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Review article

Exopolysaccharide and biopolymer-derived films as tools for transdermal drug delivery

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ABSTRACT

Microbial exopolysaccharides (EPSs) exhibit diverse functionalities and offer a variety of structural options that can be altered to fit a specific purpose. EPSs can degrade within the body via biological processes, and polysaccharides are regarded as generally safe. More so, microbial EPS is replicable from several known, inexpensive, and plentiful sources. Drug delivery-related research involving polysaccharides have continuously cited minimal to zero cytotoxicity and, where tested, sufficient drug release and a competent release profile. Transdermal drug delivery systems as films not only avoids first-pass metabolism, but also provides pain-free administration, assists patients with dysphagia, has increased patient compliance, can be self-administered, and can be removed at any time. Commonly used synthetic polymers in the field of drug delivery have been related to problems regarding toxicity and immunogenicity, escalating the need for an alternative. Ultimately, the risks while using synthetic polymers could result in serious negative influences involving physiological, physiochemical, and molecular events. Research involving exopolysaccharides from extremophiles is only recently gaining attention. However, commercial use of microbial polysaccharides in other areas as well as the positive results from preliminary research suggests microbial EPSs have a promising future in biomedical engineering and medicine, especially as an alternative to current synthetic polymers.

1. Introduction

Drug delivery can be thought of as a single definition for a plethora of subcategories: chemistry, biology, and engineering approaches come together with the goal of facilitating pharmaceuticals to achieve the desired therapeutic effect. Drug delivery systems are tasked with being an alternative to general methods of administration. Additionally, it is of interest to improve several qualities that relate to drug delivery such as prolonging drug circulation time, limiting side effects, increasing drug absorption, and even convenience of administration. All these features can be seen in the drug delivery products of today, yet limitations still exist. Although this will be covered in greater detail in the following sections of this review, drug delivery methods involving synthetic polymers commonly experience problems with harmful degradation products, problems with drug loading or interactions, and high burst release, to name a few.

Polymers that are naturally produced are termed biopolymers. In other words, they are produced by living organisms [1–3,5,6]. Recently, biopolymers have become a popular choice in drug delivery research as drug carriers. In comparison to their synthetic counterparts, biopolymers offer plenty of upside regarding their use. Possibly their most important perks considering their increasing popularity in drug delivery, biopolymers are deemed non-toxic and biocompatible. Furthermore, they are renewable, sustainable, reasonably cheap, and biodegradable [1]. Biopolymers have been seen to play roles in preserving cell viability by withholding genetic information, storing carbon-based macromolecules, fluctuating energy within a cell via production or reduction, and by acting as a defense against harmful environmental factors [18].

These natural polymers are grouped into three categories: polysaccharides, polypeptides, and polynucleotides. Polysaccharides are the most used of the three, and the presence of polynucleotides in drug

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delivery is scarce currently. Polysaccharides are complex polymeric carbohydrates that consist of monosaccharide chains connected by glycosidic bonds [1–3]. Examples of polysaccharides include cellulose, chitosan, xylan, and starch to name a few.

With regards to plant polysaccharides, they can be further divided into groups of structural polymers (cellulose), protective polysaccharides found in cell walls (pectin and hemicellulose), and polysaccharides that are stored until needed for energy (starch). Furthermore, the chemical composition of biopolymers, polysaccharides included, exhibit similarities to that of the natural extracellular environment. This adds to how polysaccharides could form glycoconjugates with proteins as well as lipids, which subsequently leads to their importance in several physiological and biochemical processes. Looking from a biomedical point of view, the utilization of biopolymers would yield a reduction in potential immunological reactions, toxicity, and inflammation [3,4].

Polysaccharides exist within microbes as well and can be mass produced in microbial fermentation. Bacterial polysaccharides involve peptidoglycans, lipopolysaccharides, and exopolysaccharides. Like plant polysaccharides, exopolysaccharides (EPSs) that are produced in bacteria serve a range of purposes from structural support within the cell wall to assisting in the survival of the microbe in what would be considered harsh environments. EPSs are mainly composed of sugar residues yet contain other macromolecules such as lipids and proteins. The cultivation of these high molecular weight carbohydrate polymers should be of great interest to research aiming to replace harmful synthetic polymers, and will one day pave the way to alternative approaches to modern drug delivery.

1.1. Indication of purpose

The purpose of this article is to highlight the advantages extracellular polymeric substances, or exopolysaccharides, have to offer to the field of drug delivery, and how their use could potentially be more beneficial than the continued use of synthetic polymers. It is important to understand the risks presented by using synthetic polymers in conjunction with drug delivery applications. As was mentioned above, and will be mentioned in greater detail in later sections, the use of EPSs not only counteracts many of the disadvantages seen with synthetic polymers, but also offers a variety of additional benefits [1–4]. Moreover, another factor for advancing drug delivery systems would involve cost. Not only have studies recorded EPS as being biocompatible and safe, but their growth and replication can be done with more ease as well as is extremely cost effective in comparison to that of creating synthetic polymers [1,4].

1.2. Commonly studied polymers for drug delivery and respective shortcomings

Several biodegradable and/or bio-based polymers are used in drug delivery-related research that are not extracellular polymeric substances. These include, but are not limited to, PLA, PLGA, PEG, polyethylene (PE), poly(trimethyleneterephthalate) (PTT), polypropylene (PPP), polyethyleneterephthalate (PET), polycaprolactone (PCL), and polyvinyl acetate (PVA). The majority of these found their initial use, and are still used today, in the field of micro and nano particle delivery. In these systems, the polymers are subjected and utilized based on such traits as their physical structure, physiochemical characteristics, and how they interact with supporting systems [7]. These polymers are widely popular in their use, but not in the realm of film-related drug delivery.

1.2.1. PLA

While all the aforementioned polymers have proven themselves as competent in methods of drug delivery, they all exhibit their fair share of limitations. PLA is one of the most utilized polymers in the biomedical discipline. This polymer degrades non-enzymatically by means of hydrolysis, and its byproducts are flushed from the body by means of specific metabolic processes like the Krebs cycle, assuming the byproducts are reasonable in amount. Additionally, PLA displays proper biodegradability traits that has made it a popular option, especially for implantable instruments or devices [9]. PLA and its bioresorbability feature make it a popular candidate for various internal biomedical applications such as bone plates. This bioresorption rate is controlled by the degree of polymerization or copolymerization [15]. PLA is a versatile polymer, and one in which its physical and mechanical properties can be easily altered by means of changes in chain stereochemistry.

PLA's ability to become increasingly flexible when polymerized with its own monomers has become a useful tool in industry. Furthermore, degradation time is increased with an increase in plasticizer, but the shelf-life of the polymer decreases with increased plasticizer content. PLA-derived microspheres are popular among tools for controlled drug delivery. Generally, a protein or peptide is homogeneously mixed into the matrix of the microsphere [15].

During production and synthesis, it is typically necessary for PLA to be polymerized with other polymers to improve physical features as well as lower total cost. As an example, VIRYL, a controlled-drug releasing agent, is composed of a 2:23 ratio of lactic acid to glycolic acid. Starch is a component often polymerized with PLA in order to increase biodegradability and reduce expenditure. Along with this, it has been seen that water is more easily absorbed, and tensile strength and elongation decreases with an increase in starch concentration.

PLA has been used in conjunction with drug-loaded scaffolds as well. In summary, PLA can be combined with hydrophobic drugs by means of electrospinning that yields homogenous distribution in the fibers. With hydrophilic drugs, PLA can be combined using a water/oil emulsification technique that offers a cost-effective method that creates drug-loaded nanofibrous scaffolds noted as to being more efficient in terms of encapsulation efficiency when comparing to other drug delivery carriers like hydrogels and liposomes [9]. However, the drug of choice can drastically alter the drug distribution within the carrier due to how well the drug interacts with PLA. Subsequently, this will change the release kinetics and often results in a burst release due to what has been determined to be a high concentration of drug near the fiber surface of the polymer carrier [9]. Another large concern with PLA involves its degradation products. Lactic acid is the primary product of PLA degradation [9,12,15]. Lactic acid is a strong acid that subsequently can result in an inflammatory response [9]. Traces of impurities found within crude lactic acid could influence the PLA properties as well as the polymerization process. Due to this, it is of importance that the lactic acid undergoes a purification process, which can be looked at as potentially a pitfall as well as a drawback that an alternative to this polymer would not need to undergo [12]. PLA also has a high crystallinity. This trait interferes with any application involving controlled degradation which subsequently reduces compatibility with soft tissues and also provides a dilemma to overcome when attempting to produce biodegradable plastics [15].

Furthermore, PLA conjugates have been known to undergo bulk erosion. A buildup of acidic products within the entirety of the material can cause an acceleration in the degradation, which would result in a rapid loss of mechanical solidarity and a hindered inflammatory response [9]. PLA also exhibits hydrophobic behavior and in such would not be qualified for use with hydrophilic drugs. Nanoparticles made up of PLA have been seen to have relatively high mechanical strength, meaning a slow degradation time and subsequent insufficient drug release for specific therapeutic needs. Additionally, PLA has been noted as to having poor thermal properties and, like poly(lactic-*co*-glycolic acid) (PLGA), are said to be difficult to conjugate when involving hydrophilic molecular probes in cases of targeted release [10,51]. Recalling that starch is commonly blended with PLA, these PLA-starch conjugates are notoriously brittle, which is viewed as a major drawback for various potential applications.

Table 1
Summary of polymers and their respective limitations.

Polymer	Properties	Applications	Limitations	EPS Advantage	References
PLA	Degrades non-enzymatically, byproducts are flushed from the body, cytocompatible, biodegradable, bioresorbable, and polymerize with other polymers	Food, medical implants, drug delivery, and scaffolds	Drug interacts with PLA, lactic acid as a degradation product, conjugates undergo bulk erosion, hydrophobic behavior, poor thermal properties, and difficult to conjugate	Highly competent thermal properties, no current knowledge of harmful degradation products, hydrophilic macromolecules	9, 12, 15, 71
PEG	Stealth properties with PEGylation, improved circulation time, higher stability, reduced immune response by host, lower administration doses, and less doses to be taken	Drug delivery, wound healing, and tissue regeneration	Repulsive nature towards proteins, purification is a crucial step following conjugation, reactive chain ends, anti-PEG antibodies, risk of blood clotting and aggregation of cells, specific and non-specific recognition by the immune system, non-biodegradable, sensitive to photooxidation, and co-formation of 1,4-dioxane.	Crude EPS contains proteins; crude EPS is naturally noncytotoxic, thus it may be competent without purification	13, 14, 71
PLGA	Can form via various ratios of glycolic acid to lactic acid, varying properties involving uptake, drug release, and degradation time depending on ratio, biocompatible, and minimal toxicity	Drug delivery, biomaterials, and tissue engineering	Previous problems with enough drug loading, large number of nanoparticles potentially needed to deliver proper dosage, high burst release of drug, and lactic acid degradation product	Biocompatible carrier for conjugate drugs, stable drug release, and no harmful degradation products	10, 71

1.2.2. PLGA

The cyclic dimers of glycolic acid and lactic acid copolymerize to form PLGA. During this process, the simple monomeric units form ester bonds between one another, forming a consecutive, linear, amorphous polyester. PLGA can form various ratios of glycolic acid to lactic acid which subsequently will also vary its properties. PLGA has been a proven compound in biomedical research and industry largely due to its biocompatibility. Furthermore, it has been reported on various occasions that PLGA can achieve continued drug release by order of days, weeks, months, or years. PLGA is considered biodegradable due to its yield of monomeric metabolites in lactic acid and glycolic acid when the polymer undergoes hydrolysis. When kept at sustainable doses, metabolic processes within the body can rid itself of these acids as carbon dioxide and water. With this, the use of PLGA is considered relatively minimal with regards to systemic toxicity.

Referring to the aforementioned differing ratios of monomeric units that PLGA can be comprised of, this value will determine the degradation time of the polymer. In short, the smaller the molecular weight, the lower the degradation time will be. Lactic acid, in comparison to glycolic acid, is more hydrophobic. That said, a PLGA copolymer with a higher count of lactic acid compounds within its network will be less hydrophilic and absorb less water, leading to a longer, steadier degradation time.

The positive or negative charge that the surface of a nanoparticle exhibits determines their interaction with specific cells as well as their rate of uptake. It is said that a positive charge on the nanoparticle is preferred so that it may promote interaction with the cell. PLGA nanoparticles have a negative charge initially, but can be altered to produce a cationic charge, or even a neutral charge, by ways of PEGylation [10]. Properties and applications of selected polymers are summarized in Table 1.

However, nanoparticles comprised of this polymer often see troubles with enough drug loading. Essentially, this means that large amount of the nanoparticle would be needed to deliver the correct dose of the therapeutic. Similar to an aforementioned deficiency with PLA, PLGA also has been noted as to exhibiting a high burst release of drug, and this has been documented with the majority of PLGA-nanoparticle use. For this to occur, there is a great chance that the therapeutic will not reach its respective target [10].

Further problems persist when it comes to the utilization of PLGA. In regard to its degradation products, PLGA breaks down into lactic acid. Although the body utilizes specific metabolism pathways to typically clear the presence of lactic acid, an increase in the necessary quantity of PLGA needed to deliver the proper amount of therapeutic due to the burst release factor, could yield an excess in acidic degradation products that the body cannot remove immediately. This has been a reoccurring problem since PLGA's initial use in drug delivery, yet the problem still exists despite research to correct it.

1.2.3. PEG

PEG is another widely used polymer and has traits of being highly water soluble, non-ionic, non-toxic, does not issue an immune response, and is noted as being non-degradable. PEG is utilized in the structure of micelles, a drug delivery vessel. The shell of the micelle is normally comprised of this polymer. The reason behind the selection of this polymer is because it inhibits any interaction between the hydrophobic micelle core and the membrane. PEG has been noted as to having the ability to reduce the affinity for particles to aggregate by a means of steric stabilization. This consequently leads to products with increased stability, important especially for storage as well as applications [14]. In most cases with drug delivery, PEG, as a part of a polymer matrix, encapsulates the drug of choice. In other cases, conjugation of PEG and the drug is applied, where the drug can be released as the bonds between the drug and copolymers are naturally cleaved [8,27].

When PEG is conjugated with a peptide or nucleotide-based drug, this is termed as PEGylation [11,23]. The purpose of this combination is due to PEG's "stealth" properties with subsequently improve circulation time, stability, and reduce the immune response of the therapeutics. Along with this, research has revealed PEG could inhibit the liver's uptake of micelles, subsequently increasing systemic circulation [23]. Today, all stealth drug delivery systems whose chemical makeup contain polymers all include PEGylated material [14]. In one study, Qi et al. (2017) determined that their EG9 exendin-C-POEGMA conjugates exhibited a successful prolonged duration of therapeutic effect illustrated by a lowering of blood glucose levels in fed mice at a duration 20 times that of unmodified exendin [13]. This finding points to a potential method to decrease the administration frequency or volume, in theory making the patient less required for continuous medical visits for treatment.

Research has shown that PEGylated drug delivery carriers have exhibited a slower uptake in regard to the liver and spleen. Along with this, PEGylated material has all showed reduced renal filtration, the aforementioned decreased uptake in some organs of the reticuloendothelial system, and lessened enzymatic degradation. All these traits add to PEGylated drugs displaying a long circulation time within the body and thereby an increase in bioavailability [11,14]. To further expand on what this means, PEGylated drugs typically have a lower administration dose and less doses need to be taken, which does improve the all-around quality of life for the patient and would reduce costs and expenses on both ends [14].

The PEG polymer exhibits a repulsive nature towards proteins, and subsequently steric hindrance is a major issue that cannot be quelled even with larger doses of the polymer [8,11,13]. Steric hindrance results in low yields of the conjugate, said to be in the 10–20% range, and will also leave an abundance of excess polymer, making purification of the product a crucial step following conjugation [13]. In essence, steric hinderance creates a concealment of charge that prevents interactions and competes with substrate binding [14]. This can result in end groups being sterically hidden, subsequently seeing a rise in unreacted polymer [13,14]. Finally, it is understood that the chain-ends of PEG react with the lysine and cysteine residues of the sidechain comprising the therapeutic biomolecule. These amino acids are sporadically located along the side-groups, which can lead to a chemically diverse products from inconsistent attachment location. Subsequently, this can greatly compromise the bioactivity of the newly formed drug [13,14,87–90].

Additionally, anti-PEG antibodies have been discovered in individuals previously not exposed to research relating to PEG materials. In other words, it is theorized that these individuals developed anti-PEG antibodies from chronic exposure to everyday items containing traces of PEG. Subsequently, this alters PEG's circulation and clearance times. Studies have introduced anti-PEG antibodies to patients prior to exposure to PEGylated material. The results concluded that the presence of these antibodies increased circulation time of these PEGylated enzymes and increased the likelihood of developing severe infusion reactions. Lastly, high levels of circulating anti-PEG antibodies have been linked to serious allergic reactions after the first exposure to a PEGylated RNA aptamer, ultimately leading to a premature end to the clinical trial being performed [13]. In 1983, Richter et al. noted an accumulation of antibodies in rabbits dosed with PEG conjugates, although the response varies between 17% and 50% [91]. In 2005, one case study yielded results that may provide evidence for specific immune response. In summary, the results depicted a large IgE antibody mediated hypersensitivity reaction to 4 kDa of PEG that was administered intravenously [92].

One of the earliest studies of PEG resulted in a finding that the polymer has the ability to induce blood clotting and the aggregation of cells, subsequently putting an individual at risk of embolism. Due to this, it has been theorized that PEG nonspecifically interacts with blood. To further draw from this theory, later studies have concluded that PEG does indeed cause specific and nonspecific recognition by the immune system, ultimately leading to a response to PEGylated drugs entering the body intravenously. Other research concluded that the benefits liposomes containing PEG may not always be present. Parr et al. (1997) documented minor differences in plasma clearance rates, reflecting the rate of drug elimination, between PEGylated and non-PEGylated forms of doxorubicin [72]. PEG incorporation did not end in improved doxorubicin delivery to the analyzed lung tumors [63]. Furthermore, Romberg et al. (2005 and 2007) acknowledged liposomes without PEG, but with poly-amino acids and poly(hydroxyethyl L-glutamine), showed approximately the same or longer circulation times as those that were PEGylated [64,65].

As mentioned before, PEG is known to be non-biodegradable. Due to this, PEG compounds with a lower mass of PEG is preferable. Although this is favored, polymers with a molar mass below 400 Da have shown to be toxic to humans by way of sequential oxidation which yields diacid and hydroxyacid products via alcohol and aldehyde dehydrogenase. Oxidative degradation, however, does decrease with increasing molar mass of the oligomer, and so there are pros and cons to both a higher and lower mass PEGylated compound. Studies have shown that regardless of the presence of antioxidants or free radical inhibitors, PEG will undergo thermal degradation at inert conditions. Moreover, PEG has shown to be sensitive to photooxidation courtesy of the α-carbon atom by color-carrying impurities.

During the synthesis of PEG, 1,4-dioxane, a cyclic dimer of ethylene oxide, is formed. According to the International Agency for Research on Cancer (IARC), 1,4-dioxane is categorized in group 2b, the most carcinogenic to humans, from evidence gathered from animal experiments. The synthesis of 1,4-dioxane is said to also yield residual ethylene oxide and formaldehyde, which IARC has both classified in group 1, meaning carcinogenic to humans. The seemingly toxic nature involving PEG adds to further care that needs to be considered when utilizing PEG for biomedical applications [14]. The limitations associated with polymers and their overcome using EPSs are summarized in Table 1.

1.3. The exopolysaccharide response for the shortcomings of common polymers

With regards to drug delivery, the aim of the field is to improve the bioavailability of the compounds to be used via innovative formulation development. In other words, no drug will be administered as itself or on its own, but rather as a delivery system given as a formulation or a series of compounds. Recent problems regarding toxicity and immunogenicity of common materials have led to the discussion of natural polysaccharides as an alternative for synthetic polymers. Exopolysaccharides have been recorded to degrade to their monomers or oligomers within the body by biological processes without actually altering their own chemical structure. In theory, this could naturally remove the drug delivery system once the active agent has been released [21]. It is well known the research with EPS has yielded results that revealed next to no toxicity of any kind [5,22–25,27]. Many studied polysaccharides have shown natural bioactivity that can direct mucoadhesion, improved specific tissue targeting, and overall inflammatory response reduction [22,23]. Additionally, certain polysaccharides have been noted to enhance circulatory stability with thanks largely to their hydrophilic nature. As an added bonus, the majority of polysaccharides are naturally antimicrobial [23].

As determined by the FDA, polysaccharides in general are largely regarded as safe, microbial polysaccharides are preferred in industry versus plant and marine sources for a few reasons. Initially, they are easily replicable from several known, cheap, and plentiful sources [22,25,26]. Microbial EPS play an important role in concealing the bacterial surface which enables an adhesive interaction with the exterior of other bacteria [22]. Next, the end result of production yields a final product of high quality. Furthermore, microbial polysaccharides exhibit unique rheological properties that allow for reproducible production despite environmental influences, and are resistant towards hydrolysis at a variety of pH and temperature parameters [21]. Lastly, they have an interesting ability to modify properties of their own aqueous environments, allowing the polysaccharides to thicken, emulsify, encapsulate, swell, and flocculate to form any of the following: gels, films, membranes, or colloidal suspensions. Film forming abilities can entrap biological compounds and, because of its oxygen barrier properties, these entrapped molecules will remain stable and with an enhanced shelf-life if discussing industry use [21,25,26].

Microbial EPS has previously been documented as providing certain health benefits in research and human trials [16–18,22]. Specific cases involving treatment for gastrointestinal, tumor, and bowl diseases [22]. Moreover, EPS has been seen to reduce blood cholesterol levels, as well as contributed to treatments for cancer, tumors, ulcers, and immune modulation [16,17,22,25]. Sulfated polysaccharides specifically have been recorded as showing a strong antiviral ability. This is because sulfated polysaccharides inhibit the virus to cell attachment, but also exhibit antiviral effects towards other viruses such as hepatitis B virus, cytomegalovirus, herpes simplex virus, and influenza virus [17,22].

Polysaccharides are easily altered and can be found in positive, negative, or neutral states [23,27]. Several polysaccharides have been found to be bioactive and subsequently can assist in enhancing the therapeutic efficacy of a drug of choice, or may improve the overall targeting ability of a drug carrier system [24]. They are diverse in their nature in that they can exist either as linear or branched, depending on the monosaccharide unit. When discussing their chemical structure, polysaccharides show several reactive functional groups, including

amino, carboxylic acid, and hydroxyl groups [23,24,27]. The reactive groups ultimately show means for chemical modification. Although these functional group backbones equal a high aqueous solubility, this trait can be attuned by modification of the monomers [23,27]. Glucose-based polysaccharides, such as cellulose and amylose, offer plenty of free reactive hydroxyl groups. Polysaccharides commonly contain both hydroxyl along with carboxylic acid moieties capable of being altered [124]. As a more specific example, quaternization of the primary amines in chitosan via numerous alkyl groups can be used as a method for enhancing solubility as well as change bioactivity [5,23]. In the case of alginate, oxidation of hydroxylic functional groups has been noted to enhance biodegradability. On the other hand, sulfonation yields anti-coagulant capabilities [124]. Polysaccharide weight has been recorded as varying between hundreds to thousands of Daltons, yet again increasing diversity [5,23].

Most polysaccharides systems undergo enzymatic degradation within the human body, mainly via lysozyme [124]. The enzymes in the body recognize the familiar biochemical properties that share similarities to human extracellular matricies, and subsequently are easily metabolized [116,125]. The result of this would yield the formation of various or oligomer building blocks, which can further be used in storage applications, structural support, or cell signaling purposes [23,24]. While the polysaccharide system breaks down, the release of a therapeutic is achievable. Nevertheless, perhaps the greatest pro of microbial EPS is their utilization in important industrial application at a high degree of purity, and at a lesser cost production and fewer production issues when compared to traditional synthetic polymers [26].

2. Challenges with utilizing EPS

As high of a potential relating to pharmaceutical use that they have, exopolysaccharides are not without their limitations. EPS synthesis primarily requires a high abundance of a carbon source combined with another nutrient such as nitrogen [26]. When performing the EPS extraction process, structural and chemical properties can be affected. Furthermore, there are a variety of extraction processes used in literature, all of which vary in the amount of pure EPS recovered [57]. Unwanted byproducts may be formed stemming from diverse metabolic pathways utilized due to the possibility of contaminants or altering makeup of nutrients. Non-reacted reagents that find their way into the broth can act as inhibitors that can lower the product yield [26]. For applications of high value, it is important to utilize EPS of high purity and quality. To accomplish this, good quality reagents and substrates are required over more cost-effective reagents to avoid the formation of different polymers and decrease impurity carryover. This ultimately can make for high production costs, especially in the production of microbial EPSs [6,26,58]. The media alone is said to represent approximately 30% of the total cost regarding microbial EPS formation. This worrying percentage has the potential to increase if using expensive, but almost necessary nutrients like yeast extract, peptone, and salts [58].

When purifying EPS, there is always the risk of experiencing unwanted reactions involving the EPS molecule reacting with the medium or solvents. Similarly, the task of avoiding co-precipitation of EPS during the removal of the proteins is of high importance [57]. With bacterial EPS, strategies involving improved strain selection and peak cultivation conditions still only allowed for minimal bioprocess improvement. It has been reported that certain purification procedures will yield a decrease in product recovery as well as negatively impact the polymer's properties [26]. Shifting to multicomponent feedstock systems to form synthetic media and reduce total cost has seen success in commercial polysaccharides, but it is uncertain if the same success will be seen with microbial EPS [58]. Additionally, the use of heat treatment or involvement of acid has been noted to cause structural damage such as cleavage to the EPS molecule [57]. Analysis of EPS has proven to be a challenge. Methods to analyze EPS are currently limited attributing to differing constraints further stemming from the enormous diversity and functionality of the many biopolymers. As a whole however, one limitation involves the low value of EPS concentration due to their high viscosity. From an instrumental perspective, this can make it difficult to obtain quality NMR analysis [59,60].

3. Microbial extremophilic extracellular polymeric substances

Exopolysaccharides (EPSs) are synthesized by microorganisms and either secreted outside of the cell or synthesized completely outside of the cell by enzymes located on the cell wall. A brief history regarding the discovery of bacterial exopolysaccharides begins in the mid-19th century when Louis Pasteur discovered a microbial polymer product in a sample of wine. Around a couple decades later, the prokaryotes responsible to produce the EPS Pasteur had discovered years earlier was acknowledged as *Leuconostoc mesenteriodes* by Philippe Édouard Léon Van Tieghem. The EPS itself was determined to be dextran [5,6].

Naturally, microbial EPS have been understood to play a major role in protection of a cell, but also assist with the adhesion of the microbe to a surface and contribute to cell-to-cell interactions. The differentiation among bacterial exopolysaccharides is diverse, however, they can be characterized by chemical composition, function, molecular weight, and physical properties. In terms of chemical composition, EPSs are said to be produced in two categories: as homopolysaccharides or heteropolysaccharides. As the titles may hint at, homopolysaccharides are composed of a single monosaccharide while heteropolysaccharides are containing various repeating units, up to the size of heptasaccharides [5]. Homopolysaccharides can be further classified as α-d-glucans, β-d-glucans, fructans and polygalactan. The differences in strain depend on the varying linking sides and degree of branching. Heteropolysaccharides on the other hand can be clustered into D-glucose, d-galactose, l-rhamnose and, in certain instances, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc) or glucuronic acid (GlcA) [5,66].

The yield of exopolysaccharides varies with each microbial species and will also depend on pH, incubation time, composition of the respective growth medium. In a separate category, EPSs have been profiled based on their functionality by Hans-Curt Flemming and Jost Wingender [7]. They serve purposes in the architecture of biofilms by assisting with the mechanical and structural stability, contributing to cell protection and water retention, sorption of nutrients and inorganic ions, acting a nutrient source itself, as a sink for excess energy, and assists with the binding interaction of enzymes.

Extreme environments have been known to host specific strains of bacteria that produce polysaccharides, but also valuable proteins and peptides. In the present day, there is a lot of focus on microbial EPS from extremophiles that resides in the extreme environments [61,62]. These sites are so uncommon that the microbes contain specialized protein adaptation structures such as chaperone systems or specific enzymes capable of operating within the surroundings without denaturing. Mesophilic proteins do not contain similar adaptations and thus could not survive in the same settings [19]. In the fields of drug delivery and cancer research, this is especially a prime concern due to recent research illustrating sustained release patterns as well as anticancer effectiveness shown by EPS stemming from extremophilic bacteria [16]. Sulfated polysaccharides have been previously studied for their promise in antiviral, anticancer, anticoagulant, antiproliferating, and immunomodulating traits [17]. Overall, extracellular polymeric substances originating from extreme environments are proving themselves quickly as promising tools in the field of drug delivery and nanomedicine.

Since the discovery of environments that yield extremophilic organisms, and how they mimic primordial Earth, it has been theorized that the existence of these microbes may date back to the earliest forms of life. Extremophiles encompass all three domains of life: Archaea, Bacteria, and Eukarya. Early research believed it was unlikely that a cell with a complex and compartmentalized interior could survive in such harsh conditions. Therefore, the Archaea and Bacteria kingdoms

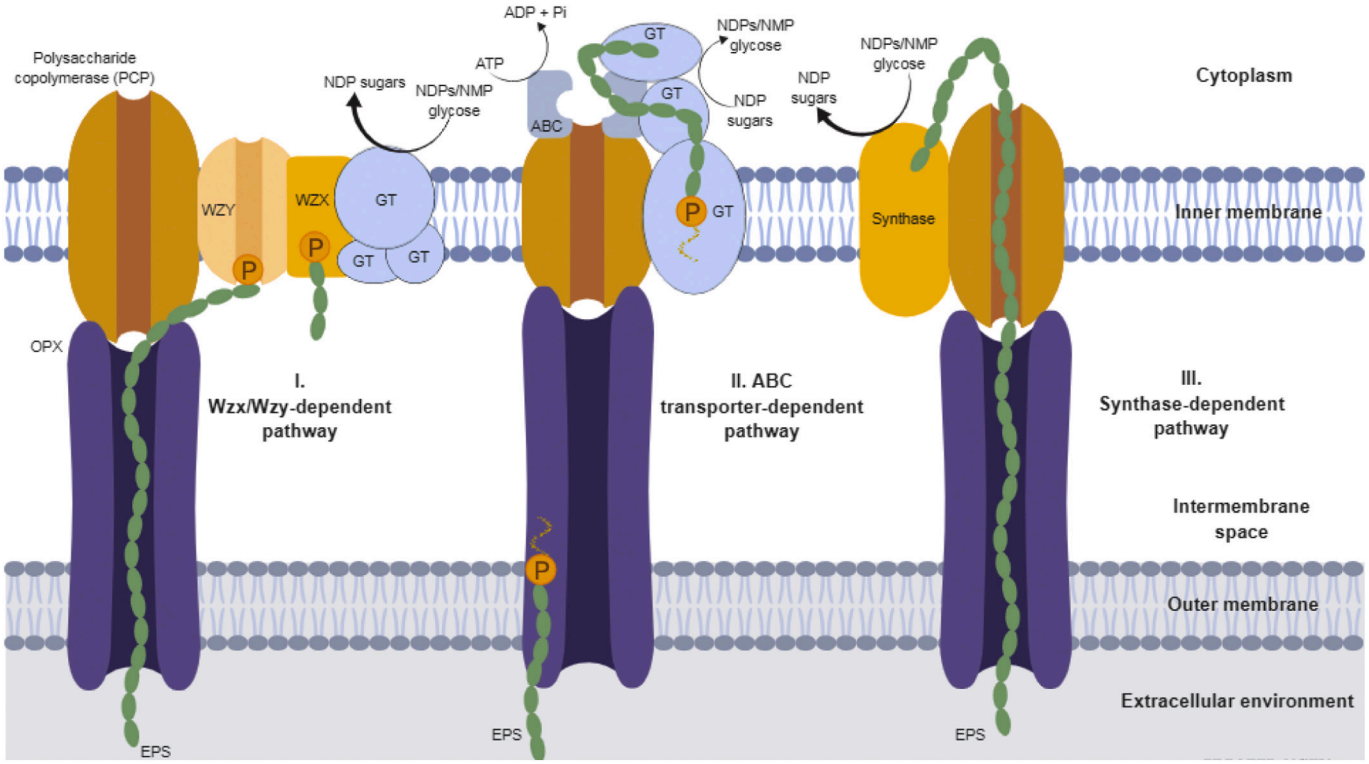

Fig. 1. General illustration of three different EPS synthesis pathways. NDP – nucleoside diphosphate; NMP – nucleoside monophosphate; GT – glycosyltransferase.

attracted much of the early attention in the scientific community. It wasn't long before several Eukaryotic cells were discovered to have evolved and learned to thrive in extremophilic conditions [18,20].

In the present day, proteins gathered from extremophiles have been used in industry, serving such purposes as reagents in molecular biology, laundry detergent, and assisting in bioremediation of contaminated environments [19]. Possibly the most commercially important microbial EPS is xanthan gum, a polymer produced by *Xanthomonas campestris*, a phytopathogen or plant disease. This complex polymer has been used for the last few decades in food additives, cosmetics, and in the oil industry. The polysaccharide pullulan is currently found in denture adhesive and pharmaceutical coatings, including those that perform extended release.

EPSs have been shown to have pharmaceutical importance as well. Pullulan exhibits no toxicity, is non-mutagenic, non-immunogenic, and non-carcinogenic. Subsequently, it has had recent interest for use in targeted drug delivery, gene delivery, and tissue engineering. Certain polysaccharides have the ability to form essential reagents of vaccines when coupled with a suitable protein [18]. As an example, meningitis vaccines have utilized this method during the formulation process. Although said to be expensive to create, multivalent polysaccharide complexes have been used in vaccines for *Streptococcus pneumoniae* as well as *Klebsiella* spp. Schizophyllan, produced by *Schizophyllum commune*, is a fungal homopolymer capable of forming a triple helix conformation which has been noted as strongly influencing biological activity. Because of this evidence, current studies have applied this polymer in conjunction with cancer studies in which it has shown to be very effective when used in the triple helical form [18,94]. In relation to human beings, polysaccharides contribute to a variety of physiological activities as antitumor, antiviral, and anti-inflammatory agents. More so, they stimulate the release of the protective protein interferon, inhibit platelet aggregation, and synthesize colony stimulating factors [18,95].

Most current research pin points its focus on the identification of EPS-producing extremophiles due to their ability to survive harsh environmental conditions ranging from unusual temperature, pressure, salinity, and desiccation to what would be considered extreme heavy metal concentration, acidity, and radiation [18,19]. The reasoning behind this focus is that if these extremophiles are able to survive and thrive in these conditions, their biopolymers are expected to contain certain unique properties in order to adapt to the aforementioned conditions [18]. However, cultivation of these microorganisms is difficult, although very much possible with the help of specialized equipment or media preparation specific to the microbe. Due to the difficulty of cultivation, much less is known about extremophiles in comparison to mesophiles [19].

3.1. Microbial exopolysaccharides and synthesis pathways

With regards to cellular EPS, most are formed within the cell and then subsequently transported to the extracellular environment as their complete macromolecule form. The biosynthesis of EPS takes place over a variety of growth phases, and is largely dependent on the environmental conditions as well as the organism producing the EPS. Currently, four separate pathways to create these polymers are known: the Wzx/Wzy-dependent pathway, the ATP-binding cassette (ABC) transporter-dependent pathway; the synthase-dependent pathway, and an extracellular synthesis that utilizes a single sucrose protein as shown in Fig. 1. The review covers only the general topic and functions of the various pathways. Readers looking for more in-depth explanations should follow-up reading the papers by Freitas et al. (2011), Schmid et al. (2015), and Madhuri and Prabhakar (2014) [26,28,29].

Initially, the substrate can be found within the cytoplasm before it is catabolized via glycolysis. Following this, a cascade event occurs as the primary metabolites formed from this step are then used as precursors to then facilitate the creation of biomolecules such as amino acids and monosaccharides. These activated precursors are required for polysaccharide synthesis, as they mainly exist as energy-rich monosaccharides, largely which are diphosphate sugars. [26].

The Wzx/Wzy-dependent pathway is utilized in Gram-negative bacteria [26]. This pathway sees its individual repeating units, linked at the inner membrane to an undecaprenol disphosphate molecule,

following assembly via glycosyltransferases (GTs), are transferred across the cytoplasmic membrane by what is termed a "flippase," or simply a Wzx protein. Following this, the Wzx protein assists with a polymerization process at the periplasm and the new molecule is then taken to the cell surface. This transportation to the cell surface is believed to be backed by the existence of additional proteins that work closely with the polysaccharide *co*-polymerase (PCP) and the outer membrane polysaccharide export (OPX) groups.

Another pathway seen in Gram-negative bacteria, the ABC transporter-dependent pathway, involves polymerization by GTs at the cytoplasm of the inner membrane [26]. It is said that this pathway synthesizes primarily capsular polysaccharides but is still grouped as an exopolysaccharide manufacturer. Homopolymers are formed when a single GT-containing operon is engaged with, and heteropolymers will be the result of multiple GTs acting on the pathway. The main difference in this pathway is the translocation across the inner membrane and to the cell surface. Here, it is accomplished by a tripartite efflux-like complex made up of ABC-transporters on the inner membrane, and PCP and OPX proteins seen in the periplasmic space.

The third pathway involved in EPS synthesis is termed the synthase dependent pathway. In this progression, complete polymer stands are secreted across both the membranes as well as the cell wall. Furthermore, this pathway is independent of the aforementioned flippase for translocating repeat units. It takes only a single synthase protein to facilitate the polymerization and subsequent translocation process. In some cases, such as those of alginate and cellulose, this synthase protein is a subunit of a multiprotein complex spanning the entire envelope. This pathway traditionally is seen to utilize only one type of sugar precursor to assemble homopolymers [28].

Interestingly, the genes involved in the pathways will ultimately determine the chemical structure as well as the material properties of the final polymers. These genes encode for a variety of GTs, polymerizing and branching enzymes, and also the enzymes responsible for the addition of functional groups or modifications of sugar moieties. All of the aforementioned as well as other enzymes and regulatory proteins involved are not solely seen in EPS production and secretion [29,30]. Uridine diphosphate (UDP)-glucose and UDP-galactose, two sugar nucleotides have been documented as playing key roles in EPS production. These two sugars activate further sugar production, which is ultimately necessary for the polymerization of monosaccharides and the modification of sugars. These monosaccharides and disaccharides are the primary sources of carbon utilized by microorganisms [29]. It has been seen that when these sugar nucleotides are not allowed to be synthesized, a decrease in EPS production follows [30].

3.2. Cultivation and engineering strategies for EPS

Researchers have developed methods to increase production of EPS by the microorganism to go along more cost-effective productivity. As mentioned previously, sugar nucleotides act as the primary source of carbon in EPS production. Cheaper substrates have already been constructed from byproducts and industrial wastes, and have shown as a suitable carbon source for the production of bacterial EPS. However, other applications regarding alteration of metabolic pathways offer prospect for increased EPS production. These applications target specific genes that encode for enzymes relevant to the EPS producing pathways, like glycosyltransferases, Wzx, and Wzy [26,28,29]. Expression modification of these genes, individual or grouped, can fluctuate the conversion efficiency of the chemicals directly involved in the pathway, thus increasing EPS yield. This technique has been proven to work in EPS-producing strains to yield xanthan, gellan, bacterial cellulose, and levan [26].

As seen in Fig. 1, NMPs and NDPs initiate primary metabolism. Studies have examined the use of altered sugar precursors via metabolic engineering to fluctuate enzyme activities as well as metabolite levels that are associated with EPS formation. In all cases, the mutant responsible for the greater EPS yield exhibited higher activities of phosphoglucomutase, UDP-glucose pyrophosphorylase, UDP-glucose dehydrogenase, and UDP-galactose-4-epimerase [67–69]. This strategy has also been examined in EPS-producing mesophiles. Huang et al. determined the over-expression of phosphoglucomutase resulted in a 17% increase in EPS production [70]. Duel overexpression of phosphoglucomutase and UDP-glucose pyrophosphorylase saw in increase in EPS production in both *Streptococcus thermophilus* and *Bacillus licheniformis* [71,72].

The recovery of the EPS during research is typically done by (i) removing the cells from the culture broth by means of centrifugation or filtration; (ii) precipitating the polymer from the subsequent cell-free supernatant via the addition of a precipitating agent such as absolute ethanol or methanol; and lastly (iii) drying the precipitated polymer with methods of freeze drying or drum drying. The polymer can be extracted from the precipitant by centrifugation at high rotational speeds before it undergoes the drying process. Prior to the cell removal and after the fermentation process, the culture can be placed in heat, sometimes up to 90–95 °C, that act on killing any existing bacteria as well as inactivating specific enzymes that have the potential to degrade the polymer during the synthesis process. Purification processes can be performed along the way as well, but has been known to decrease final product yield as well as alter the composition of the EPS in cases where chemical reagents involved in the purification process react directly with the polymer [26,53–56]. Subsequently, it is important for research to improve current methods of extraction and purification in order for the final polymer to be tailored for to requirements.

3.3. Classification of extremophiles and respective environments

Extremophiles that cope and thrive in highly unusual temperatures are further subcategorized into different types. Perhaps the most studied environments are those of high temperatures and home to the thermophiles. These thermophiles exist in temperatures greater than 55 °C. Microbes that have been found to exist in temperatures ranging from >70 °C to 113 °C have been further categorized as hyperthermophiles. Hyperthermophiles are generally Archaea due to their structure containing ether bonds versus the ester bonds that make up traditional bacterial and eukaryal lipid structures. Furthermore, the glycerol moieties found within the monolayer membrane of the Archaea membrane are linked by covalent bonds, adding to the stability against high temperatures [18]. The enzymes that have been extracted from thermophiles to this point are considered valuable, largely for their unique thermotolerance. Perhaps the most well-known of these enzymes is *Taq* polymerase, a key contributor in PCR technique [19]. Sources of these thermophiles include hot springs, oceanic vents, mudpots, geysers, and deep biospheres. Some famous locations around the world that are home to thermophilic organisms include Yellowstone National Park with it's over 10,000 geothermic features, Iceland, the Kamchatka Peninsula in Russia, the Echo Crater located within the Waimangu Volcanic Rift Valley, and Boiling Lake of Morne Trois Pitons National Park within Dominica, to name a few.

Psychrophiles are capable of growing and surviving in low temperatures, said to be less than 15 °C. These locations are typically that of glaciers, polar regions, alpine soil, the deep sea, and places of high altitudes [20]. Enzymes that have been extracted from psychrophiles have been seen to exhibit high catalytic activity in addition to their ability to effectively function in cold temperatures [19]. When looking into the structure of these extremophiles, it has been seen that high levels of polyunsaturated fatty acids are found to comprise the membrane of some of these organisms [18].

Piezophiles exist in extreme barometric pressures ranging from approximately 117 mPa to where they are most commonly found, at a pressure of around 38 mPa. The bottom of the Pacific Ocean has yielded piezophilic life forms and has registered a maximum pressure of near 117 mPa. It has been noted that among the specific adaptions that are

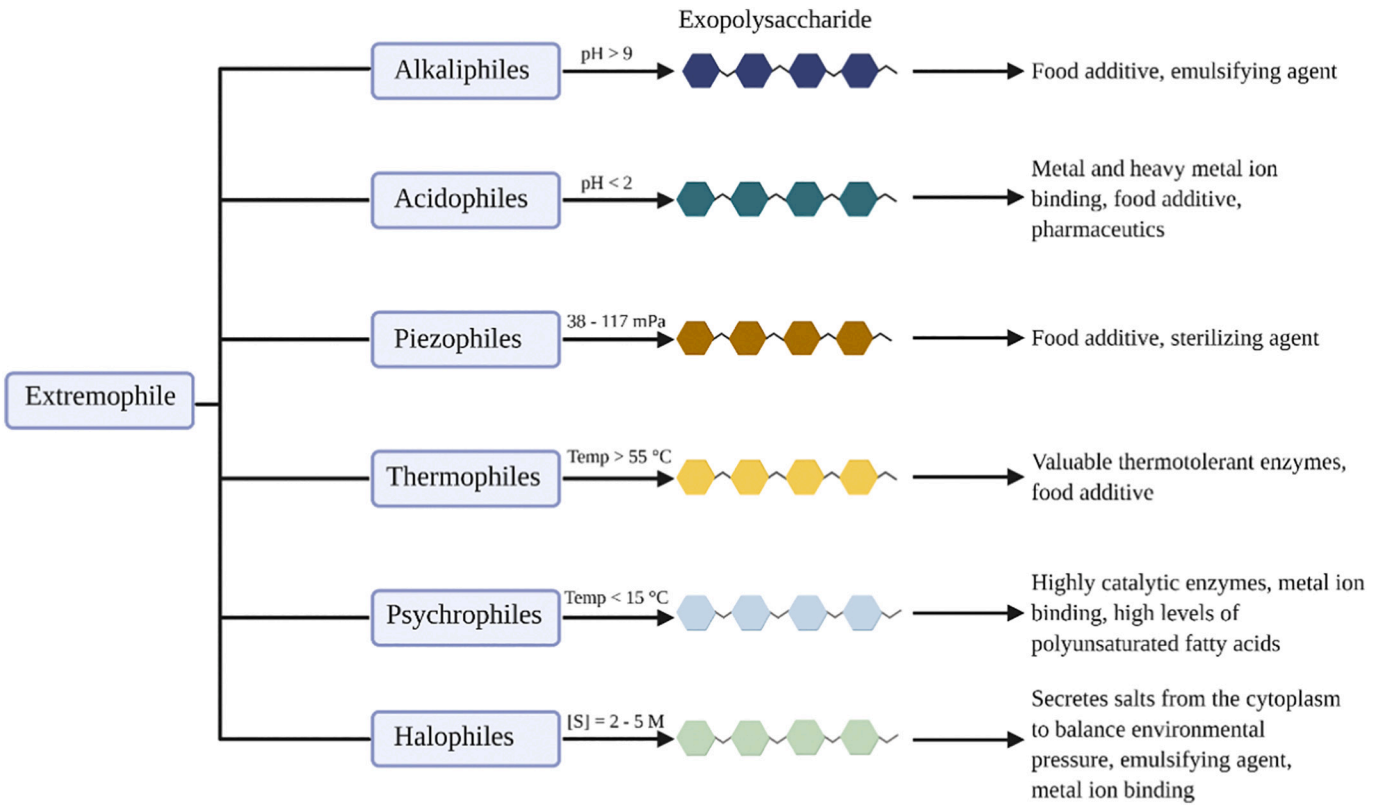

Fig. 2. EPS-producing extremophiles and varying EPS properties.

seen in piezophiles, dense hydrophobic cores surrounding their respective proteins as well as the ability to form multimeric proteins to counteract the pressure are among the more key. Recently, it has been proposed that enzymes comprised by piezophiles could assist with the high sterilization of foods e.g. in the process of ripening of certain cheeses [19].

Organisms that thrive in low pH values are termed acidophiles. These locations include acidic caves, sulfuric geysers, hot springs, and man-made drainage ditch systems. It is said that acidophiles are able to retain the neutral pH values within the cell by the low permeability allowed by their membranes to the excess protons found in their environments [18]. In industry, acidophiles are of value due to their ability to bioleach metal and heavy metal ions that are considered toxic to the majority of organisms. Enzymes from acidophilic microbes have shown commercial uses in starch, baking, fruit juice processing, animal feed and pharmaceuticals [93]. Alkaliphiles exist in strong alkaline environments are that exhibiting a pH value greater than 9, such as the soda lakes, and are commonly used as emulsifying agents [19].

Halophilic, or those able to grow in a variety of salt concentrations by means of either intracellular accumulation of salt concentrations close to that which exists outside the medium, or by excluding salts from its cytoplasm as well as producing solutes like ectoine and glycine-betaine in order to balance out the osmotic pressure of the medium. It is said that the majority of halophilic EPS exists as heteropolysaccharides, and that mannose and glucose have been found to be the most common sugar moieties within the structure [62]. Research with halophiles have also examined the competency of emulsifying activities and metal binding activity. Extremophiles are known to live in several other types of environments as well. These include, but are not limited to, radiotolerant microorganisms resistant to radiation by way of remarkable DNA repair systems. Furthermore, it has been seen that organisms exist to counter desiccation, in the case of xerophiles; osmotic pressure, in the case of osmophiles; and those which thrive in high concentrations of heavy metals, or metallophiles [18,19]. Fig. 2 focuses on the unique mechanisms and properties of certain extremophiles that have been recognized as having promising EPS use. Table 2 summarizes the classification of a broad scope of various extremophiles and the unique properties of the EPS for those that have been studied. Wang et al. provide a detailed description of EPSs produced by a variety of extremophiles as well as their respective advantages and properties [62].

4. Transdermal drug delivery systems as films

The skin is easily the most accessible organ of the body, and yet oral delivery is the most common and convenient route of drug administration. However, drugs delivered this way must go through first-pass metabolism and are additionally put at risk of degradation via specific enzymes and/or deactivation associated with a decrease in pH within the stomach [31,32]. Another method of drug delivery is by way of injection, which can be difficult for reasons of patient compliance, pain, and sometimes must be administered by a trained professional. Transdermal drug delivery systems (TDDS) as patches or films not only avoids first-pass metabolism, but also provides pain-free administration, assists patients with dysphagia, has increased patient compliance, and can be self-administered. Additionally, the patch or film can be removed at any time to cease the transfer of the drug to the body.

Semisolid drug delivery methods like ointments have their drawbacks too. These creams can yield an oily and greasy texture, and can easily be wiped off, including unintentionally by clothing. Subsequently, these methods often require continuous applications [33]. Films and patches offer a novel approach that maintains skin contact with less frequent dosing and a more desirable texture or less residue.

The skin is said to cover approximately 2 m^2 of surface area on an average adult human body and receives around one-third of the total volume of blood that circulates within the body [31,33]. With that, the skin provides a variety of placement options for patch use. The first TDDS was approved in 1979 and was a patch that delivered scopolamine

Table 2
Classification of extremophiles.

Environmental conditions	Extremophile category	Parameters	Unique Properties
pH	Alkaliphiles	pH > 9	Calcite dissolution
	Acidophiles	Low pH; typically pH 2 or lower	Protect against low pH and metals
Radioresistance/ radiotolerance	Radiophiles		***
Pressure	Piezophiles	High pressure; ~38–117 mPa	Contain multimeric proteins
Temperature	Hyperthermophiles	>70 °C	Short fermentation, proposed EPS adaption to high temperatures
	Thermophiles	55–70 °C	
	Mesophiles	15–50 °C	***
	Psychrophiles	<15 °C	Polyanionic structure binds to metal cations
Gravity	Hypergravity	> 1 g	***
	Hypogravity	< 1 g	***
Vacuum		Vacuum conditions; extreme dehydration	***
Desiccation	Xerophiles	Low availability of water	***
Salinity	Halophiles	Variety of salt concentrations; 2–5 M	Charged structure due to significant amount of uronic acids
Oxygen fluctuation	Anaerobes	oxygen-free conditions	***
	Microaerophiles	Low levels of oxygen	***
	Aerobes	Requires oxygen	***
Osmotic pressure	Osmophiles	High osmotic pressure; high sugar concentration	***
Chemical	Metallophiles	High concentrations of heavy metals	***
	Toxitoletants	Can withstand high levels of damaging agents; benzene-saturated; nuclear core	***

*** More research required to improve scientific understanding.

to the user for up to three days to treat motion sickness. From 1979 until 2002, a novel TDDS patch was approved on average of every 2.2 years. From 2002 to 2007, that average was reduced to every 7.5 months [34]. Since then, the rate of approved transdermal systems has only increased as knowledge increases, roles are more understood, and techniques become more modern.

Absorption of the drug itself occurs primarily through the stratum corneum. This is seen as a difficulty when working with transdermal systems, because of the poor permeability of the stratum corneum and its 15–20 μm thickness [32–34]. Once the drug penetrates the stratum corneum, it passes through the underlying epidermis and dermis as shown in Fig. 3. Here it is important that the drug does not accumulate in these layers. It is only after the drug passes through the epidermis and dermis that the drug can be available for systemic absorption by means of dermal microcirculation [31].

Transdermal drug delivery systems often require permeation enhancers to penetrate the stratum corneum. The chemicals chosen for these enhancers essentially disrupt the stratum corneum while avoiding damage to deeper tissues beneath. However, it is not uncommon for these chemicals to also yield skin irritation [34]. The list of permeation enhancing chemicals, recently said to be over 360, include fatty acid, fatty alcohol, glycol, laurocapram, azone, terpenoids, terpenes, sulphoxides, pyrrolidones, surfactants, and urea [31,35]. Further limitations regarding these penetration enhancers include their inability to achieve the sought after skin disruption as well as their ability to transport across the skin is still seen as low and inconsistent. Furthermore, adding on to the aforementioned skin irritation problem, there have been cases involving symptoms of inflammation, erythema, swelling, and dermatitis [31]. Microneedles (MNs) are used as means for physical pore formation for the delivery of drugs through the stratum corneum. However, a common drawback seen in all techniques utilizing MNs is the possibility that part of the membrane will break off from the MN and be left behind under the skin [96,97]. Therefore, these MNs must be established from biocompatible materials as to avoid unintentional inflammatory responses [96–98]. Chen et al. developed a microneedle patch from a mixture of polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) that allowed passage of anti-programmed death-ligand 1 antibody (aPDL1) using a cold atmospheric plasma (CAP) technique. The MN patch acted as a facilitator to allow the CAP to pass through the skin. The authors noted a microneedle size of 700 μm in length, 300 μm at the base, and a sheath thickness of approximately 50 μm. The MN patch successfully induced a local cancer treatment determined by a CD8+ and CD4+ T cell increase in mice [126]. In a separate study, Yu et al. approached the regulation of blood glucose with an insulin-loaded glucose-responsive polymeric microneedle patch. Specifically, this matrix was comprised of poly(Nvinylpyrrolidone-*co*-2-(dimethylamino)ethyl acrylate-co-3-(acrylamido) (PBA) and ethylene glycol dimethacrylate (EGDMA) via in situ photopolymerization at 4 °C. The authors described the MN patch as a 20 × 20 array with each needle measuring 900 μm in height and a width of 400 μm. Fluorescence imaging indicated that rhodamine B-labelled insulin was uniformly distributed among the needles of the MN patch, with in vivo studies confirming bioactivity of insulin for up to 8 weeks. More over, normal glucose levels of minipigs were able to be maintained for over 20 h at normal feeding conditions using the MN patch [127].

Similarly, another method for physical disruption of the stratum corneum is via microdermabrasion. This is a widely used method that can be facilitated with such simple tools as sandpaper [99]. All of the aforementioned transdermal drug delivery systems can be further visualized in Fig. 4.

Skin hydration is important to drug uptake through the skin, but this can be accomplished with the addition of humectant during the film or patch casting. Research on Natural polymer thin films have documented ease and varying methods of application, flexibility, and improved cosmetic appearance [36]. A comparison of transdermal drug delivery using films and ointments is shown in Table 3.

5. Current applications and relevant research

The primary role of extremophilic EPS is to provide adhesion as well as protection to the bacteria of its origin [5,62]. These polymers exhibit the cohesive strength required for biomedical applications and are competent in their ability to issue a biocompatible response to cells and tissues [62,100]. Polysaccharide-based films can be formed with minimal requirements: the polymer, water, and a plasticizer. Polysaccharides used in these films are typically water-soluble, thus a plasticizer sharing similarities to the polysaccharide structure are used. An effective plasticizer in these cases is glycerol [101].

Halophilic bacterium, *Halomonas smyrnensis* sp. nov. AAD6^T, produces EPS levan that has been previously documented to form a multilayer film by a method of electrostatic adsorption [100]. Costa et al. documented the cells used in this study exhibited adherence to this film in high number. Furthermore, the film displayed good adhesiveness and the authors reported levan-incorporated films required higher strength

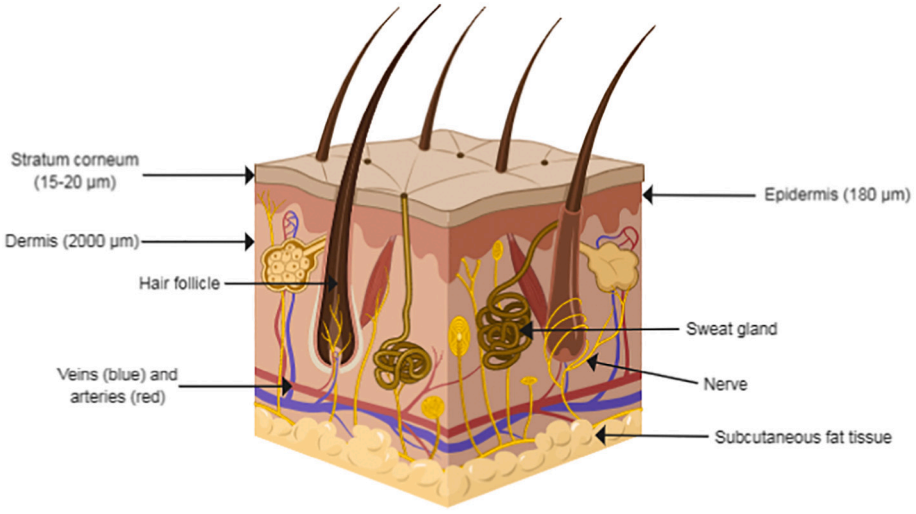

Fig. 3. Anatomy and labeling of the skin.

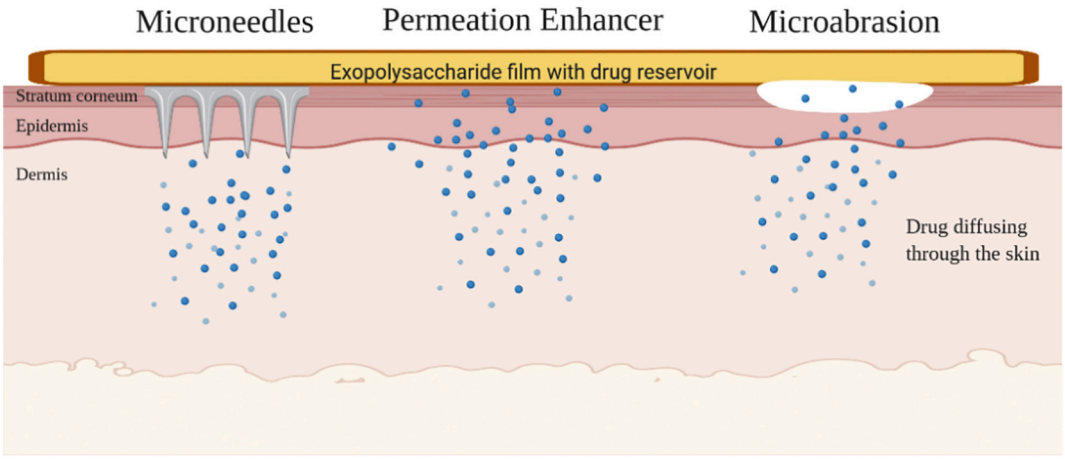

Fig. 4. Drug delivery methods using a film device.

Table 3
Transdermal drug delivery: Films vs. Ointments.

	Films/Patches	Semisolid Ointments
Appearance	Transparent, semi-transparent	Visible, slight sheen
Texture	Adjustable adhesion, dry	Oily, greasy
Application	Convenient, can form to the skin	Messy, sticky, cause stains
Residue	Possible	Not often
Wash/rub off resistance	Yes	No
Dose frequency	≥1 day	≤1 day
Sustained release	Capable	No

for detachment. Overall, the biocompatibility seen by this EPS-based film provides initiative to explore novel applications of cell-substrate and cell-material interactions [62,100]. In a separate, but related case, the EPS levan was blended with chitosan and PEO to form a film using a solvent casting technique [102]. Bostan et al. prepared stocks solutions of each polymer in 1% acetic acid in water (*v*/v). The film mixtures were stirred at room temperature for a reported 12 h prior to degassing a subsequent addition into a polystyrene petri dish. Traces of water and acetic acid were removed by storage in a vacuum oven set to 40 °C for 2 days. Cell viability and proliferation studies confirmed the addition of levan to the ternary blend films exhibited better biocompatibility compared to chitosan-PEO films [102]. Another method of film construction using levan was performed by matrix-assisted pulsed laser evaporation (MAPLE). The levan thin films in one study utilizing the MAPLE method reported an increase in cell proliferation and adhesion [103].

The fungus, *Aureobasidium pullulans,* produces an extracellular polymer called pullulan (PU). Lima et al. produced a pullulan-based film using a casting method [104]. The authors described the PU was initially dissolved in water, heated to 50 °C, and added to a polymethacrylate dispersion. The homogeneous mixture was poured into Teflon molds and placed in a 50 °C oven for 24 h to complete solvent evaporation. The film was concluded to be resistant to premature release of drugs within a simulated setting of the upper gastral intestinal tract [104].

Alginate (ALG) and carboxymethyl cellulose (CMC) are two common polysaccharides used commonly in wound treatment materials. Maver et al. developed a multilayered thin film from the two mentioned polysaccharides using spin-assisted layer-by-layer (LbL) coating method [105]. During the spin-coating, the ALG-CMC combination was mixed with lidocaine (LID) or diclofenac (DCF) to form drug-coated layers of the film. The ALG-CMC film exhibited a rapid burst release of both drugs before settling into a prolonged release profile for the 24 h analyzed. Moreover, the films, both with and without drug added, did not have a cytotoxic effect on human keratinocytes and fibroblasts [105]. Xu et al. layered silica nanocapsules between alginate/chitosan/alginate

multilayer films using electrostatic interactions, and monitored the effect pH had on swelling under physiological conditions. The films were loaded with fulvestrant, a selective estrogen receptor down-regulator agent, and studies confirmed the film's high loading capacity for the anticancer drug. Additionally, the LbL films displayed proper physical stability, good structure, and a long-term drug release profile at the examined physiological conditions. Overall, the film's ability for reversable swelling indicates the potential as a tool for a smart, on-demand drug-delivery system [128]. In a following study, Xu et al. developed temperature-responsive block copolymer micelles (BCM) to be conjugated with a hyaluronic acid (HA) film via hydrogen bonding. The BCM/HA multilayer films proficiently absorbed Osimertinib, a lung cancer treatment agent, into the hydrophobic cores of the micelles. A temperature-sensitive response, by way of protonation and deprotonation of the micelle core, triggered either a swelling or deswelling of the LbL film [129].

Halomonas maura has been discovered to produce a sulfated polysaccharide termed Mauran (MR) and it is noted as to having a high sulfate and uronic acid content. Furthermore, MR has a high molecular weight, reportedly an average of 4.7×10^6 Da, and is comprised of repeating units of mannose, galactose, glucose, and glucuronic acid. Because of the aforementioned high sulfate and uronic acid content, MR is highly anionic naturally. It also has viscoelastic, pseudoplastic, and thixotropic properties and behaviors, which are signs of being a good choice as a molecule for material sciences. MR has an ability to withstand severe conditions, such as high temperature, thawing from a frozen state, pH values, and high salt conditions. Once bound to a variety of metal ions, MR can form a type of gel that allows for a removal of toxic ions from its surroundings. Attributing to its high sulfate content, which prevents cells from obtaining glucose, MR is said to have immunomodulating and anticancer properties [16].

Polymeric materials such as chitosan can be functionalized with MR to enhance its functional properties. Raveendran et al. fabricated MR/chitosan (MR/CH) nanoparticles and reported sustained release of antitumor drug fluorouracil (5FU). Additionally, these nanoparticles exhibit an overall positive charge, alluding to potential adsorption properties of negatively charged peptides or drugs, as well as being able to bind to negatively charged sites. Nanoparticles containing MR have previously been recorded as being non-cytotoxic and biocompatible. In this study, with the authors' highest concentration of nanoparticles, an in vitro cytotoxicity assay performed on their mouse fibroblast L929 cells revealed 85% cell viability. Another experiment involving the MR/CH in this same study reported a gradual killing of cancer cells. With regards to free drug, 83% of cells found to be viable after a 24-h period, although were reduced to 45% after a 48-h incubation period. These MR/CH nanoparticles appear to be a possible answer for reducing repeated exposure of free drug and the subsequent side effects, and offer use in the field of drug delivery [16].

A separate study again using MR/CH nanoparticles showed that they do not induce reactive oxygen species, which may otherwise oxidize polyunsaturated fatty acids as well as fatty acid membranes. Briefly, the presence of reactive oxygen species would also be a threat towards DNA damage, oxidation of protein amino acids, cell/tissue death, and the oxidation of cofactors that would subsequently inactivate their respective enzymes. Further with this study, the authors concluded that free MR/CH nanoparticles were relatively non-toxic when tests revealed an approximate 85% cell viability under the highest concentration of nanoparticles stated at 1 mg/mL. Although nanoparticles loaded with the drug 5FU showed a cytocidal effect of approximately 30% on the mouse fibroblasts at its highest concentration, it was seen that these same 5FU-MR/CH nanoparticles were able to kill the glioma cells to a certain extent, and the breast adenocarcinoma cells to a much greater extent. The data can further be examined within the article [17]. Overall, MR/CH nanoparticles further prove to be a useful polysaccharide of extremophilic bacterial origin for purposes of biomedical and drug delivery applications. The nanoparticles have the potential to

Table 4
Current applications of natural polymers in research.

Polymer	Formation	Comments	References
Levan	Film	Multilayer film displayed enhanced cell adhesive property. Levan-chitosan-PEO blend films saw the addition of levan exhibited better biocompatibility compared to chitosan-PEO films. Levan films constructed via MAPLE displayed sustainable cell adhesion and proliferation	62, 100, 102, 103
Pullulan	Film	Sufficient resistance to GIT fluids and potential resistance to premature release of drugs	104
Alginate – Carboxymethyl Cellulose	Multi-layered Film	Sustained release of lidocaine and diclofenac. Minimal to no cytotoxicity on human keratinocytes and fibroblasts.	105
Silica microcapsule – alginate & chitosan	Multi-layered Film	pH-sensitive LbL film loaded with fulvestrant. Displayed physical stability, good structure, and a long-term drug release profile at physiological conditions.	128
Hyaluronic acid	Multi-layered Film	Block copolymer micelles (BCM) and HA combined to form a temperature-sensitive LbL film capable of absorbing Osimertinib into the micelle core. Fluctuation in proton concentration of the BCM triggered swelling and deswelling of the film.	129
Mauran (MR)	Nanoparticles	Immunomodulating and anticancer properties. MR/chitosan nanoparticles reported sustained release of antitumor drug fluorouracil (5FU).	16, 17
Sodium alginate	Film	Good bioadhesive character that retains a high bioadhesion value. Limonene permeation enhancer disrupted pig stratum corneum, delivering a reasonable amount of 3% donepezil	37
Starch nanocrystals (SNCs)	Hydrogel patch	Hydrogels derived from potato SNCs and maize SNCs were noted as having better stability during storage in comparison to the SNC formed from cassava	38
Chitosan	Matrix film	Conjugated with Transcutol as a plasticizer and terpenes as a permeation enhancer had good mechanical properties as well as bioadhesiveness. Released a realistic flux of nortriptyline hydrochloride into human abdominal skin samples. Silver nanoparticles ultimately increased flexability, stability, and prolong lifespan of the film. Silver nanoparticles were incubated with *Ganoderma lucidum* extract and the release of this compound	39, 40, 41

(*continued on next page*)

Table 4 (*continued*)

Polymer	Formation	Comments	References
		was measured over time, yielding consistent release values during in vitro drug release studies.	
Sodium alginate-L-cysteine	Matrix patch	Drug permeation was higher in patches that contained conjugated sodium alginate, no matter the varying concentration of sodium alginate, in comparison to patches that contained only sodium alginate. Opening of the polymer chains loosened the matrix, allowing for greater permeability and rapid release of drug	42
Cordia dichotoma fruit mucilage	Film	Loaded with alfuzosin hydrochloride, in vitro studies illustrated a greater release of the loaded alfuzosin HCL as the concentration of *Cordia dichtoma* mucilage increased.	43
Sodium locust bean gum (LBG)	Film	12:80.5 w/w% LBG to sodium alginate mixture was noted as yielding the highest percentage of drug permeation as well as holding the highest percentage of drug in this study.	44
Pectin	Matrix patch	Transdermal patch loaded with verapamil hydrochloride. Systolic blood pressure decreased significantly with and without permeation enhancers. Patches loaded with nerolidol and D-limonene were capable of maintaining a decrease in blood pressure for a span of 360 min. A preparation containing 3.5% (*w*/w) of pectin was found to display strong mechanical strengths, flexibility, and high bioadhesiveness.	45, 47
Amidated pectin	Hydrogel matrix patch	Immunohistochemical studies revealed that the pectin hydrogel insulin patch potentially has the ability to transmit insulin, including controlled release, throughout the skin and into the blood stream.	46
Tamarind/ xyloglucan	Patch	Tamarind seed extract and clindamycin were used to construct a patch that was further used as a gelling agent. Tests concluded respectable drug release and promising efficiency in a performed antibiotics test.	48
Xanthan gum	Grafted matrix film	An in vitro release study acknowledged a 1:7.5 grafting ratio of xanthan gum to acrylamide resulted in high drug release profiles of atenolol.	49
Cellulose	Matrix film/ nanowhiskers	Film containing solely HPMC and glycerol (the plasticizer) yielded the greatest value for drug release versus various combinations including ratios of HPMC and Eudragit RS100 combined. Lower concentrations of HPMC would in turn lower the drug release percentage. Nanowhiskers yielded cytotoxic effects on human bronchial epithelial BEAS 2B cells, but not in nine other cell lines, and induced pro-inflammatory cytokine production in human monocyte-derived macrophages. Nanocellulose was not reported to produce cytotoxic or inflammatory effects on mouse and human macrophages.	50, 78–80
Bacterial cellulose	Matrix patch	Positive results for skin tolerance and permeation rate, comparable to commercial patches. The inclusion of glycerin to the compound resulted in a moisturizing effect.	51
Starch	Film/polymer material	Induces immune activation, positive cell adhesion, zero change to cell viability, can induce cytokine production.	74–77
Chitin	Beads, porous matricies, nanoparticles	Immune responses and cytokine profiles depend on physical characteristics. Non-cytotoxic to mouse and human fibroblasts.	81–86

reduce side effects of drugs in the body, and have been proven to do just that with at least the general cancer drug 5FU. More so, it has been suggested that they can also inhibit damage caused by reactive oxygen species.

Galipoğlu et al. utilized the acetylcholinesterase inhibitor donepezil in conjunction with a sodium alginate-based film. The sodium alginate base results in good bioadhesive character that retains a high bioadhesion value. Along with the worthy mechanical properties, the film, combined with a limonene permeation enhancer, delivered a feasible amount of 3% donepezil during in vitro studies. Furthermore, the authors stated the stratum corneum of their pig skin sample was more fluidized when in the presence of limonene, indicating a disruption of the barrier [37].

Bakrudeen et al. utilized starch nanocrystals (SNCs) as drug carriers in conjunction with hydrogel-based transdermal patches made from three different starches: maize, potato, and cassava. Briefly, SNCs are crystalline platelets formed during the hydrolysis step in starches when exposed to an acid concentration. The size and yield of SNCs are determined by the hydrolysis condition, or more specifically, the type of acid concentration, temperature, and time. The polymeric hydrogels derived from potato SNCs and maize SNCs were noted as having better stability during storage in comparison to the SNC formed from cassava. During this study, this was documented that the sample used in the release study resulted in a 95% release value during a time period of 17 h [38].

Chitosan has also been used as a polymer matrix for transdermal delivery. Can et al. described their findings of chitosan films, conjugated with Transcutol as a plasticizer and terpenes as a permeation enhancer, as having good mechanical properties as well as bioadhesiveness [39].

Escobar-Chávez et al. developed a patch that released a realistic flux of nortriptyline hydrochloride into human abdominal skin samples [40]. Paul et al. introduced silver nanoparticles to chitosan films, which ultimately increase flexibility, stability, and prolong lifespan of the film due to the silver's role as a binding agent. Furthermore, within this study, the silver nanoparticles were incubated with *Ganoderma lucidum* extract and the release of this compound was measured over time, yielding consistent release values during in vitro drug release studies [41].

Applications of natural polymers are shown in Table 4. Sodium alginate is said to be biocompatible, hydrophilic, biodegradable, and have bioadhesive properties. Babu and Rao (2015) synthesized a sodium alginate-L-cysteine transdermal patches and noted the effect it had on drug permeation in conjunction with losartan potassium as the example drug. The authors noted that drug permeation was higher in patches that contained conjugated sodium alginate, no matter the varying concentration of sodium alginate, in comparison to patches that contained only sodium alginate. It was theorized that this may be due to the conjugation itself, which replaced the primary groups with thiol groups, subsequently forming a loose polymer matrix, which then allowed for a quicker uptake of moisture at a larger amount. Furthermore, the conjugation, and its subsequent opening of the polymer chains that loosened the matrix, also will no longer resist the drug diffusion, allowing for greater permeability and a rapid release of the drug [42].

Cordia dichotoma fruit mucilage was reported as to having positive pharmacological and film-forming properties, and thus one study took this transdermal film and loaded it with alfuzosin hydrochloride, an α(1)-adrenergic blocker. In vitro studies illustrated a greater release of the loaded alfuzosin HCL as the concentration of *Cordia dichtoma* mucilage increased. Sodium alginate was used in this study to increase tensile strength, and it was found that increasing concentrations of sodium alginate in conjunction with increasing concentrations of mucilage potentially contributed to the enhanced dissolution [43].

Keshavarao et al. (2011) utilized sodium locust bean gum (LBG) and sodium alginate as biopolymers to construct a transdermal film loaded with piroxicam. A 12:80.5 *w*/w% LBG to sodium alginate mixture was noted as yielding the highest percentage of drug permeation as well as holding the highest percentage of drug. Further testing revealed this same ratio with 3% methanol exhibited impressive anti-inflammatory properties [44].

Güngör et al. (2008) used pectin to develop a matrix-type transdermal patch loaded with verapamil hydrochloride. Interestingly, the in vivo experiments depicting percutaneous absorption through rat skin showed that the verapamil hydrochloride patches, both with and without the permeation enhancers, significantly decreased systolic blood pressure after 30 min. Additionally, the patches loaded with nerolidol and D-limonene were capable of maintaining a decrease in blood pressure for a span of 360 min [45]. Hadebe et al. (2014) utilized amidated pectin-derived hydrogel matrix patches for transdermal delivery of insulin. Immunohistochemical studies revealed that the pectin hydrogel insulin patch potentially has the ability to transmit insulin throughout the skin and into the blood stream. More so, studies illustrated the patch can offer a controlled release of insulin, ultimately leading to concomitant alleviation of particular diabetic symptoms. Data revealed too that the patch caused change in blood glucose and plasma insulin concentration [46]. Bektas et al. (2014) developed a patch from pectin and focused on the transdermal delivery of nifedipine. A preparation containing 3.5% (*w*/w) of pectin was found to display strong mechanical strengths, flexibility, and high bioadhesiveness. However, further studies are required to determine the potential of pectin-based transdermal films loaded with nifedipine and their therapeutic use [47].

A clindamycin patch was created using extracts of tamarind seeds. Duangjit et al. (2015) used this patch as a novel gelling agent for transdermal drug delivery. A patch including a 4:6 ratio of glycerin to propylene glycol as a plasticizer and permeation enhancer, respectively, showed respectable drug release and promising efficiency in a performed antibiotics test [48].

Xanthan gum is an exopolysaccharide, largely produced by the bacterium *Xanthomonas campestris*, and can exist with a molecular weight ranging from 200 to 2000 kDa. Mundargi et al. (2007) grafted a matrix film from xanthan gum to focus on transdermal delivery of atenolol. The film carried a slightly amorphous, but uniform drug distribution. An in vitro release study acknowledged a 1:7.5 grafting ratio of xanthan gum to acrylamide resulted in high drug release profiles compared to other tested ratios performed in this study. It was noted that an increasing value for drug load also yielded an increasing value for drug release. Interestingly, atenolol, the hydrophilic drug used in this study, was seen to perform a type of interaction with the xanthan gum, leading to swelling and the formation of large pores. This consequently led to increased drug loading and increased release rate [49].

Cellulose is a commonly studied polysaccharide with high abundance in plant cell walls. Parhi and Suresh (2016) developed a diltiazem hydrochloride matrix film and performed various in vitro and in vivo studies. Results concluded that the film containing solely hydroxypropyl methylcellulose (HPMC) and glycerol (the plasticizer) yielded the greatest value for drug release versus various combinations including ratios of HPMC and Eudragit RS100 combined. Furthermore, data illustrated lower concentrations of HPMC would in turn lower the drug release percentage [50]. In Almeida et al.'s study (2014), cellulose derived from bacteria was utilized to formulate a matrix patch. Positive results for skin tolerance were recorded, further backing the general interest in bacterial cellulose as role players in transdermal or topical drug delivery. In addition to the patch's studied drug release ability, it was also seen that the inclusion of glycerin to the compound resulted in a moisturizing effect, giving thought to potential cosmetic uses as well [51]. Dong et al. (2012) examined the cytotoxicity of cellulose-derived nanowhiskers on nine separate cell lines. Zero cytotoxic effects were documented [78]. Conversely, Catalán et al. (2015) reported 100 μg/mL cellulose NWs and 50 μm microcrystalline cellulose (MCC) particles caused 55% of their human bronchial epithelial BEAS 2B cells to perish within 48 h [79]. In the latter study, the authors also reported the release of pro-inflammatory cytokines TNF-α and increased LPS-induced IL-1β release in human monocyte-derived macrophages where 300 μg/mL MCC's were involved. Vartiainen et al. (2012) reported no cytotoxicity or inflammatory responses from human and mouse macrophages when exposed to nanocellulose produced by fibrillating the fibers to produce particles [80].

Research involving starch nano materials has suggested they can induce immune responses, while still being considered non-toxic [73]. Torres et al. (2015) produced study utilizing Andean potato starch films concluded the films have the ability to interact with THP-1 monocyte membranes and induce immune activation. Additionally, it was shown the six tested starches did not affect cell viability [74]. Marques et al. (2002, 2004, 2005) examined the biocompatibility of starch-based polymers [75–77]. In summary, the authors tested two separate blends: starch/ethylene vinyl alcohol (SEVA-C) and starch/cellulose acetate (SCA), along with their composites conjugated with hydroxyapatite (HA). The results indicated the starch-based materials exhibited proper cytocompatibility as well as good adhesion to L929 mouse fibroblasts, with the authors noting the SCA surface experiencing more of an affinity from the aforementioned cells. The group then went on to compare a starch/polycaprolactone (SPCL) material and its HA composite with poly-L-lactide (PLLA) to examine cytokine production in vitro [77]. Production of both IL-6 and TNF-α was noted as high in all the tested conditions. IL-1β was produced in considerably low amounts, while no expression of IL-2 or IFN-γ was detected over the course of the 14-day experiment. Specifically, the authors concluded the cytokine production by the starch materials were lower in all conditions compared to PLLA. Interestingly too, the presence of HA resulted in a major reduction of inflammatory cytokines.

Chitin is a biopolymer that shares a similar chemical structure to cellulose, with the difference being acetamido groups in chitin where

hydroxyl groups in cellulose exist [73]. It has been reported that the size, source, purification method, and other traits pertaining to physical characteristics of chitin ultimately affect immune responses and cytokine profiles [73,81,82]. Van Dyken et al. (2014) reported chitin exposure induced expression of epithelial cytokines interleukin-25 (IL-25), IL-33 and thymic stromal lymphopoietin (TSLP). This in turn activated innate lymphoid type 2 cells (ILC2) to then express IL-5 and IL-13 [83]. Reese et al. (2007) similarly recorded chitin provoking the buildup of IL-4-expressing innate immune cells in mice tissues [84]. Outside of claims relating to immune responses, chitin matrices and nanoparticles have been documented as being non-cytotoxic to mouse and human fibroblasts, and L929 mouse cells, respectively [85,86].

5.1. Clinical studies involving polysaccharides

It is encouraging to see a large number of publications dedicate themselves to examining the potential benefits of polysaccharide-based drug delivery systems. Clinical results from drug-delivering polysaccharide formulations in the form of a film or patch are scarce. However, it seems feasible that research can build upon clinical data acquired by polysaccharides in other forms and then determine a suitable polymer for designs involving films. Kim et al. utilized sodium hyaluronate microparticles for a sustained-release formulation of recombinant human growth hormone (SR-rhGH). This Phase IV clinical trial had its patients receive a weekly SR-rhGH injection for a total of 12 weeks. The authors report serum IGF-levels had increased considerably while the patients received their weekly injection. More so, they concluded the results were comparable to data involving patients receiving daily growth hormone injections. Overall, the authors confirmed the efficacy and safety of these sodium hyaluronate microparticles [106].

Bacterial cellulose (BC) is a promising polysaccharide that has seen its use focused largely within the realm of permeable materials and wound healing. Clinical trials have treated patients experiencing non-healing lower extremity (LE) ulcers with BC wound dressings, known as Dermafill, following what the authors denoted as a standard care procedure. It was reported the application of the BC wound dressings reduced the time for 75% epithelialization down to 81 days on average, versus an average of 315 days [107,108]. Additionally, another clinical trial compared the wound healing of diabetic foot ulcers when utilizing either a BC wound dressing or Xeroform™ Petrolatum gauze. The application of the BC wound dressing on 15 separate ulcers all resulted in a faster rate of wound healing as well as a shorted time for epithelialization [107,109].

A level one randomized controlled trial (RCT) aimed to compare the effects on treatment with a silver-releasing hydroalginate dressing and an alginate control in patients with colonized chronic wounds in the form of pressure ulcers and venous leg ulcers. 99 patients were divided into two groups: those treated with Silvercel (test group) and those treated with Algosteril (calcium alginate dressing control group). The authors concluded the Silvercel dressing resulted in a faster closure rate as well as fewer cases of infection [110]. In a separate study, similar results were seen. This four-week randomized study evaluated the effect of a wound dressing comprised of silver alginate powder on patients with leg and foot ulcers. The test group ($n = 24$) would be treated with a foam dressing and silver alginate powder, and the control group ($n = 10$) was treated with the foam dressing and without the silver alginate powder. The authors reported the patients in the test group were seen to exhibit a significant reduction in symptoms relating to localized wound infection and wound surface area reduction. Additionally, those in the test group were not required to receive treatment for their wounds, whereas the control group were given antibiotics for treatment [111].

A recent clinical trial also utilized silver and its antimicrobial properties, but in conjunction with a hyaluronic acid topical spray powder in human lesions that showed signs of bacterial colonization. This 28-day experiment required patients to apply the spray daily on their own. On days 1, 7, and 28 of the experiment, such factors as wound size and bacterial load were evaluated. The authors denoted daily spray application begins to be effective after the first spray application. Furthermore, following the 28 days, not only did the test group exhibit a greater reduced area of the respective wound, but bacteria contamination, odor, exudate, and erythema also improved [112].

Zuckerman et al. reported the results of a phase I trial where the authors investigated the use of cyclodextrin-polyethylene glycol copolymer (CDP) nanoparticles initially formulated with siRNA as a human therapeutic [113,114]. This complex ultimately entered human trials under the name CALAA-01 with the goal of delivering RNAi to extrahepatic tumors [114]. A total of 24 patients received at least one dose of CALAA-01 to treat tumor types such as melanoma, gastrointestinal, and prostate. Ultimately, the trial was discontinued due to the progression of disease in seven patients, as was made evident by increased tumor size. While the full potential of the tool may not have been recognized in this study, the authors concluded that CALAA-01 is capable of targeted delivery of siRNA and delivery was found to be well tolerated during the initial dose [114]. Another example of CDP nanoparticles is seen in CRLX101, a conjugate of CDP and camptothecin (CPT). CPT is known as an anti-cancer agent that inhibits topoisomerase I [115]. In a phase I study conducted to investigate the safety, pharmacokinetics, dosing parameters, dosing toxicities, and maximum tolerated dose, CRLX101 was confirmed to regress disease with intravenous administration to a patient group [115]. Clinical studies further confirmed CRLX101 saw a 100-fold increase in solubility, increased circulation time, and reduced toxicity when compared to CPT alone [115,116].

Self-assembled cholesterol-modified pullulan (CHP) has shown great potential in forming complexes with and delivering peptides. A phase I clinical trial involved CHP as a carrier for HER-2 antigens to be introduced to nine HER2-expressing cancer patients. The CHP-HER2 vaccine was injected three times in a two-week window at a dose of 300 μg. The authors reported the vaccine was well tolerated with a lone patient experiencing a side effect in the form of a skin reaction at the site of injection. More so, the CHP-HER2 vaccine prompted HER2-specific $CD8^+$ and/or $CD4^+$ T-cell immune responses [117]. Uenaka et al. employed the CHP platform to deliver NY-ESO-1 antigen to esophageal cancer patients. Again, $CD4^+$ and $CD8^+$ T cell responses were noted in patients who were given the vaccine [118]. Kageyama et al. similarly examined the effect of the CHP-NY-ESO-1 complex vaccine on patients with advanced/metastatic esophageal cancer or melanoma. The authors confirmed the safety of the vaccine along with concluding a 200 μg dose induced antigen-specific immune responses [119].

A phase I trial utilizing dextran in the form of a product called DE-310 aimed to evaluate the maximum-tolerated dose, toxicity in a test group experiencing advanced solid tumors. DE-310 specifically acts as a topoisomerase I inhibitor. 86 administrations were given over 27 total patients as 3-h infusions once every 2 weeks. A single patient from this study with metastatic adenocarcinoma succeeded in complete remission, while another patient with metastatic pancreatic cancer achieved partial remission. The authors noted 14 other patients within their test group had stabilized disease progression [120].

Chitosan and radioisotope holmium-166 are the primary components to a compound termed Milican. A phase IIb clinical study oversaw 40 total patients with single hepatocellular carcinoma. The patients were noted as to either having refused surgery or were considered a poor surgical candidate. The results indicated exceptional efficacy and long-term safety for patients experiencing the aforementioned carcinoma. However, it was determined a phase III trial that included a larger population would be warranted to determine the effectiveness of treatment with Milican [121].

Diabecell contains insulin-producing porcine islet cells within alginate-based microcapsules to combat type-1 diabetes [116,122]. OligoG CF-5/20 is an alginate-based nanotherapeutic utilized with the goal of treating cystic fibrosis [123]. The preliminary information of Diabecell indicates the safe use of the drug delivery system, as well as

Table 5
Examples of Polysaccharide-based Drug Delivery Systems in Clinical and Human Trials.

Polysaccharide	Formulation/Administration	Disease/Condition	Development stage	References
Sodium hyaluronate	Intravenous	Growth-hormone release	Phase IV	106
Bacterial cellulose	Wound dressing	Lower extremity ulcers	Open label study	107, 108, 109
Silver alginate/hydroalginate	Wound dressing Powder	Lower extremity ulcers	Open label study	110, 111
Hyaluronan	Topical spray	Pressure/Venous leg ulcers	Open label study	112
Cyclodextrin	Intravenous	Solid tumors, Varying forms of cancer	Phase I, II	113–116
Pullulan	Intravenous	Peptide delivery, Varying forms of cancer	Phase I	117–119
Dextran	Intravenous	Advanced solid tumors	Phase I	120
Chitosan	Intravenous	Hepatocellular carcinoma	Phase IIb	121
Alginate	Xenotransplantation	Type-I diabetes	Phase I, II, III	116, 122
	Inhilation	Cystic fibrosis	Phase I, II	123

the suggestion that there exists a long-term benefit of better blood glucose control. Additionally, those behind Diabecell report the possibility of reduced insulin requirements [122]. A clinical study employing OligoG CF-5/20 as an inhaled polymer therapy for cystic fibrosis patients confirmed the device's ability to alter the structure of mucus, modulate mucin assembly, and diminish the adhesive and viscous properties of the patient's sputum [123]. (See Table 5.)

6. Conclusion

Microbial exopolysaccharides offer an incredibly diverse range of functions and uses. Their physical and chemical compositions offer flexibility and can be easily altered to adapt to requirements. Furthermore, the ease behind reproducibility stands above current polymers utilized in the field of drug delivery. Individually, the varying types of extremophiles that can produce polysaccharides are all naturally equipped with specific benefits and abilities that can make sure any biomedical requirement can be covered by at least one type of extremophile. Perhaps the most important feature utilizing exopolysaccharides has to offer is that they are generally regarded as safe. Synthetic polymers currently used in drug delivery applications have been noted to being related to toxicity and immunogenicity issues. Exopolysaccharides have been recorded to degrade within the body by biological processes without actually altering their own chemical structure. Indeed, there are still current issues revolving around the use of exopolysaccharides in relevant fields. It is of high importance that all natural polymers being considered for use in drug delivery demonstrate their in vivo bioavailability pertaining to humans, as well as their properties relating to absorption, distribution, metabolism, and elimination. Likewise, production methods capable of producing an industrial-sized quantity of exopolysaccharides and exopolysaccharide conjugates are currently a limiting factor. Research today involves searching for microbes proficient in producing a large consistent quantity of extracellular polymeric substances. Nevertheless, exopolysaccharides have shown promising therapeutic use, as films and in other formations, and have been recorded as to being strong contributing factors to positive results involving present medical situations.

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Declaration of Competing Interest

None.

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