium* (Jung et al., 2007; Ullah et al., 2017) and *Glucanacetobacter hansenii*-based cell-free systems (Khan et al., 2015; Ullah et al., 2016b). It is produced within the microbial cells in the

form of β-1,4-glucan chains which are excreted across the terminal complexes (TCs), present at the outer membrane of bacterial cells, into the culture medium where these crystallize and form high-order structures such as protofibrils, ribbons, and bundles and ultimately form of a hydrogel at the air-medium interface (Endler et al., 2010; Kim et al., 2019) (Fig. 2).

In bacteria, BC plays different functional roles, such as facilitating plant attachment and flocculation. Compared to plant cellulose, which is one of the most abundant polymers on earth, BC has several distinct advantages; including high purity, hydrophilicity, and a finer 3D fibrous structure (Ul-Islam et al., 2019a). Furthermore, it demonstrates a high tensile strength, shear-thinning capacity, flexibility, and chemical stability (Gao et al., 2017, 2016). It is highly porous, non-toxic, and biocompatible allowing not only the attachment and proliferation of different mammalian cells such as pluripotent stem cells (de Oliveira, 2012; Dourado et al., 2017) and human keratinocytes (HaCaT) (Khan et al., 2018a) but also allows the infiltration of cells (osteoblasts MC3T3-E1) into its 3D matrix (Khan et al., 2018b). This has led to BC being explored in a diverse array of biomedical applications; the greatest success has been seen in its use in wound dressings with several commercial BC-based wound dressings available (BioFill™, XCell) and sustained drug delivery applications (Li et al., 2018). Its capacity to form a protective layer over a wound is due to the small pores in the nano-fibrillar network, which prevent bacteria from entering a wound and promote healing (Czaja et al., 2007; Fontana et al., 1990; Sulaeva et al., 2015). Being a hydrogel, BC resembles the natural ECM. Its 3D nanofibrous network structure and morphological similarities with collagen (Lamboni et al., 2019; Lee et al., 2015), make it an attractive material for cell immobilization, cell support, and natural ECM scaffolds (El-Hoseny et al., 2015). Natural ECM contains several signals that are received by cell surface receptors and contribute to cell adhesion and fate by influencing cellular

activities such as proliferation, migration, and differentiation. As pristine BC provides a less adhesive surface to the growth of cells due to the absence of adhesive ligands seen in natural ECM, the immobilization of different ECMs (e.g., collagen, elastin, hyaluronan), growth factors such as basic fibroblast, human epidermal growth factor, and keratinocyte growth factor (Fu et al., 2013), RGD (Llacua et al., 2018), and its compositing with other biocompatible polymers such as gelatin (Khan et al., 2018a) and chitosan (Ul-Islam et al., 2019b), significantly improve its biocompatibility to support the adhesion, proliferation, and migration of cells within its interconnected porous structure (Halib et al., 2019; Martínez Ávila et al., 2016). However, beyond creating a physical barrier, pristine BC lacks innate antibacterial and antifungal properties; this has led to the development of enhancement strategies whereby it is impregnated with different antimicrobials or nanoparticles such as silver (Maneerung et al., 2008), gold (Khan et al., 2018b), zinc oxide (Ul-Islam et al., 2014), and titanium dioxide (Ullah et al., 2016a), as well as cationic peptides (Fürsatz et al., 2018) to improve the anti-infective capacity of BC-based wound dressings (Di et al., 2017; Ul-Islam et al., 2011). In bioprinting, the application of cellulose has been dominated by the generation of ductile films or mats produced through electrospinning, a technique used to produce one-dimensional (1D) fibrous materials (Maria Manzine Costa et al., 2012). The direct use of BC in bioprinting has been limited by its poor solubility in common solvents owing to the presence of regular intra- and inter-molecular hydrogen bonding that stabilizes its reticulate structure. Nevertheless, it is used as a component of bioinks, for example with alginate, where the excellent shear thinning properties of BC are combined with the rapid crosslinking activity of alginate, to print anatomically accurate cartilage structures loaded with human chondrocytes using electromagnetic jet printing technology (Markstedt et al., 2015). One of the most recent methods involves the incorporation of BC

producing strains such as *A. xylinum* into the already established hydrogel-based inks. These inks are then printed over a given surface in a defined geometry and incubated for a defined period. The ink constituents can then be washed out, leaving only a network of nanofibrillated BC (Schaffner et al., 2017).

2.3 Hyaluronic acid

Hyaluronic acid (HA) is a linear polysaccharide composed of β-(1→4) linked *D*-glucuronic acid and *N*-acetyl-β-(1→3) linked *D*-glucosamine. It is commonly found in the ECM of vertebrate epithelial, neural, and connective tissues. HA possesses a wide range of features that make it amenable to bioprinting, such as high viscoelasticity, degradability, and low immunogenicity (Aljohani et al., 2018b). Owing to these features, it has been used in biomedical applications since the 1950s. It is; however, also produced by different bacteria including *Streptococci spp., Pasteurella multocida,* and *Cryptococcus neoformans* where it is believed to play a role in immune evasion, encapsulating the cells to allow them to escape detection from the host's immune system (Sze et al., 2016). Due to the high levels of proteinaceous contamination, time, and cost associated with the extraction of HA from eukaryotic tissues, biotechnological production methods using bacterial or cell-free systems is the preferred method of production. Synthetic biology approaches have been used to express the *Streptococci* HA biosynthetic cluster in industrial bacterial strains such as *Bacillus subtilis*. This organism is capable of being grown in fermenters allowing large-scale production of HA (Widner et al., 2005). Currently, HA is widely used in a variety of biomedical applications such as wound healing, surface coatings, and sustained/targeted release formulations (Moscovici, 2015). Its physical properties and prior use in biomedical applications have led to HA becoming one of the most popular polymers used in bioprinting. It is typically blended with dextran to overcome stability issues that derive from its

high hydrophilicity (Aljohani et al., 2018b; Pescosolido et al., 2011a). Numerous examples have demonstrated how bioprinted scaffolds based on HA can be used to mimic the native ECM, allowing cellular adhesion, growth, and proliferation (Bian et al., 2016a; Ning et al., 2018). Like many of the bioink polymers in general use, HA does not possess any intrinsic antimicrobial properties other than its capacity to impede the passage of bacteria in the pericellular space of eukaryotic tissues. However, it has been doped with gold, silver, copper, and palladium nanoparticles as well as with antimicrobials to prevent bacterial attachment and the colonization of tissue scaffolds (Cárdenas-Triviño et al., 2017; Matsuno et al., 2006). As more HA crosslinking variants are discovered and explored, the capacity to have more control over features such as the gelation process and subsequent degradation kinetics facilitating greater functionality and the eventual development of smart bioinks (Bian et al., 2016a, Bian et al., 2016b). Various strategies to improve the functionality of HA based bioinks have been developed these include the introduction of hydrophobic moieties and crosslinking with various chemical functional groups such as with photo cross-linkable dextran derivatives, hydroxyethyl methacrylate derivatized dextran (Pescosolido et al., 2011b), thiolation and gelatin-modification (Skardal et al., 2010), functionalization of thiolated HA and gelatin (Aleksander Skardal et al., 2010), grafting of poly(lactic-co-glycolic acid) with incorporated bone morphogenesis protein-2 (BMP-2) (Park et al., 2011).

Table 2. Application of different bacterial polysaccharides in 3D printing

Bacterial polysaccharides	Crosslinker/ reinforced material	Printing method	Improved features	Cells type	Applications/ Functions	Ref.
Alginate	Calcium sulfate	Extrusion	Improved mechanical properties (Young's modulus, degradation rate)	Mesenchymal stem cells	Controlled growth factor delivery for differentiation of stem cells	(Freeman and Kelly, 2017)
Bacterial cellulose	Alginate	Stereolithography	Shape and size stability, cell-laden, patient-specific auricular constructs, high cell density, and homogenous distribution	Human nasal chondrocytes	Patient-specific auricular cartilage tissue engineering	(Markstedt et al., 2015)

Bacterial polysaccharides	Crosslinker/ reinforced material	Printing method	Improved features	Cells type	Applications/ Functions	Ref.
	---	Extrusion	Electrical conductivity, low diameter, and improved adhesion, proliferation, and differentiation of cells	Human neuroblastoma cells	Neural tissue engineering	(Kuzmenko et al., 2018)
	Alginate	Extrusion	Improved cell viability, non-toxicity	Human nasoseptal chondrocytes	Organ printing: human ear and sheep meniscus	(Markstedt et al., 2015)
Hyaluronic acid	Poly(ethylene glycol), thiol-HA and gelatin	Extrusion	Improved viability and mimicking the biochemical and mechanical properties of	Tissue-derived decellularized	Artificial liver	(Skardal et al., 2015)

Bacterial polysaccharides	Crosslinker/ reinforced material	Printing method	Improved features	Cells type	Applications/ Functions	Ref.
			native tissue	ECM		
Gellan	Calcium chloride and Dulbecco's Modified Eagle Medium (DMEM)	Hand-held printing (manual)	Peptide-modification, improved cell proliferation, and ECM formation	Primary cortical neurons	Formation of complex and layered structures such as brain-like, cell behavior studies, and treatment of neural disorders	(Lozano et al., 2015)
Dextran	Gelatin	Extrusion	Tunable gelation time and phase separation, simple and spontaneous fixation,	Human dermal fibroblasts	---	(Du et al., 2017)

Bacterial polysaccharides	Crosslinker/ reinforced material	Printing method	Improved features	Cells type	Applications/ Functions	Ref.
			thermal stability	(HDFs)		
Xanthan	Mashed potato	Extrusion	Improved viscosity, storage modulus (G′), and loss modulus (G″)	---	Nutritious food systems	(Z. Liu et al., 2018)
	Vitamin D and wheat starch blends	Extrusion	Improved viscosity, storage modulus (G′), and loss modulus (G″)	---	Fortified 3D printed foods with diversified physical properties	(Azam et al., 2018)

2.4 Gellan

Gellan gum is an anionic extracellular polysaccharide produced by the bacteria *Sphingomonas elodea*. It is composed of repeating units consisting of α-*L*-rhamnose, β-*D*-glucose, and β-*D*-glucoronate. It has been used in a wide variety of applications in the food industry, including as a gelling/stabilizing agent. In the biomedical industry, it has been used in ophthalmic treatments and sustained drug release formulations (Ferris et al., 2013; Posadowska et al., 2016; Yu et al., 2017). It has also been explored in wound dressings; however, it has not enjoyed the success of other bacterial polymers such as BC due to its soft texture and low thermal stability. These issues are for the most part being overcome with the advent of 3D printing technology. Gellan gum has a number of properties that make it amenable to use in inks for bioprinting, including its capacity to be crosslinked by cation concentrations in the low millimolar range, high monodispersity, low immunogenicity, excellent rheological properties, and a high gelling efficiency at 37°C (Ferris et al., 2013; Silva-Correia et al., 2011; Smith et al., 2007). These properties have allowed gellan gum to be used successfully to create scaffolds for bone, fibroblasts, and neural cultures (Lozano et al., 2015; Silva-Correia et al., 2011). One significant disadvantage hampering the further development of gellan gum-based inks, however, is that significant degradation of structural integrity has been observed over time *in vivo*. This is being overcome by the utilization of different crosslinking approaches such as UV photo-crosslinking; however, this requires chemical modification of the polymer to add methyacrylates, but this has not been shown to impact the cytotoxicity of gellan (Silva-Correia et al., 2011). It has also been shown that the degradation properties of gellan gum in the synthetic body fluid can be altered by changing the ratio of surface area per mass, demonstrating that this must be careful consideration when designing scaffolds for *in vivo* use (Yu et al., 2017). Gellan has also been

assessed as part of a polymer blend with alginate where it was shown to improve several features such as shape fidelity, mechanical strength, and cell attachment as opposed to a pure alginate gel (Akkineni et al., 2016). A study reported that the addition of glycerol significantly improved the mechanical properties by overcoming the brittleness caused by the rigid interconnection among the polymeric chains , it also improved the muco-adhesion capacity (Paolicelli et al., 2018). Further, the addition of TiO_2 nanoparticles not only improved the mechanical strength and swelling, but the small shielding effect of TiO_2 prevented the degradation and retained the stability of gellan-TiO_2 film. Further, the gellan-TiO_2 film generated reactive oxygen species (e.g., H_2O_2, $OH^{\bullet}$, and O_2^{-}) at low wavelength ($\leq$ 400 nm) which possess antibacterial activity (Ismail et al., 2019; Ullah et al., 2016a). Similarly, formulations have been blended that contain compounds with antibacterial activity such as zinc- and strontium-loaded glass microparticles (Douglas et al., 2018). This remains an area for potential exploration to improve the transition of 3D printed structures using gellan into the clinic.

2.5 Dextran

Dextran is a neutral polymer with α-(1→6) and α-(1→4) glucopyranosyl linkages produced by several lactic acid-producing bacteria including *Leuconostoc mesenteroides* and *Streptococcus mutans*. It was initially discovered by Louis Pasteur as a fermentation by-product of wine and went on to become one of the first microbial polysaccharides to be used in a clinical setting when it was approved for use as a plasma volume expander in the 1950s (Moscovici, 2015; Pasteur, 1861). It has been used as a key component of hydrogels in burn wound dressings, where it has been shown to promote rapid functional neovascularization and wound healing processes (Sun et al., 2011). Its use in bioprinting; however, has been relatively limited and largely confined to being used as a component of polymer blends. An oxidized form of dextran

has also been used in combination with gelatin to create ink for bioprinting with a tuneable gelation time based on the thermal sensitivity of gelatin and subsequent Schiff-base crosslinking of oxidized dextran (Du et al., 2017). Dextran modified with hydroxyethyl methacrylate (to be made photosensitive), has been used as a blend with HA to overcome its stability issues associated with its high hydrophilicity. By varying the concentration of modified dextran in ink, it was possible to alter key features such as the mechanical properties and degradation time (Pescosolido et al., 2011a). Dextran does not possess any antibacterial activity but has been modified through the addition of aldehyde groups or by blending with bioactive compounds to exhibit antibacterial and anti-biofilm activity, highlighting its potential as a component of antimicrobial inks for bioprinting (Aziz et al., 2012; De Cicco et al., 2014).

2.6 Xanthan

Xanthan is an exopolysaccharide (EPS) produced by the plant pathogen *Xanthomonas campestris* through the aerobic fermentation of glucose or sucrose. It is a heteropolysaccharide composed of glucose, mannose, glucuronic acid, acetate, and pyruvate. It has been used as a food additive for almost 50 years due to its ability to function as a thickener. Due to its long term use as a food additive and biological inertness, much of the focus of the applications of xanthan gum to 3D printing technology has focused on 3D food printing, where its shear-thinning capacity and viscosity at low concentrations are properties that allow it to act as a rheological modifier, improving the 3D printing properties of a given food (Azam et al., 2018; Z. Liu et al., 2018). These properties have also led it to be a component of some hydrogels used for tissue regeneration studies (Elizalde-Peña et al., 2017). Like many of biologically inert bacterial polysaccharides, its functionality has been improved by the incorporation of antifungals and

antibacterial elements allowing the targeted treatment of infections using hydrogel formulations (Silva Santos et al., 2016; Singh et al., 2019).

2.7 Bioactive bacterial polysaccharides

Bacteria are known to produce a diverse range of polysaccharides. The primary use of bacterial polysaccharides in bioprinting is to confer structural properties. However, many have been shown to also have additional bioactivities. Recently the number of bacteria identified that are capable of producing polysaccharides with antibiofilm activity has risen sharply, suggesting this is an under-identified strategy employed by bacteria to secure a favourable environment from the competing species (Table 3) (Bernal and Llamas, 2012; Junter et al., 2016; Rendueles et al., 2013). These polysaccharides typically have broad-spectrum activity against both Gram-positive and Gram-negative pathogens without impacting their growth (Abu Sayem et al., 2014; Bendaoud et al., 2011; He et al., 2010; Jiang et al., 2011; Kanmani et al., 2011; Karwacki et al., 2013; Li et al., 2014; Spanò et al., 2016; Valle et al., 2006; J. Wang et al., 2015). This suggests that the capacity to develop resistance to these antibiofilm polysaccharides is low as compared to traditional antibiotic therapies (Travier et al., 2013).

Several different potential mechanisms of action for these anti-biofilm polysaccharides have been proposed, including biomasking, the disruption of gene expression, the alteration of biotic/abiotic surface properties and the activation of biofilm degrading agents (Junter et al., 2016; Rendueles et al., 2013). r-EPS obtained from *Lactobacillus acidophilus* A4 has been shown to inhibit biofilm formation by downregulating the expression of genes required for chemotaxis and curli formation in enterohemorrhagic *Escherichia coli* (Kim et al., 2009). Significantly, a number of these polysaccharides have been shown to be capable of dispersing the already established biofilms (Jiang et al., 2011; Wu et al., 2016), suggesting the biomedical

implication for such a polysaccharide may not be just prophylactic. Some of these antibiofilm polysaccharides have also been shown to exhibit further biologically relevant activities such as antioxidant activity and metal ion chelation activity as well as possessing features amenable to incorporation into bioinks such as high levels of thermostability, a pseudoplastic rheology, emulsifying activity, and water solubility (Abid et al., 2018; Li et al., 2014, 2015; Sardar et al., 2015; Spanò et al., 2016; Wu et al., 2016).

In comparison to antibiofilm polysaccharides, only a small number of bacteria-derived polysaccharides have been identified that display antibacterial activity (He et al., 2010; J. Liu et al., 2018). Of these, HS-P03, a polysaccharide composed of glucose, mannose, and galactose derived from *Streptomyces virginia* H03 has been shown to be active against both Gram-negative and Gram-positive bacteria. The precise mechanism of action for this polysaccharide is yet to be determined, although it is proposed to disrupt the cytoplasmic membrane and cell wall leading to cell death (He et al., 2010). The capacity for these polysaccharides to be functionally integrated into ink for bioprinting as either bioactive constituents or core conveyors of form is dependent on further investigation of their biophysical properties. This collection of bioactive bacterial polysaccharides is consistently expanding particularly as the likelihood of finding functionally active and biologically relevant polysaccharides is higher among bacteria due to the close proximity that exists in microbial communities and the evolution of antimicrobial and antibiofilm polysaccharides that may offer competitive advantages within these environmental niches. The amenability of many of these polysaccharides to being utilized as a bioink is yet to be determined, but many have the potential to form the starting blocks for bioactive inks.

Table 3. Antibiofilm and antibacterial polysaccharides

Name	Source	Main components	Bioactivity	Additional features	Ref.
EPS1-T14	*Bacillus licheniformis T14*	Fructose, Fucose	Antibiofilm	Soluble in water, non-cytotoxic	(Spanò et al., 2016)
LPS	*Marinobacter litoralis*	Fucose, Xylose, Mannose	Antibiofilm	Membrane-bound, non-toxic	(Sardar et al., 2015)
dLPS	*Vibrio vulnificus*	Glucosamine, galactosamine	Antibiofilm	Activity-dependent on O-antigen, Gram-negative only	(Lee et al., 2016)
TB-PS	*Psuedoalteromonas ulvae strain TC16*	Glucose	Antibiofilm	---	(Brian-Jaisson et al., 2016)
B4-EPS	*Arthrobacter sp. B4*	Galactose	Antibiofilm	Impacts Gram-negatives and Gram-positives. Optimized for low-cost and large-scale production	(Li et al., 2014)
r-EPS	*Lactobacillus acidophilus A4*	Unknown	Antibiofilm	Impacts both Gram-negatives	(Kim et al.,

				and Gram-positives	2009)
EPS-SP1	*Bacillus licheniformis SP1*	Glycerol, Galactose	Antibiofilm	Impacts both Gram-negatives and Gram-positives	(Abu Sayem et al., 2014)
Ps1	*Pseudomonas aeruginosa*	Glucose, Mannose, Rhamnose	Antibiofilm	Only demonstrated for Gram-positives	(Qin et al., 2009)
Pe1	*Pseudomonas aeruginosa*	Galactosamine, Glucosamine	Antibiofilm	Only demonstrated for Gram-positives	(Qin et al., 2009)
EPS-BK6	*Oceonobacillus iheyensis BK6*	Mannose, Glucose Arabinose	Antibiofilm	Demonstrates thermostability, pseudoplastic rheology, and emulsifying activity. Only tested for Gram-positives	(Kavita et al., 2014)
EPS-BMS	*Leuconostoc citreum BMS*	Glucose, Mannose, Fructose	Antibiofilm	High thermostability, active against Gram-positives and negatives, and disrupts established biofilms.	(Abid et al., 2018)

EPS-TMS	*Leuconostoc mesenteroides TMS*	Glucose, Mannose, Fructose	Antibiofilm	High thermostability. Active against Gram-positives and negatives	(Abid et al., 2018)
EPS-DPS	*Pediococcus pentosaceus DPS*	Glucose, Mannose, Fructose	Antibiofilm	High thermostability, active against Gram-positives and negatives	(Abid et al., 2018)
EPS-CM	*Leuconostoc pseudo-messenteroides CM*	Glucose, Mannose, Fructose	Antibiofilm	High thermostability, active against Gram-positives and negatives	(Abid et al., 2018)
PI80 EPS	*Streptococcus phocae PIS0*	Arabinose, Fructose, Galactose	Antibiofilm	Active against Gram-positives and Gram-negatives. Antioxidant activity	(Kanmani et al., 2011)
EPS-YW32	*Lactobacillus plantarum YW32*	Mannose, Fructose, Galactose	Antibiofilm	Active against Gram-positives and negatives	(J. Wang et al., 2015)
EPS-	*Lactobacillus plantarum*	Xylose,	Antibiofilm	Active against Gram-positives	(Liu et al., 2017)

WLPL04	*WLPL04*	Glucose, Galactose		and negatives	
EPS4	*Lactobacillus plantarum EPS4*	Galactose, Ribose, Fructose	Antibiofilm	Active against Gram-positives and negatives. Inhibits cell-surface and cell–cell interaction	(Pradeepa et al., 2016)
EPS-MB2-1	*Lactobacillus helveticus MB2-1*	Galactose, Glucose, Mannose	Antibiofilm	Displays emulsifying, antioxidant, and metal ion chelating activities. Active against Gram-positive and negatives.	(Li et al., 2014)
A101	*Vibrio sp. QY101*	Galacturonic acid, Glucuronic acid, Rhamnose and Glucosamine	Antibiofilm	Active against Gram-positives and negatives	(Jiang et al., 2011)
EPS273	*Pseudomonas stutzeri 273*	Glucosamine,	Antibiofilm	Exhibits antioxidant potential,	(Wu et al., 2016)

		Rhamnose, Glucose, Mannose		inhibits virulence factor production in *P. aeruginosa*, tested in Zebrafish, no cytotoxicity.	
PAM galactan	*Kingella kingae PYKK081*	Galactose	Antibiofilm	Active against Gram-positives, Gram-negatives, and *Candida*	(Bendaoud et al., 2011)
CPS-IA5	*Actinobacillus pleuropneumoniae serotype 5*	Unknown	Antibiofilm	Capsular, thermostable, and active against Gram-positives and negatives. Inhibits cell-to-cell and cell-to-surface interactions	(Karwacki et al., 2013)
CPS	*Klebsiella pneumoniae*	Galactose, Glucose, Rhamnose, Glucuronic acid, and Glucosamine	Antibiofilm	Capsular, thermostable, and active against Gram-positives and negatives	(Dos Santos Goncalves et al., 2014)

Ec300p	*Escherichia coli EC300*	Mannose, Glucose, Galactose, Glucuronic acid	Antibiofilm	Thermostable, Gram-positive only	(Rendueles et al., 2013)
K2 (G2cps)	*Escherichia coli CFT073*	Galactose, Glycerol, Phosphate, Acetate	Antibiofilm	Capsular, active against Gram-positives and negatives	(Valle et al., 2006)
Ec111p	*Escherichia coli Ec111p*	Mannose, Glucose, Galactose, Glucuronic acid	Antibiofilm	Thermostable, Gram-positive only	(Rendueles et al., 2013)
qCurdlan	*Agrobacterium spp.*	Glucose	Antibacterial	Must be quaternized, water-soluble, thermostable, and active against Gram-positives and negatives	(Chen and Liang, 2017)

PS-H03	*Streptomyces virginia H03*	Glucose, Mannose, Galactose	Antibacterial	Thermostable, and active against Gram-positives and negatives	(He et al., 2010)
ECP	*Enterobacter cloacae CMCC45301*	Glucose	Antibacterial	Antibacterial activities mediated through cell membrane damage. Activity only tested on drug-resistant *E. cloacae*	(J. Liu et al., 2018)

3. Limitations to emerging methods

Many of the limitations of current bioprinting procedures are associated with the preparation of bioinks, which usually takes a few days to several weeks and requires complex preparation procedures. For instance, the preparation of multicomponent bioinks includes the development of appropriate materials with desired structural, shear-thinning, and cyto-compatible properties (Ashammakhi et al., 2019). Moreover, limited shelf-life and storage difficulties are major challenges, which compromise the efficacy of printing procedures. For example, most hydrogels of heterogeneous and biomimetic structures are degraded relatively fast and lose their structures in two to three weeks. This issue has been addressed to some extent by introducing reinforcing fibres (Narayanan et al., 2016) or particles (Sawkins et al., 2015; Visser et al., 2015). Further, the shelf-life of bioinks is increased through lyophilization and cryomilling and their subsequent reconstitution before use (Yu et al., 2019). However, the reconstitution of bioinks or their components from the lyophilized state compromises their shelf-life and local working time (Hornick and Rajan, 2015; Murphy and Atala, 2014). Further, the introduction of new features into printers to preserve the newly printed regions, designing of advanced parallel printers, and refining the printing process such as through introduction of continuous liquid interface production (CLIP) (Tumbleston et al., 2015) can help resolve the major issues associated with the limited shelf-life of a bioink. Another major challenge in bioink preparation is defining the balance between the different components of bioink (i.e., materials, cells, and biomolecules). The use of materials with specialized properties, such as smart materials with stimuli-response abilities or shape memory, further complicates the preparation of bioinks.

Another key limitation associated with the current bioprinting technology is the requirements of all hydrogels to be in liquid or semi-liquid state for printing. This indicates that

the viscosity of printable bioink must be controlled according to the requirements of a bioprinter as well as the desired features of the scaffold to be printed. Difficulties can arise when attempting to control the transition from a liquid to a more rigid structure. In general, all bioinks should form quasi-scaffold structures supporting the adhesion and proliferation of cells after printing, which can be achieved by using hydrogel pre-polymer solutions which are photo- or chemical crosslinking polymers (Araujo et al., 2014; Bajaj et al., 2014). A simple printing process requires that the different printed layers remain connected and provide mechanical support to each other during the printing process. However, the introduction of voids in one layer usually results in the collapsing of subsequent layers, thus resulting in a cascade of offset features and deformed geometry of the printed scaffold. The incorporation of sacrificial materials, such as carbohydrate glass (Miller et al., 2012), Pluronic F-127 (Kolesky et al., 2016, 2014), and gelatin microparticles (Hinton et al., 2015) overcome this discrepancy by providing mechanical support to the subsequent layers during the layer-by-layer printing process. This sacrificial material is removed as soon as the desired geometry is attained. This approach has been successfully used in the printing of microelectrochemical system (MEMS) devices (Luiz E Bertassoni et al., 2014); however, this strategy complicates the overall printing process, such as the requirements of using multiple nozzles as well as the post-printing processing of the printed scaffolds. This indicates that the any substance used as sacrificial material should not only provide mechanical support to the printing scaffold but should also be printable under the same experimental conditions as well as non-toxic to the cells.

The limitations associated with the use of bacterial polysaccharides in bioinks are common with the integration of any new polysaccharide into an ink for bioprinting with the aim to improve its existing features such as thermostability, rheology, water solubility,

biocompatibility, and degradative capacity, or impart additional features. Such properties govern the fabrication and stability of bioprinted complex structures. While microbial polysaccharides are being exploited for additive manufacturing technologies, their uptake is still limited. The limited uptake of bacterial polysaccharides as biomaterials is at least partly due to costly production methods, difficulty in scalability, and the availability of cheaper synthetic or plant/algal alternatives. However, the emergence of antibiotic resistance has led to an increased interest in bacterial polysaccharides as potential biomaterials for use in a range of medical applications (wound dressings, tissue regeneration, and bone repair) (Moscovici, 2015; Rendueles et al., 2013). This has been supported by the exponentially growing field of synthetic biology where the polysaccharide synthesizing gene clusters can be inserted into the synthetic scaffolds or workhorse bacterial strains that can optimize the production, reduce the contaminants, and streamline the purification procedures (Widner et al., 2005). Production and engineering of structures composed of bacterial polysaccharide have also been hampered by a lack of suitable technology. This limitation is being eroded by advances in additive manufacturing and the diversity of 3D printing technology, allowing the high speed and high throughput manufacturing of prototypes to test in a biomedical setting. There is an issue, however, with cross-platform integration whereby the specific polysaccharides used in a bioink may only be compatible with specific customized printing facilities. This can hamper the general uptake of these prospective bioinks but also makes it more attractive as a commercial venture given the intellectual property that may be associated with the production procedures.

Although the printing of various simple tissue constructs has been achieved with considerable success, the printing of complex tissue constructs and full-scale organs is still not feasible. This is due to the lack of reliable printing techniques and metabolic complexity of full-

scale organs. A full-scale organ requires a complex and embedded vasculature and mechanically vigorous conduits associated with the host blood circulatory system. Further, the extended time required for printing of large organs risks the viability of cells within the bioink as well as in the first printed regions (Mandrycky et al., 2016). The less efficient and slow assembly of vascular features and high risk of necrosis during the early printed regions further limit the printing of large organs. These issues can be addressed to some extent through the development of high-speed and advanced printers and exploring new combinations of cells and materials with better structural features and compatibility. In response to the limitations of core 3D printing technologies (inkjet/droplet, extrusion, and laser-induced transfer), refinements, modifications, and hybrid models are developing a greater precision and mechanical control of bioprinting parameters. These models include pneumatic valve actuation, drop-on-demand micro-valve bioprinting, and cell sedimentation. These techniques facilitate the printing of stacking cellular monolayers, high output precision, and focused cell seeding for directed tissue growth (Shi et al., 2018). Scaling up is another major challenge for industrialization of 3D printing technology. With 3D bioprinting technologies forecast to reach a value of US$1.9 billion by 2028, more complex technologies such as microfluidics, 2-photon polymerization, and polymeric fibre electro-spinning are advancing the 3D bioprinting application markets (Colosi et al., 2016; Z. Liu et al., 2018; Miri et al., 2019).

The acceptance of 3D printed material by the general public is another major issue. Although the 3D printed constructs are produced from the same microbial polysaccharides commonly used by the people, the 3D printed constructs need to go through comprehensive evaluation prior to their general use in clinic and routine life. Such regulatory issues have delayed the wide applications of bioprinted constructs in clinical applications. To date, the

clinical use of 3D printed constructs is only limited to few sporadic cases. Although the use of 3D implants varies from country to country, wider acceptance and common consensus need to be developed by establishing appropriate regulations by the regulatory bodies to enhance their industrial-scale production and general applications.

4. Future perspectives

The capacity for many bioactive polysaccharides to be incorporated into inks for bioprinting is dependent on further investigation of their biocompatibility and printability. Many of the bacterial polysaccharides that are currently used in bioprinting have been augmented by the addition of antimicrobials (Fürsatz et al., 2018; Matsuno et al., 2006; Sulaeva et al., 2015; K. Yue et al., 2015). However, the possibility of integrating next-generation antimicrobials, that do not actively kill bacteria, but suppress the key virulent mechanisms they use to establish infection, such as the capacity to form a biofilm, is an underexplored area and one that could have the biggest impact in the shortest time frame. Particularly, as many compounds possessing nonbiocidal antibiofilm activity have been identified as phytochemical components of food such as ajoene in garlic and coumarin in cinnamon. This means that they can be fast-tracked through further development as much of the pharmacokinetics are already determined (Gutiérrez-Barranquero et al., 2015; McCarthy and O'Gara, 2015; Reen et al., 2018). They are also effective in doses that are not likely to significantly impact the structural integrity of a given bioink while also reducing the probability of developing resistance as compared to the integration of traditional bactericidal antibiotics.

The key to developing the use of bacterial polysaccharides is identifying high-value applications that can necessitate the further development of bacterial polymers as bioinks and highlight their use in the biomedical sector. Using 3D printing with bacterial polysaccharides,

particularly those with bioactivity, to tackle the emergent threat of antibiotic resistance may be the high-value application needed to drive their development. This is being helped by the discovery of more and more polysaccharides that display antimicrobial properties but could be improved as the potential applications of bacterial polysaccharide-based bioinks has the capacity to act as a key battlefront in the war against antibiotic resistance.

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Fig. 1. Bioprinting Technologies: Extrusion-based bioprinting uses pneumatic and mechanical force to dispense the bioink. The bioink is extruded as a continuous filament by one of three techniques, (a) pneumatic pressure, (b) mechanical piston, and (c) rotating screw. Droplet/inkjet bioprinting enables accurate ink deposition with droplets generated by thermal (d), or piezoelectric (e) techniques. The bioink is generated by a short electric pulse to the heating element, forming a bubble, which exudes the ink droplet onto the substrate. Similarly, a droplet is formed when a charge is applied to piezo crystals in the piezoelectric inkjets, the resulting vibration forces out the ink droplet. Laser-assisted bioprinting deposits an individual cell with a laser pulse from a donor bioink coated source layer. The laser pulse creates a bubble in the energy-absorbing layer, forcing the cells in the donor layer to be deposited to the substrate enabling nozzle-free, high-resolution precision printing with more viscous bioinks. Stereolithography polymerizes photosensitive polymers (resin). An XY digital scanner and mirror array focuses UV light onto the platform. As each surface layer is polymerized, the platform drops allowing the resin to wash over the print. The UV light then polymerizes this new layer. This cycle continues until the object is printed.

Fig. 2. Cellulose Hydrogels: Schematic illustration of (A) synthesis of β-1,4-glucan chains and their excretion from the bacterial cells across the cell wall through TCs, involving the (B) synthesis and aggregation of fibrils, (C) formation of pellicles, (D) movement of pellicle towards the air-medium interface due to density gradient (cell-free system), and (E) and formation of BC sheet at air-medium interface in the form of a (F) hydrogel, which is seen as a (G) reticulated fibrous structure forming a network of cellulose fibres. The figure has been adapted from (Ul-Islam *et al.*, 2015; Kim *et al.*, 2019).

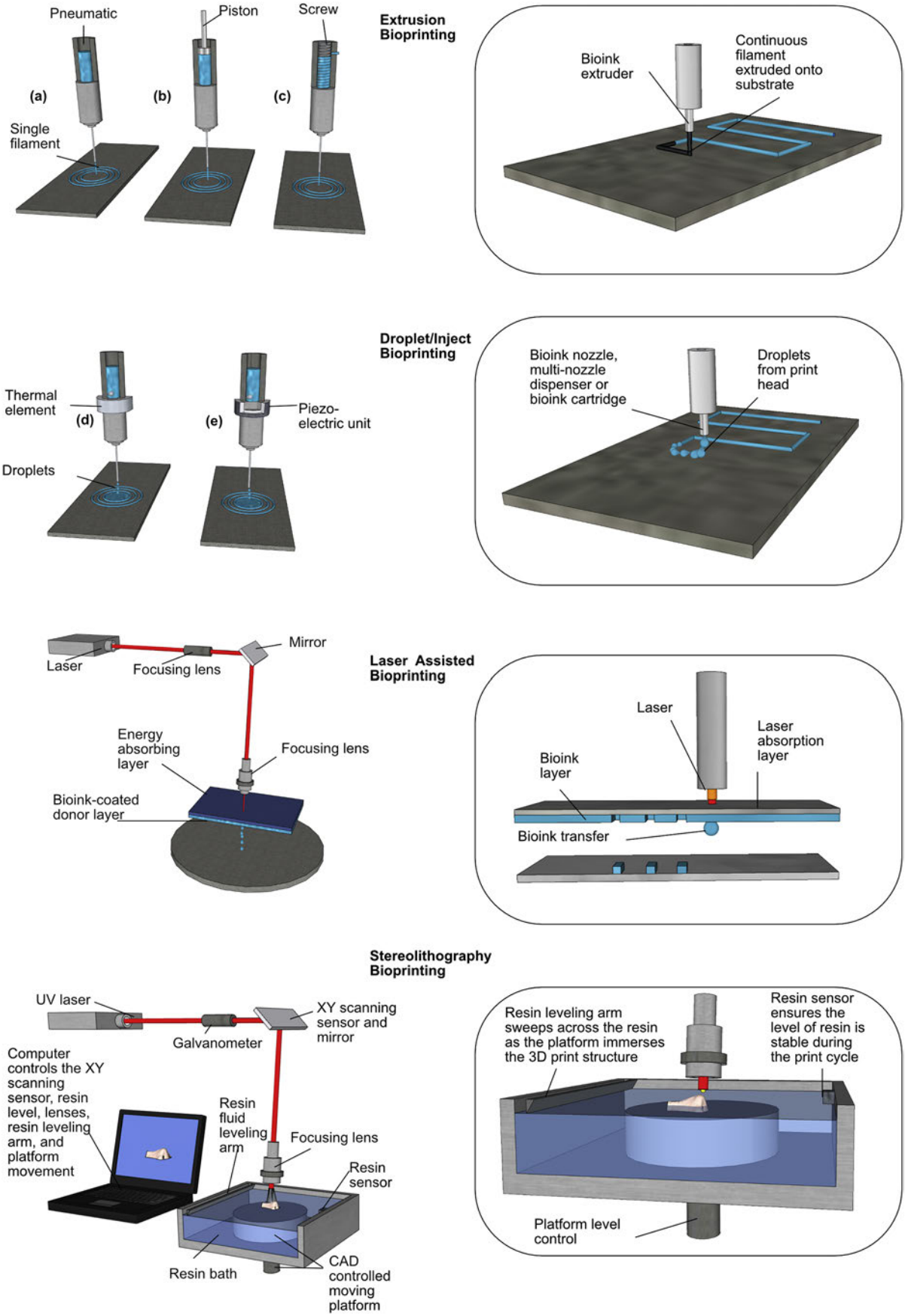
Pneumatic
Piston
Screw
(a)
(b)
(c)
Single filament
Extrusion Bioprinting
Bioink extruder
Continuous filament extruded onto substrate
Thermal element
(d)
(e)
Piezo-electric unit
Droplets
Droplet/Inject Bioprinting
Bioink nozzle, multi-nozzle dispenser or bioink cartridge
Droplets from print head
Laser
Mirror
Focusing lens
Energy absorbing layer
Focusing lens
Bioink-coated donor layer
Laser Assisted Bioprinting
Laser
Laser absorption layer
Bioink layer
Bioink transfer
Stereolithography Bioprinting
UV laser
XY scanning sensor and mirror
Galvanometer
Computer controls the XY scanning sensor, resin level, lenses, resin leveling arm, and platform movement
Resin fluid leveling arm
Focusing lens
Resin sensor
Resin bath
CAD controlled moving platform
Resin leveling arm sweeps across the resin as the platform immerses the 3D print structure
Resin sensor ensures the level of resin is stable during the print cycle
Platform level control

Figure 1

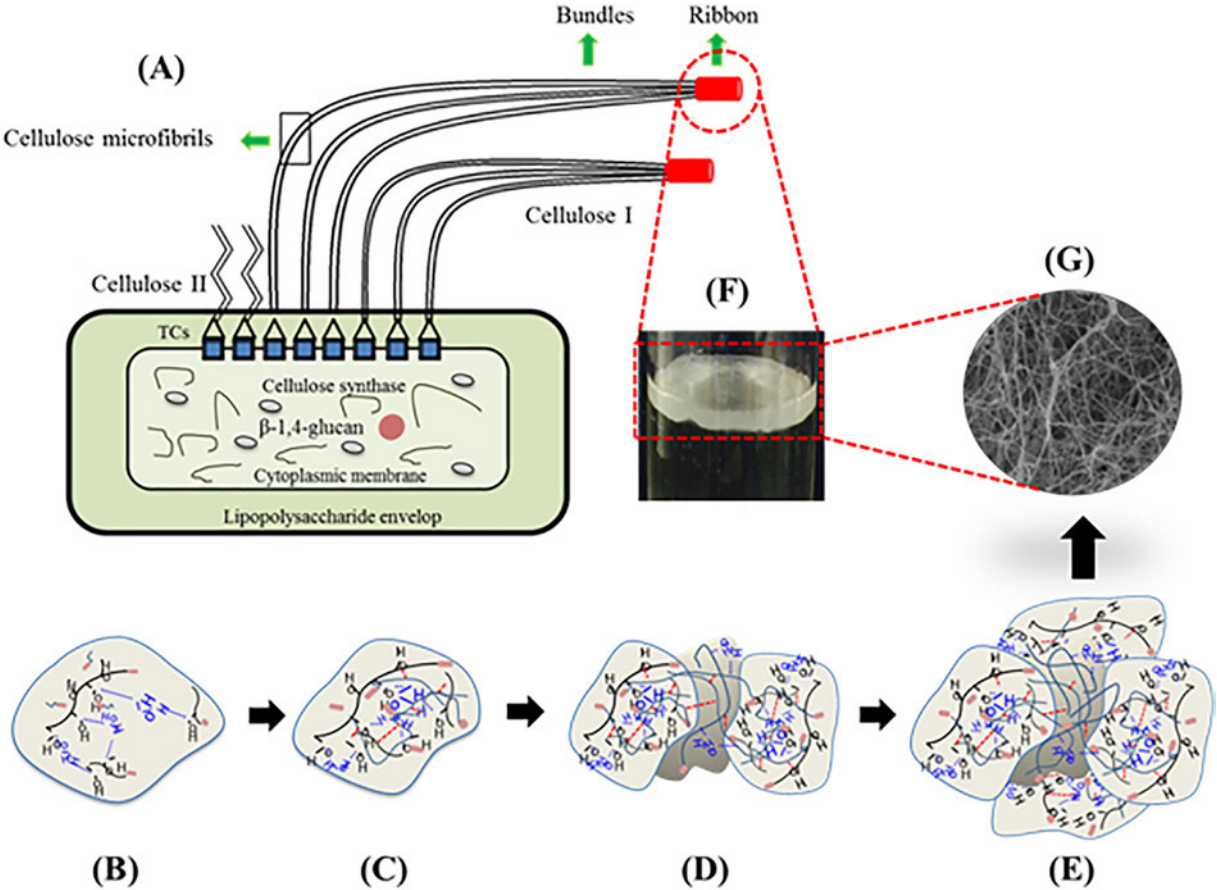
(A)
Bundles
Ribbon
Cellulose microfibrils
Cellulose I
Cellulose II
TCs
Cellulose synthase
β-1,4-glucan
Cytoplasmic membrane
Lipopolysaccharide envelop
(F)
(G)
(B)
(C)
(D)
(E)

Figure 2