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Antimicrobial Inks: The Anti-Infective Applications of Bioprinted Bacterial Polysaccharides

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Bioprinting is a rapidly emerging technology with the potential to transform the biomedical sector. Here, we discuss how a range of bacterial polysaccharides with antibiofilm and antibacterial activity could be used to augment current bioink formulations to improve their biocompatibility and tackle the spread of antibiotic-resistant infections.

Printing Bacteria and Bacterial Polysaccharides

Additive manufacturing or 3D printing has spearheaded a revolution in the biomedical sector for rapidly prototyping medical devices and personalized therapeutic solutions. The field of 3D bioprinting stemmed from the idea of combining 3D printing, which uses layer-by-layer fabrication techniques, with living organisms and biomaterials to produce complex tissues *in vitro*. Bioprinting can be classified into four main process categories: material jetting, laser-assisted printing, stereolithography, and material extrusion. Material jetting is a droplet-based technique that provides a high-throughput method with the ability to precisely control the displacement of biological material. It is compatible with a range of hydrogel formulations, including alginate, agarose, collagen, and fibrinogen [1]. Laser-assisted printing uses laser-induced forward transfer to pattern cells over a given surface. This allows the positioning of small volumes of cell suspensions with high-resolution accuracy. This technology has been used to print a range of cell types, including embryonic stem cells, with a limited impact on cell viability. Stereolithography is a modified form of laser-assisted printing that uses an energy source, usually through laser curing or ultraviolet (UV) light to selectively initiate the polymerization process within a vat containing the photosensitive polymer, such as polyethylene glycol–diacrylate (PEGDA) and gelatin methacryloy [2]. Material extrusion is the most commonly used method of bioprinting. It utilizes physical forces, such as pneumatic pressure, to force a bioink through an extrusion nozzle and deposit it on a surface substrate in a coordinated fashion. Applications, including bone, tendon, skin, cardiovascular, and other types of tissue engineering, can be realized using material extrusion processes. In addition, extrusion processes enable adjustable pressure settings to accommodate the processing of materials with a range of viscosities [2]. Such recent advances in bioprinting have significantly affected the development of potentially new applications for tissue engineering and regenerative medicine (Figure 1).

Bacterial polysaccharides have emerged as a key component of many of the inks used in bioprinting [3]. These bacterial polysaccharides can influence key features, such as the mechanical and thermal properties, printability, biocompatibility, and biodegradability. However, implanting any foreign structure in the body comes with an increased risk of bacterial infection and, in particular, bacterial colonization of the implant itself [4]. Pathogenic bacteria can form communities called biofilms on these implanted structures and, when growing in a biofilm, bacteria are more tolerant to the rigors of the host immune system and antimicrobial therapy. Most hospital-related bacterial infections involve biofilm formation, with bacteria attaching to implanted foreign objects, such as prosthetic joints, dental implants, catheters, or intravenous lines, being a leading cause of morbidity [4]. Integrating bacterial polysaccharides with native anti-infective properties into bioink formulations can reduce the risk of infection and have a role in removing a key barrier to the further uptake of 3D bioprinting technology within the biomedical sector. Anti-infective polysaccharides can also ease some of the pressure on the healthcare system caused by antibiotic-resistant infections.

Bioactive Bacterial Polysaccharides

Bacteria are rich reservoirs for polysaccharides and, while the primary use of bacterial polysaccharides in bioprinting is to confer structural properties [3], many have been shown to have secondary functionalities. An increasingly diverse array of bacterial polysaccharides has been identified that display antibiofilm activity both *in vitro* and *in vivo*. The functional capacity of these polysaccharides to inhibit bacterial adhesion and subsequent biofilm formation has been proposed to be a key competitive strategy to allow a producer species to occupy a given environmental niche [5].

The structural variety seen in these antibiofilm polysaccharides is diverse, ranging from monosaccharide to heteropolysaccahride polymers, with no consistent feature linked to antibiofilm activity. Both exopolysaccharides and capsular polysaccharides have been identified with antibiofilm activity [6]. Most antibiofilm polysaccharides identified so far have broad-spectrum activity against both clinically relevant Gram-positive and Gram-negative pathogens. Critically, this activity is mediated without impacting growth, ruling this out as a mechanism for their antibiofilm properties. Potential mechanisms of action include biomasking, signal disruption, gene expression disruption,

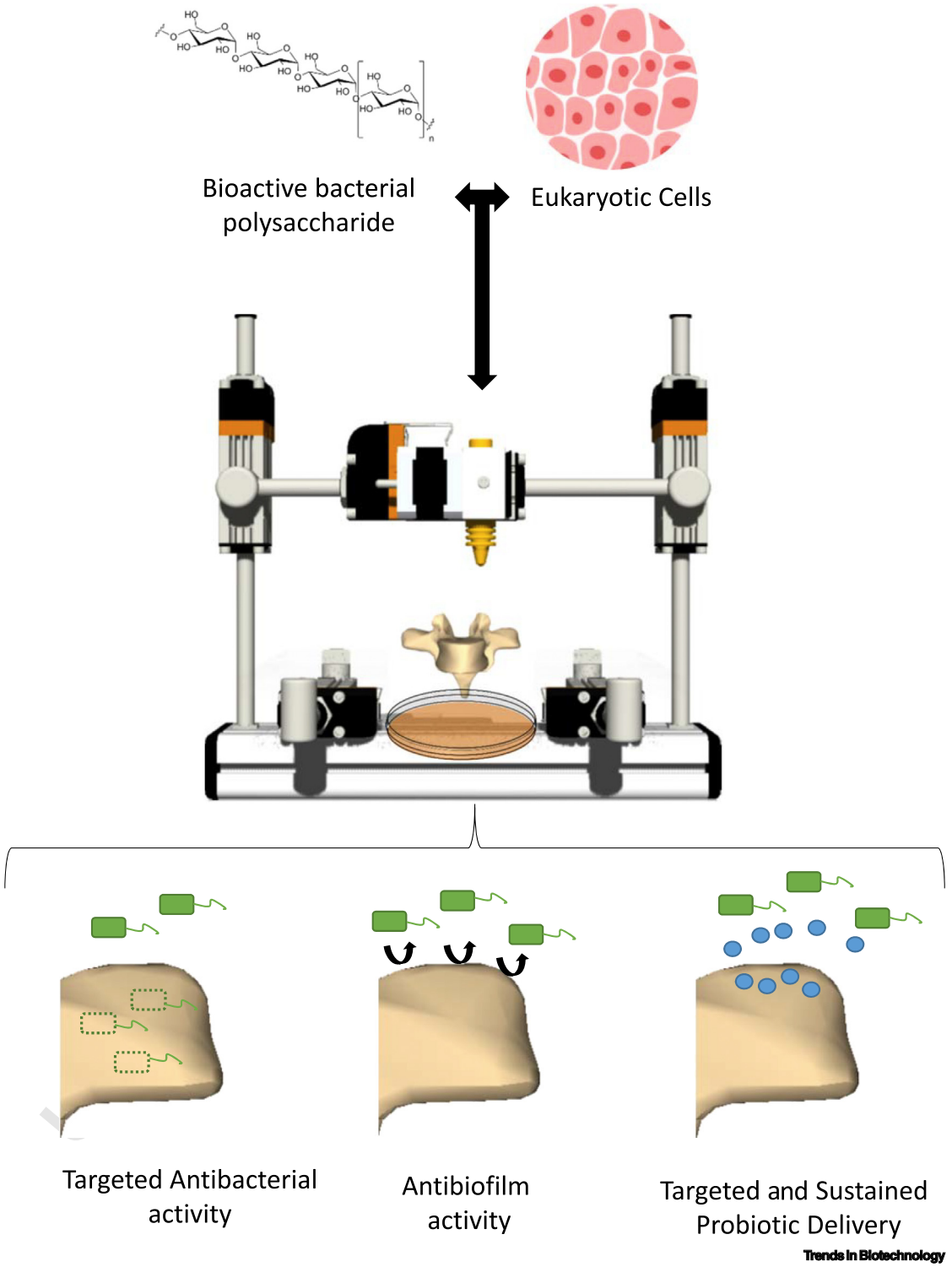

Figure 1. Role of Bioactive Bacterial Polysaccharides in Bioprinting. Functional bacterial polysaccharides can be integrated into current bioink formations to confer antibiofilm or antibacterial activities to 3D bioprinted structures, such as bioprinted bone grafts or prosthetic implants. Integrating these bioactive polysaccharides can reduce the probability of implant reject by preventing bacterial attachment and biofilm formation. Depending on the proposed implantation site, integrating probiotic Q1 bacteria into bioprinted structures can facilitate targeted probiotic delivery.

and alteration of biotic/abiotic surface properties [5]. Biomasking is the ability of a bacterial polysaccharide to bind to and occlude native bacterial lectins or sugar-binding proteins that are necessary for biofilm initiation. Indeed, in antibiofilm polysaccharides rich in fucose and fructose, such as EPS1-T14, produced by a marine thermophilic species of *Bacillus*, biomasking may be a potential mechanism of action because these are known inhibitors of surface lectins [7]. Significantly, several bacterial polysaccharides can disperse already established biofilms. This includes a range of glucose-rich polysaccharides secreted by food-borne lactic acid bacteria and EPS273, a polysaccharide secreted by a marine isolate of *Pseudomonas stutzeri*. The underlying mechanism of action of this biofilm dispersal activity remains to be uncovered [8,9]. However, this suggests that the biomedical applications for such a polysaccharide are not purely prophylactic but could be used to control and disperse established biofilm-associated infections.

Compared with antibiofilm polysaccharides, only a few bacterially derived polysaccharides display antibacterial activity [9,10]. ECP, a polysaccharide derived from *Enterobacter cloacae*, was recently shown to exhibit antibacterial activity against a multidrug-resistant isolate of *E. cloacae*. While the precise mechanism of action of this polysaccharide remains unclear, it significantly damages the cell membrane [10]. Some of these antibiofilm and antibacterial polysaccharides exhibit further biologically relevant activities, such as antioxidant activity and metal ion chelation activity [8]. Of the anti-infective polysaccharides identified to date, many can also be incorporated into bioinks because of their high levels of thermostability, pseudoplastic rheology, emulsifying activity, and water solubility [3–5]. Critically, most of these anti-infective polysaccharides retain their eukaryotic biological inertness and are considered noncytotoxic [7,8]. Given that antibiofilm polysaccharides are nonbiocidal, the capacity for evolved resistance to their activity is significantly diminished. Indeed, in *Escherichia coli,* resistance to nonbiocidal antibiofilm polysaccharides is rare and requires numerous mutations that significantly alter the surface physiochemical properties of the bacteria [11]. However, the potential for resistance to develop to antibacterial polysaccharides has yet to be explored.

Limitations

The limited uptake of bacterial polysaccharides as biomaterials is due, at least partly, to costly production methods, difficulty in scalability, and the availability of cheaper synthetic or plant/algal alternatives. However, the emergence of bioprinting has led to an increased interest in bacterial polysaccharides as potential biomaterials for use in a range of medical applications (e.g., wound dressings, tissue regeneration, and bone repair). The capacity for both antibiofilm and antibacterial polysaccharides to be functionally integrated into ink for bioprinting to treat and prevent infection clearly depends on further investigating their biophysical properties. However, they do represent a diverse panel of anti-infective agents that can be used to augment the biocompatibility of traditional bioinks. Rapid advancements in synthetic biology can be utilized to overcome the scalability and production cost issues, whereby the bioactive polysaccharide-synthesising gene clusters can be inserted into synthetic genetic scaffolds to optimize production in workhorse bacteria. This synthetic biology approach may also overcome the issue of minor variations or polysaccharide modifications that can occur in native strains, leading to a loss of homogeneity and potentially bioactivity. The bacteria producing these anti-infective polysaccharides could also be functionally integrated into the bioink itself. Similar methodologies have been used to functionalize a bioink by integrating strains of bacteria capable of degrading pollutants or producing cellulose into already established bioink formulations. These inks can then printed over a given surface in a bespoke geometry and incubated for a defined period to achieve a desired outcome, such as bioremediation or the formation of a cellulose-based synthetic skin scaffold [12].

Future Directions

The advent of 4D bioprinting, where the added fourth dimension is the capacity to alter the shape of a 3D printed structure over time or exposure to specific stimuli, also has the potential to transform bioprinting and to have a key role in tackling bacterial infections in the future. Hydrogels have already been developed that have shape-morphing capacity [13]. This technology could be used to create programmable wound dressings comprising antibiofilm polysaccharides that release antimicrobials upon exposure to the molecular determinants associated with a specific pathogen. However, for this to be implemented, a new mathematical modeling approach is necessary to strategically control the sequence of stimulus to act on the stimulus-responsive material and, consequentially, for targeted drug delivery [14]. 3D printed bioinks could also be used as vectors to influence the microbiome. Constructing scaffolds or seeder population reservoirs that can be implanted into locations such as the gut during procedures such as bariatric surgery might pave the way for intelligent microbiota delivery systems. Bioinks need to be regarded not only as a vehicle for cells, but also as being equally important to the cells themselves in terms of biological impact; the drive to use inert polysaccharides will be superseded by the need for polysaccharides with additional bioactivities, such as antibacterial, antibiofilm, antioxidant, immunostimulatory, or metal chelation activity [8,15]. Thus, integrating anti-infective polysaccharides into bioprinting technology

has the potential to reduce the incidence of implant infection in the clinic and mitigate the spread of antibiotic-resistant isolates.

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