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Review

The production and application of bacterial exopolysaccharides as biomaterials for bone regeneration

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ABSTRACT

Bacterial exopolysaccharides (EPS) are water-soluble polymers consisting of repeating sugar moieties that serve a wide range of functions for the bacterial species that produce them. Their functions include biofilm matrix constituent, nutrient retention, protection from environmental threats and even pathogenicity. EPS have also been exploited for use in various applications in the biomedical field: most notably as viscosupplements, drug delivery vehicles and in tissue engineering constructs. The use of EPS in bone tissue engineering has increased in recent years due to the wide range of compounds available, low cost, and ease of production on an industrial scale. This review discusses the extraction and purification methods employed to produce bacterial EPS. A particular focus is on bone-related tissue engineering applications where EPS is the primary active agent, or as a scaffold matrix, as well as a carrier for osteopromotive agents.

1. Introduction

Bacterial exopolysaccharides (EPS) are water-soluble polymeric sugars produced by bacteria that are either attached to the external cell membrane or exported outside of the bacteria and associated with the external surface. As a whole, EPS show a great diversity in monomer composition and molecular weight (MW) (Nwodo et al., 2012) as well as biosynthesis pathways (Schmid et al., 2015). EPS fulfills many different roles for the bacteria depending on the species and the environmental conditions in which they are growing. The most commonly ascribed functions of bacterial EPS is a food and water reserve, and protection from environmental stressors (Freitas et al., 2017). For pathogenic bacteria, EPS can offer protection from host defenses (Limoli et al., 2015), or increase its pathogenicity (Rudolph et al., 1994). Moreover, EPS are one of the main constituents of bacterial biofilms, which ensure bacterial adhesion to surfaces and are particularly important in device-associated infections (Limoli et al., 2015).

Despite their microbial origin, some bacterial EPS have very similar or identical structures to the polysaccharides contained in the human body, which contains many polysaccharides for example in the glycocalyx of its cells, with new research showing its importance in cell biology (Mockl, 2020).For example, hyaluronic acid (HA) is naturally produced by both bacteria and mammals and have similar biosynthetic pathways in both. However, the functionality of any given EPS can vary depending on the species from which they were extracted (Yoshioka et al., 2019). In the human gut for example, bacteria play a crucial role in digestion and help protect the organism against pathogenic bacteria (Van Zyl et al., 2020) and play a role in systemic inflammation (Mazmanian & Kasper, 2006). Many of these activities are at least partially mediated by EPS (Schiavi et al., 2016).

Critical size bone defects originating from pathology or trauma can cause functionality impairment in patients (Nauth et al., 2018). Autograft is most often employed to replace the missing bone tissue (Fernandez de Grado et al., 2018) due to its biocompatibility and osteoconductivity (Faour et al., 2011). However, comorbidity at the donor site (Fernandez de Grado et al., 2018) limits the amount of material available. Allograft and xenograft, bone from a human or animal donor respectively, can be used as an alternative, but they have a high cost (Saikia et al., 2008) while both carrying risks of disease transmission (Campana et al., 2014; R. Gunzburg et al., 2002), and immunogenicity in the case of xenografts (Saikia et al., 2008). Synthetic bone substitutes based on various calcium phosphates ceramics, bioglasses and cements as well as polymeric scaffolding are alternatives (Fernandez de Grado et al., 2018) which can also be used as carriers for pro-healing

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biologics such as stem cells, growth factors and drugs. Polysaccharides have been exploited in a number of biomedical applications related to tissue engineering such as osteoarthritis, wound healing and drug delivery (Shih, 2010). Their application in bone applications most commonly involves their use as tissue engineering scaffolds, or as a carrier encapsulating calcium phosphate or bioglass particles, as well as osteogenic compounds and drugs.

EPS may also be used in these applications, as an inert component of the construct, chosen for their suitability rather than their biological effects. However, the great structural and biological variability of bacterial EPS, combined with similarity in composition to the human extracellular matrix (ECM), leaves the possibility that some EPS may have a direct positive biological effect *i.e.* as the active agent itself (Moscovici, 2015). The possibility to harvest bacteria in controlled cultures and produce EPS on an industrial scale, without relying on animal sources, makes bacterial EPS a sustainable option for large scale production and, therefore, for clinical implementation (Freitas et al., 2017). As is the case for any form of tissue regeneration, the immune response to the implanted material plays a critical role in bone regeneration. An excessive or chronic inflammation in response to an immunogenic material can prevent the tissue from entering the resolution phase, thus impairing the formation of new tissue. While bacterial exopolysaccharides can have a structure close to the human extracellular matrix, some other bacterial products in certain strains can be very immunogenic, like it is the case with lipopolysaccharides (endotoxin). For this reason, the production and purification process of bacterial EPSs are paramount because small contaminations can greatly influence the immune response of the body and thus bone healing in a negative way. This review investigates the use of bacterial EPS, with a particular focus on their application in bone tissue engineering and regeneration. Early stages in the processes, including production and purification will be briefly described as they can greatly influence EPS properties and their biological functionality. Subsequently, the uses of bacterial EPS for bone applications are reported with discrete sections covering their use as scaffolds, the chemical modifications used to increase functionality, as well as the direct biological effects on cells of the skeletal and immune systems.

2. Bacterial EPS production

Bacteria produce EPS in periods of nutrient abundance to serve as a water or carbon reserve and can help the bacteria adhere to surfaces and survive nutrient limitation (Delattre et al., 2016; Donot et al., 2012). They can also serve as a protection from environmental stressors such as temperature, pH, osmotic stress, UV light, desiccation, oxidants and heavy metals (Delattre et al., 2016; Donot et al., 2012; Moreno et al., 1998; Moscovici, 2015; Papinutti, 2010). Based on their monomeric units and linkage bonds, bacterial homopolysaccharides can be divided in 4 groups: a-D-glucans, b-D-glucans, polygalactan and fructans, whilst heteropolysaccharides can be composed of D-glucose, L-rhamnose, D-galactose, glucuronic acid, *N*-acetylgalactosamine and *N*-acetylglucosamine repeating units, and include non-carbohydrate substituents (Nwodo et al., 2012).

The production of EPS by bacteria is both strain/species and polysaccharide dependent and is achieved through one of 4 different pathways. The synthase pathway is most often used to produce homopolymers within the cell and excrete them into the extracellular space, as is the case for cellulose (Rehm, 2010). In this pathway, the formation of the polymer and the crossing of the inner cellular membrane is achieved by a synthase protein, part of a multi-protein complex glycosyltransferase (Czaczyk & Myszka, 2007). The second pathway, the sucrase pathway, is primarily used to produce homopolysaccharides in the extracellular space through the use of glycansucrase enzymes (Rana & Upadhyay, 2020). Disaccharides are first converted to monosaccharides and then attached to a growing polymer chain through the transfer of their glycosyl residues. The glycansucrase catalyzes the reaction using energy released from sugar hydrolysis (Kumar et al., 2011). Dextran is an example of an EPS produced through the sucrase pathway (Dols et al., 1998). The ATP-Binding cassette (ABC) pathway is a pathway used to produce capsular EPS (Nwodo et al., 2012), which are bound to the membrane (Cescutti, 2010) and often involve heteropolysaccharides. Glycosyltransferases first assemble the polysaccharide at the inner cellular membrane, then a tripartite efflux pump complex facilitates the migration of the polymer at the cell surface (Zhang, Fan, et al., 2011). The particularity of this pathway is the integration of a glycolipid at the end of the polymer chain, keeping it attached to the cell membrane (Rana & Upadhyay, 2020). The last pathway is the Wzx/Wzy pathway, which starts with a nucleotide activated sugar forming a phosphate linkage with a membrane associated lipid carrier. More sugars are sequentially linked to it by glycosyltransferase to create repeating units, which are transported through the cytoplasmic membrane by WZx flippase. Wzy proteins then polymerize the oligosaccharides into polysaccharides before their transport outside of the cell (Sutherland, 1990). This pathway allows the production of highly diverse EPS such as xanthan (Vorhölter et al., 2008).

Numerous factors influence bacterial EPS production, such as the cell growth phase, nutrient availability, and the presence of environmental stressors (Fig. 1). For most strains, EPS production occurs at the end of the exponential growth phase or during the stationary phase of growth (Sengupta et al., 2018). However, for some EPS such as levan (Rutering et al., 2016), the EPS is produced during the growth phase as EPS secreting enzymes are produced concomitantly with cell replication (Esawy et al., 2013). Two of the biggest factors influencing EPS production are the presence of carbon and nitrogen in correct ratios, with the highest production generally occurring with abundant carbon and limited nitrogen availability (Freitas et al., 2017). During the stationary phase, the carbon source is used for EPS production and a higher carbon availability usually leads to a higher yield. Simple sugars are more easily broken down by bacterial enzymes than complex ones, leading to an easier utilization and higher yield (Celik et al., 2008). A nitrogen source is necessary, however in high quantity, nitrogen can favors cell growth with lower EPS yield like in the case of FucoPol production by *Enterobacter A47* (Torres et al., 2014). Phosphorus, potassium, magnesium and metal cations are also necessary for EPS production and bacterial growth (Freitas et al., 2017; Survase et al., 2007a, 2007b). Temperature and pH also play an important role in EPS production but are both strain dependent, and can differ from the optimal conditions for cell growth. For example, Glucunobacter hansenii grow best at pH 4 and 30 °C, while its highest EPS yield is at pH 5 below 30 °C (Valepyn et al., 2012). However, for many species, such as *Streptococcus thermophilus* (Zisu & Shah, 2003), an acidic stress can increase EPS production. Other factors influencing EPS production have been explored, such as the presence of light for symbiotic and photosynthetic strains (Zisu & Shah, 2003), microwave radiation (Kothari et al., 2014; Kushwah et al., 2013), magnetic field (Xu et al., 2014) and sound frequencies (Sarvaiya & Kothari, 2015; Shah et al., 2016). Culture parameters can also influence the composition and MW of the produced EPS. For example, the pH and temperature of culture both influenced sugar composition, acyl group composition and MW of the EPS in *Enterobacter A47* (Torres et al., 2012), and magnetic field changed both the charge and adsorption properties of an EPS from *Bacillus cereus CrA* (Xu et al., 2014).

Bacterial EPS are produced on a large scale for use in medical, cosmetic and food applications. Generally, EPS production takes from 0.5 to 7 days, but can be as short as 8 h for *Streptococcus zooepidemicus* (Vazquez et al., 2009) and as long as 20 days for *Ganoderma lucidum* (Papinutti, 2010). The yield varies significantly between species and can be as low as 0.29 g/L for *Streptococcus zooepidemicus* (Benedini & Santana, 2013) and 100 g/L for *Bacillus methylotrophycus* (Zhang et al., 2014). The sugars most used for culture are glucose and sucrose, as their simplicity make them easily usable by the bacteria. However it is possible to use alternative sources for some bacteria, such as by-products and waste products from food processing (Antunes et al., 2015; Antunes

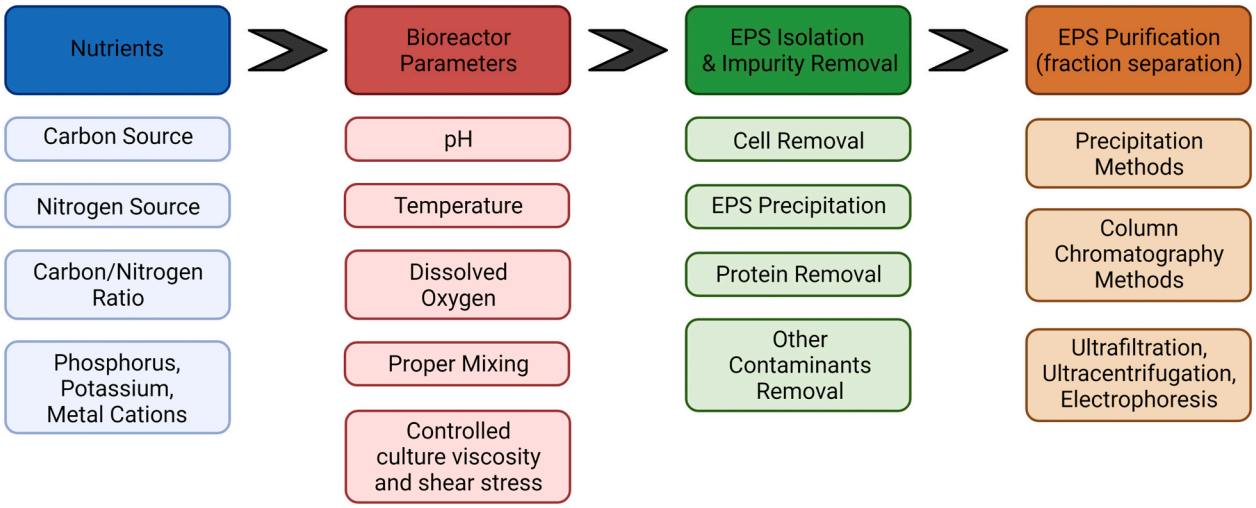

Fig. 1. Scheme of the main parameters involved in bacterial EPS production and purification optimization.

et al., 2017), agriculture (Mehta et al., 2014) and other industries, such as glycerol (Freitas et al., 2009). Another major factor for EPS production by certain species is ensuring proper aeration and agitation of the culture, leading to a better nutrient and oxygen distribution. In some species, strong agitation was shown to increase EPS production, as reported for HA (Liu et al., 2008) and gellan (Prajapati et al., 2013), while in others, growth in low oxygen levels was beneficial, *i.e.* alginate production (Cimini et al., 2012). However, excessive agitation can damage the bacterial cell (Palaniraj & Jayaraman, 2011) and change the properties of the resultant EPS (*e.g.*, reduced MW (Galindo et al., 2007)).

Several technologies can be employed for EPS production. At laboratory scale, the bacteria can be cultured in shake flasks (Aman et al., 2012; I. L. Shih et al., 2009; Yang et al., 2016), where nutrients ratio are optimized to encourage EPS production after bacterial growth and nitrogen depletion (Seviour et al., 2011). While easy to apply, this approach lacks dynamic control over culture conditions. Solutions to overcome this include replacing part of the media regularly which was done to produce levan from *Bacillus subtilis* (I. L. Shih et al., 2010) or continually supplementing the culture with new media, which can however lead to more risk of contamination and reducing yield (Seviour et al., 2011). For larger scale or industrial production, bioreactors are often used as they bring the possibility to control nutrient, oxygen and heat diffusion (Freitas et al., 2017). One of the most used bioreactor design is the continuous stirred tank reactor, in which the mixing of the broth is ensured by a stirring turbine, however the high shear can sometime lead to a degradation of the EPS (Freitas et al., 2017). Air lift reactors are another commonly used design which allows to work in lower shear conditions, for example to produce pullulan from *Aureobasidium pullulans* (Ozcan et al., 2014). Some major challenges in using bioreactors are properly controlling the shear rate inside the tank and the viscosity of the solution, as it influence mixing efficiency, heat and mass transfer in the broth (Freitas et al., 2017). This is particularly the case for high yield strains where the viscosity of the broth dramatically increases as the production progresses. The challenges related to operating bioreactors have been described in great detail (Seviour et al., 2011).

The last steps of bacterial EPS production are their purification and removal of impurities, as the final broth contains bacterial cells, proteins, minerals, other polymers, and contaminants that are generally not desired in the final product. The first step usually involves the removal the bacterial cells from the solution through centrifugation or filtration (Freitas et al., 2017). The EPS can then be precipitated from the supernatant, usually through the addition of a water-miscible polar solvents such as ethanol, isopropanol or acetone (Freitas et al., 2017). However, precipitation is not specific to the desired polysaccharide and can cause *co*-precipitation of other polysaccharides and proteins, and may also decrease yields. A viable alternative to the precipitation method is to use ultrafiltration with a membrane with an appropriate cutoff MW (Ziadi et al., 2018). Further details on polysaccharide purification are described in detail in the following review (Shi, 2016). The removal of proteins is also possible through chemical methods combined with centrifugation, such as the Sevag method taking advantage of chloroform for protein denaturation (He et al., 2012; Zhu et al., 2016), the trichlorotrifluorethane method and the trichloroacetic method (Chambi et al., 2021; Oliveira et al., 1999; Pintado et al., 2020) leading to protein precipitation. It can also be achieved through enzymatic methods, where proteases digest or hydrolyze the proteins in solution (Wugeditsch et al., 1999; K. W. Yu et al., 2004). Deproteinization can also be achieved by membrane-based processes (Freitas et al., 2011). Other impurities such as monosaccharides, oligosaccharides, inorganic salts and low MW non polar substances can be taken out of the solution by using dialysis methods (Chaplin & Kennedy, 1994; J. Fang & Wang, 1997). Whilst an alternative technique for the removal of inorganic salt can also be the use of ion exchange resins (Shi, 2016).

In addition to impurity removal, it is possible to purify mixed polysaccharides into several homogenous polysaccharides through various techniques. One method uses the formation of metallic coordination compounds, where various metal ions such as copper, barium, calcium or lead are added to precipitate with the polysaccharide, which is latter decomposed with an acid (Shi, 2016). Due to different ion concentrations needed to precipitate polysaccharides, they can be selectively precipitated (Chaplin & Kennedy, 1994; H. L. Chen & Fang, 1997; J. Fang & Wang, 1997). The gradual precipitation method takes advantage of the lower solubility of high MW polysaccharides in acetone and ethanol compared to low MW polysaccharides. This allows successive precipitations and separation of polysaccharides by their MW, by gradually adding solvent and collecting the precipitate (Chaplin & Kennedy, 1994). The salting out method uses a similar principle by taking advantage of the different solubilities of polysaccharide fractions with different MW when in solution with a given salt concentration. The use of neutral salts such as potassium chloride, sodium chloride, ammonium sulfate allows different polysaccharide fractions to precipitate as they are added to the solution, which can be used to separate fractions by MW (Shi, 2016), the salts being removed latter through dialysis. Another method uses long chain quaternary ammonium salts to form coordination compounds with acidic or high MW polysaccharides, which cannot dissolve in low ionic strength solutions. By adjusting the solution ionic strength, pH or solvent, it is possible to selectively dissolve

different polysaccharide fractions, which can then be isolated from the various supernatant fractions (Shi, 2016). This method is very effective at low polysaccharides concentrations (Liu et al., 2015; Zhang et al., 2016). Ultracentrifugation can be used to separate polysaccharides with different MW due to different sedimentation speed, either by centrifuging and collecting the polysaccharide pellet at gradually higher speed, or by centrifuging at extremely high speed and separating the pellet in different zones afterwards (H. L. Chen & Fang, 1997; J. Fang & Wang, 1997). Ultrafiltration can be used to separate polysaccharides by choosing a membrane with an appropriate cut-off MW (Baptista et al., 2022), but can be limited by the adsorption of the polysaccharide on the membrane and the crossing of linear polysaccharides with MW above the cut-off MW (Shi, 2016). The electrophoresis method, which is explained in detail here (Shi, 2016), uses an electrical field to separate polysaccharides based on their shape, charge and MW, and was used for EPS purification (Volpi, 2004). Finally, column chromatography methods are currently the most used for polysaccharide purification due the simplicity of the process and their efficient purification (Shi, 2016). Cellulose column chromatography is efficient at separating polysaccharides based on MW as low MW fractions elute before high MW fractions (Li et al., 2018). Anion exchange column chromatography is widely used to separate acidic and neutrally charged EPS (Zhu et al., 2022), where acidic polysaccharides are preferentially adsorbed in the columns due to their acidic group, but can be later eluted by adapting the pH and ionic strength of the buffer (Shi, 2016). Size-exclusion chromatography can be used as a secondary tool to purify polysaccharides based on their shape and size (Shi, 2016). Affinity chromatography takes the advantage of the reversible binding of certain polysaccharides with another specific molecule (Steinmetz et al., 1995). If the proper column is found for one of the polysaccharides in solution, it will bind to the column while the other polysaccharide fractions are eluted, and can later be dissociated by changing the pH and ionic strength of the buffer (Shi, 2016). The choice of the purification method may influence the final composition (Ziadi et al., 2018), MW, functionality and impurity profile of the EPS which in turn can significantly impact its biological effect as seen with HA (Yoshioka et al., 2019). The purification method was also shown to have a great impact on properties of other types of biopolymers (Ghori et al., 2017), and its effect on the final polysaccharide functionality should carefully be assessed.

Taken altogether, bacterial EPS production is a complex process that needs to be adapted specifically for each polysaccharide or bacterial strain, but it can lead to high yield and pure product when all parameters are optimized.

3. Bacterial EPS for bone applications

A relatively small number of bacterial EPS have been extensively used in the biomedical field (Mohd Nadzir et al., 2021). The most commonly used include cellulose, dextran, xanthan gum, hyaluronic acid, alginate, kefiran, gellan, levan and curdlan. Their applications are numerous, some examples being tissue engineering scaffolds, carriers for various drugs and biological factors, intra-articular injections, anti-tumor agent, wound dressing, mucosal adjuvant, hypocholesterolonic agent and adhesive, cancer therapeutic agent or antioxidant. The following section summarizes studies where bacterial EPS have been utilized for bone regeneration purposes. Several EPSs have been applied as a structural scaffold for bone defect filling, but also as delivery vehicle carrying and releasing osteogenic factors, cells and inorganic particles. These include hyaluronic acid, bacterial cellulose, gellan and dextran. The following section summarizes studies where bacterial EPS have been utilized for bone regeneration purposes. Several EPSs have been applied as a structural scaffold for bone defect filling, but also as delivery vehicle carrying and releasing osteogenic factors, cells and inorganic particles. These include hyaluronic acid, bacterial cellulose, gellan and dextran.

3.1. Overview of the most commonly used bacterial polysaccharides in the biomedical field for bone application

Hyaluronic acid (HA) is an anionic, non-sulphated heteropolysaccharide of the glycosaminoglycan family, with a repetitive motive composed of D-glucuronic acid and *N*-acetyl-D-glucosamine linked with alternating β-(1 → 4) and β-(1 → 3) glycosidic bonds (Fig. 2) (Witzler et al., 2019). It is naturally present in the extracellular matrix of humans - only in small quantities in the bone ECM (Vejlens, 1971a) - and is particularly important in epithelia, synovial fluid and articular cartilage, where it supports lubrication, cell migration (Litwiniuk et al., 2016) and hydration (DeVore et al., 1994). These beneficial effects are the reason HA is commonly applied as visco-supplement or for cosmetic purposes. It has, therefore, become an important macromolecule industrially, where it is most often produced by the bacterium *Streptococcus zooepidemicus* (Witzler et al., 2019). It is usually sterilized by optimized high-temperature steam sterilization since its properties are negatively affected by gamma irradiation and ethylene oxide (Haridas & Rosemary, 2019). The strong moisturizing effect is due to the presence of the hydroxyl group (Zhai et al., 2020) and functional groups such as carboxylic acid, hydroxyl and amide group allow for easy chemical modification (Hemshekhar et al., 2016). The chemical modification of HA has been discussed in length elsewhere (Schanté et al., 2011), but typically proteins and drugs have been tethered to HA through covalent bonding and ionic interactions (Schanté et al., 2011).

Bacterial cellulose is a homopolysaccharide composed of linear chains of β-(1 → 4) linked glucose residues (Fig. 2) that differs from plant cellulose in MW, and has higher crystallinity and purity (Shih, 2010). It is usually produced on an industrial scale by *Acetobacter xylinum*, but can also be produced by *Gluconacetobacter, Pseudomonas, Sarcina, Azobacter, Rhizobium*, and *Agrobacterium* species (Torgbo & Sukyai, 2018). The bacteria generally use cellulose for biological, mechanical or chemical protection from their natural environment, or increasing cell adhesion necessary for pathogenic and symbiotic purposes (Ross et al., 1991). It is biocompatible, can be made porous, and sterilized by steam or gamma radiation without damaging its structure (Czaja et al., 2007; Shih, 2010). It showed mild antibacterial-properties against *Staphylococcus aureus* and *Escherichia coli* (Zhang et al., 2010), two strains commonly involved in human infections, including bone infection. It also has a slow degradation rate *in vivo*, which may be useful for certain biomedical applications. Degradation of bacterial cellulose may be accelerated *in vivo* by a limited periodate oxidation, forming dialdehyde bacterial cellulose which is significantly more degradable in body fluid (Hutchens et al., 2009). Moreover, bacterial cellulose fibers have a similar morphology to the collagenous fibers contained in bone (Shi, Aid, et al., 2012), high mechanical strength and very good water retention capabilities (Helenius et al., 2006) and allows cell attachment and growth (Zverlov & Schwarz, 2008), all of which make it an attractive material for bone regeneration purposes. Its ordered fibrous structure can ensure good nutrient and oxygen diffusion as well as metabolic waste removal, which is essential to avoid local apoptosis of cells (Shi, Li, et al., 2012).

Gellan is a linear tetrasaccharide whose repeating unit is composed of D-glucose, D-glucuronic acid and L-rhamnose with a 2:1:1 M ratio, and is produced by *Sphingomonas paucimobili (*Shih*, 2010)*. It has been used for tissue engineering mainly because of its biocompatibility, biodegradability, similarity with the human ECM structure and ease of modification (Costa et al., 2018). While it lacks specific motifs for cell adhesion (Stevens et al., 2016), the presence of hydroxyl and carboxyl groups in its structure enables functionalization to overcome this issue (Costa et al., 2018). Gellan is a thermo-responsive polymer, allowing gellan/calcium chloridemixtures to be injected and form a gel when cooling down to body temperature, with the possibility of incorporating cells in the mixture slightly above 37 °C (Oliveira et al., 2010).

Dextran is an homopolysaccharide of D-glucose molecules which are connected by consecutive α(1 → 6) linkages, and it has various degrees of branching in the (1 → 4) and (1 → 3) positions (Fig. 2) (Shih, 2010). It

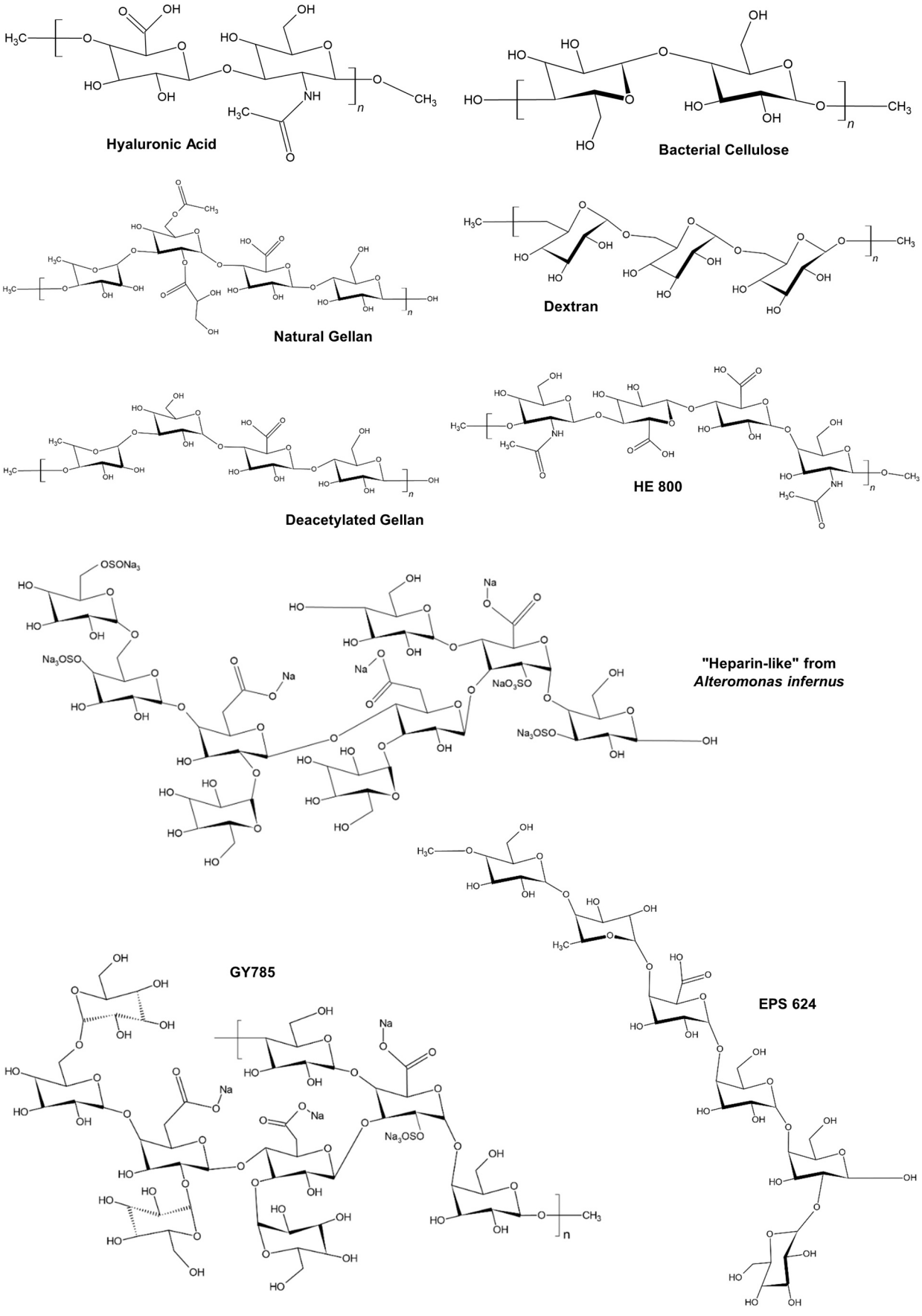

Fig. 2. Chemical structure of bacterial exopolysaccharides used for bone regeneration

is mainly produced by *Leuconostoc mesenteroides*, however it is also produced in industrial scale by *Streptococcus mutans* and *Lactobacillus brevis* (Bacakova et al., 2014). It has been used extensively for biomedical applications as a plasma volume expander, rheological enhancer of artificial tears or as an antithrombolytic compound (Johnson, 1990; Maia et al., 2014). It has a good biocompatibility and is slow to degrade by human enzymes. Dextran contains a large number of hydroxyl groups, making derivatization and further chemical or physical cross-linking simple. Moreover, it showed bacterial and protein inhibition properties, and its hydrophylic groups can provide anti-adhesive properties (Holland et al., 1998; Vacheethasanee & Marchant, 2000). It also has several surface binding sites in its polymeric chain, giving it the potential for a high binding surface concentration of bioactive molecules (Shi et al., 2009). Therefore, dextran hydrogels have regularly been used as drug carrier agents.

3.2. The use of bare unmodified bacterial EPS as a bone defect filler or tissue engineering scaffold material

The most commonly reported application of EPS in tissue engineering is as a scaffold material. This approach involves producing a porous EPS matrix that can be introduced in a defect and allow cells from the body to progressively colonize the material and start building new bone in the porosities offered by the scaffold, whether it is degradable or not. Scaffolds can act as carriers for calcium phosphates, bioglass or osteogenic factors, but this section focuses on studies using bare and unmodified bacterial EPS. Section 3.5 will focus on bacterial EPS with direct effect on bone. Table 1 summarizes the advantageous properties and direct biological effects for bone applications of the bacterial EPS described in this review. Fig. 3 summarizes the use of bacterial EPS for bone tissue engineering and tissue regeneration including bare unmodified EPS as well as unmodified EPS carriers, modified EPS carriers and EPS with biological properties as discussed later.

Several studies used dextran in combination with pullulan, an EPS produced by the fungus strain *Aureobasidium pullulans*. When studied *in vitro*, dextran/pullulan fibers supported the growth of endothelial cells (Shi, Aid, et al., 2012), and dextran/pullulan scaffolds supported the viability, proliferation and function of human endothelial progenitor cells, required for the vascularization of the scaffold (Lavergne et al., 2012). Another study used water insoluble polyvinyl formal sponges coated with dextran, which were air dried and seeded with rat bone marrow cells (BMCs) before being subcutaneously implanted in rats for 4 weeks (Yoshikawa et al., 2010). Dextran coating was used to counteract the low cell adhesion properties of the polyvinyl formal sponge by providing easily accessible functional groups to the cells. When the dextran coating was used, both osteocalcin and calcium levels were significantly higher, moreover osteogenesis inside the sponges could be observed. This study suggests a direct positive influence of dextran on polyvinyl sponges for bone tissue engineering when used as a surface coating, mainly through increased adhesion of BMCs.

Bacterial cellulose was also studied in a bone guiding construct *in vivo*. Cellulose membranes have been used to cover a bone defect filled with calcium phosphates particles (Lee et al., 2015; Lee et al., 2017), supporting bone repair by keeping the calcium phosphate particles in place. Due to their microporous fibrous structure, resorbable bacterial cellulose membranes with optimized thicknesses can have a good permeability (Lee et al., 2017) ensuring a good fluid, nutrient and gas exchange and act as guide for bone regeneration as effectively as collagen membranes (Lee et al., 2015).

3.3. The use of bacterial EPS as a carrier

Bacterial EPS have also been researched as carrier materials for a variety of materials and compounds, such as calcium phosphates or bioglass, as well as osteogenic compounds such as growth factors, and enzymes. The most common rationale for the use of bacterial EPS rather

Table 1
List of EPS used for bone regeneration, their advantages and positive effects on bone biology.

Exopolysaccharide	Advantageous properties	Direct biological effects
Hyaluronic acid	• Natural part of the ECM (Vejlens, 1971b) • Strong moisturizing effect (hydroxyl groups) (Zhai et al., 2020) • Easy modification (M. Hemshekhar et al., 2016) • Easy combination with proteins or drugs (Carole E. Schanté et al., 2011) • Effective to load calcium phosphate and osteogenic factors	• Enhance osteoblast precursor and early osteoblast differentiation (Hempel et al., 2012; Hempel et al., 2014) • Enhance osteogenic effect of BMP-2 (Kawano et al., 2011) • Degradation products enhance angiogenesis (West et al., 1985)
Bacterial cellulose	• High crystallinity and purity compared to plant cellulose (Shih, 2010) • Biocompatible, porous, easy to sterilize (Czaja et al., 2007; Shih, 2010) • Slow but adjustable degradation rate (Hutchens et al., 2009) • Similar morphology to collagenous fibers contained in bone (Shi, Aid, et al., 2012) • High water retention (Helenius et al., 2006) • Good mechanical properties (Helenius et al., 2006) • Allow cell attachment (Zverlov & Schwarz, 2008) • Easily combined with calcium phosphate and osteogenic factors	• None described
Gellan	• Biocompatible, biodegradable (Costa et al., 2018) • Similar with the human ECM (Costa et al., 2018) • Easy modifications (Costa et al., 2018) • Form gel when cooling down to body temperature (Oliveira et al., 2010) • High affinity with calcium ions (carboxyl units) (Douglas,	• Increases HDMECs growth when added to alginate/calcium phosphate scaffolds (Ahlfeld et al., 2017)

(*continued on next page*)

Table 1 (*continued*)

Exopolysaccharide	Advantageous properties	Direct biological effects
	Wlodarczyk, et al., 2014) • Easily crosslinked (Douglas, Wlodarczyk, et al., 2014) • Forms mineral layer when put in body-fluid (Douglas, Wlodarczyk, et al., 2014)	
Dextran	• Good biocompatibility (Johnson, 1990; Maia et al., 2014) • Slow degradation rate (Johnson, 1990; Maia et al., 2014) • Easy chemical modification and crosslinking (hydroxyl groups) (Johnson, 1990; Maia et al., 2014) • Bacterial inhibition and anti-adhesive properties (Holland et al., 1998; Vacheethasanee & Marchant, 2000) • High binding site concentration, allowing high drug loading concentrations (Shi et al., 2009)	• Increases osteocalcin and calcium levels in polyvinyl scaffolds (Yoshikawa et al., 2010)
HE 800	• Composition close to the human ECM (Senni et al., 2013) • Linear structure and anionic properties which could encourage interaction with the rod-like cationic structure of fibrillar collagen (Senni et al., 2013) • High calcium binding capacity (Zanchetta et al., 2003a) • High molecular weight could encourage growth factors binding and protection from enzymatic degradation (Zanchetta et al., 2003a)	• Promotes collagen structuring and ECM settling by fibroblasts (Senni et al., 2013) • Enhanced bone healing, osteocyte inclusion and bone neovascularization compared to collagen in 5 mm rat parietal defect (Zanchetta et al., 2003a)
GY785	• Its negatively charged structure favorizes interactions with the cationic amino-acid groups of proteins and thus their adsorption (Rederstorff et al., 2011)	• Increases MC3T3-E1 and C28/I2 cell viability more than hyaluronic acid when added to hydroxypropylmethylcellulose scaffolds (Rederstorff et al., 2011) • Favorize MC3T3-E1 and C28/I2 cell attachment (Rederstorff et al., 2011)
MK1 EPS	• None described	• Increases bone and angiogenesis when added to calcium phosphate in a rat calvarial defect model (Park et al., 2016)
K5 EPS	• None described	• K5 EPS modified by *N*-deacetylation and N-sulfation inhibits bone resorption activity of human osteoclasts *in vitro*, reduces osteolytic lesion area and tumor burden in bone when inoculated daily to mice in an *in vivo* mouse breast cancer model. (Pollari et al., 2012)
EPS624	• None described	• Prevents osteoclast formation from murine bone marrow precursors through a TLR-2 dependent pathway, both under normal and TNF-a induced inflammatory conditions (Wallimann et al., 2021)
"Heparin-like" from Alteromonas Infernus	• None described	• Increases survival rate from osteosarcoma in mice (Velasco, Baud'huin, et al., 2011)

than other excipients are: improved mechanical cohesion and injectability, biodegradation or dissolution for tissue repair, drug loading and release.

3.3.1. *EPS as a carrier for calcium phosphate particles*

Due to their osteoconductive, and sometimes osteoinductive capacities, and a chemical composition close the mineralized part of the bone, calcium phosphate-based granules are the gold standard at the present time for bone regeneration. Bacterial EPS have been combined with calcium phosphate particles and used as an additive to provide physical cohesion, enhance mechanical properties, provide degradable channel between particles and thereby modulate subsequent cell invasion, and additional drug delivery capacity.

For example, in a dental application, hyaluronic acid/beta-tricalcium phosphate (HA/BCP) particles loaded with recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) were placed in a rat tibial defect (Lee et al., 2014) before inserting a screw implant into the defect. The choice for HA in this application was due to it being anionic and capable of forming ionic bonds with the rhBMP, which is cationic. The rhBMP group showed an increased osseointegration of the implant, however the HA/BCP group did not show any improvement compared to the control group, implying that HA itself did not have osteoinductive properties apart from its ability to carry osteogenic factors. In another study, a cement with an aqueous liquid phase based on HA, citric acid and sodium phosphate dibasic and a solid phase containing α-tricalcium phosphate, calcium carbonate and monocalcium carbonate could be injected as a paste for a time window of 8–17 min and self-cured after 10 min at 37 °C (Landeck et al., 2021). Hyaluronic acid was added because its anionic carboxylate groups can chelate dissolved calcium ions and hydrogen bond with protonated phosphate ions, forming an organic mineral interface and because its affinity with calcium ions stabilizes early stage calcium phosphate crystallization, preventing aggregation. It reached a maximum compressive strength of 8.20 ± 0.95 MPa, which is similar to cancellous bone. When used *in vivo* in a critical sized distal femur defect in rabbits, it showed biocompatibility, osteoconductivity, new bone formation and normal bone architecture after 6 weeks and up to 26 weeks. In another study (Cui et al., 2021), the addition of 1% of HA to a tetra-calcium phosphate/dicalcium phosphate cement significantly increased its compressive strength and conversion rate to hydroxyapatite, by accelerating dissolution of the initial calcium phosphates subsequently increasing hydroxyapatite growth. It also promoted ALP activity, osteogenic related mRNA and protein expression of hBMSCs, with an optimal enhancement of their osteogenic differentiation with a 4% HA addition. When implanted *in*

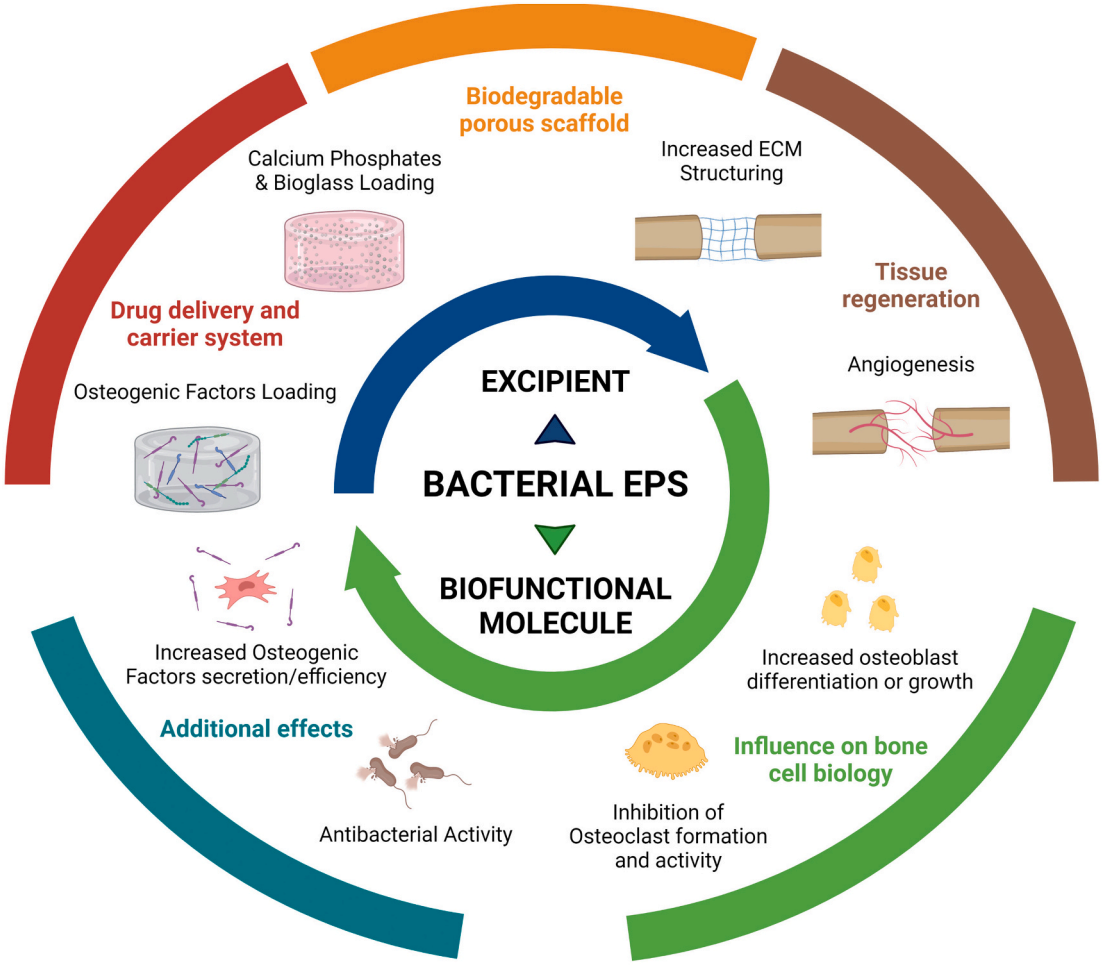

Fig. 3. Overview of bacterial EPS use in bone TE and regenerative applications.

vivo in rat tibial defects, the 4% HA group showed an increase in bone repair and osteogenic gene expression, which the author attributed to a more favorable environment for cell attachment and differentiation and the increase in osteogenic promoting factors secretion and osteogenic genes expression.

Bacterial cellulose was also successfully combined with various type of calcium phosphates mineral phases. In one study, a laser patterning technique was used to prepare a porous honeycomb structure (300 μm) with bacterial cellulose (Favi et al., 2016). When this process was combined with periodate oxidation and nano-hydroxyapatite coating, mechanical properties similar to the cancellous part of the bone were obtained. Human mesenchymal stem cells (MSCs) adhered and were viable both on the bacterial cellulose and the composite scaffolds. The influence of bacterial cellulose on the mechanical properties and bioactivity of calcium phosphate cements has also been studied (Zhang, Lei, et al., 2019). Addition of a small amount of bacterial cellulose doubled the cement compressive strength, due to the high strength of its fibers and the strong interaction between the bacterial cellulose hydroxyl group and the calcium(II) ion in calcium phosphates, leading to a robust matrix/fiber interface. The composite group showed spreading of osteoblasts (MC3T3s) and a higher metabolic activity after 5 days *in vitro*. The addition of hydroxyapatite to bacterial cellulose membranes (Tazi et al., 2012) has been shown to increase osteoblast adhesion and growth *in vitro*. An *in vivo* study in rats used a bacterial cellulose/nano-hydroxyapatite membrane to cover 4 mm holes in the cortical bone of the tibia and filled with blood clots (Saska et al., 2011). Histological measurements showed a higher bone formation after 1, 4 and 16 weeks for the membrane group, with the bacterial cellulose being partially degraded and replaced by new bone. Another study used an *in vivo* rat calvarial defect either filled with bacterial cellulose or bacterial cellulose coated with hydroxyapatite (Ahn et al., 2015). Both groups showed new bone formation after 4 and 8 weeks, however, the addition of the hydroxyapatite again improved outcomes as expected.

The combination of gellan with various calcium phosphates was investigated in several studies. In one example, gellan crosslinked by calcium chloride was combined with hydroxyapatite (Manda et al., 2018). Mineral deposition of the scaffolds in simulated body-fluid was observed after 14 days and increased with calcium chloride crosslinking. Raw gellan scaffolds also supported the adhesion and spreading of human stem cells incubated in osteogenic media for 21 days, and the bioactivity was both increased by the addition of hydroxyapatite and calcium chloride. Another study mixed gellan with alpha-tricalcium phosphate (α-TCP) (Douglas et al., 2018). Due to the release of calcium ions by the α-TCP, the researchers could obtain a gelation time of 30 min of the gellan matrix and observed a conversion of α-TCP to hydroxyapatite. A study reported the use of 3D printed scaffolds combining a calcium phosphate ink (inorganic phase made of α-TCP, calcium hydrogen phosphate, calcium carbonate and precipitated hydroxyapatite), alginate and gellan loaded with Vascular endothelial growth factor (VEGF), which successfully increased human dermal microvascular endothelial cells (HDMECs) growth when exposed to release media (Ahlfeld et al., 2017).

Dextran was also combined with calcium sulfate or phosphate. In an *in vitro* model of rat parietal defects, combining calcium sulfate hemihydrate and negatively charged dextran beads led to a better fibrous and bone repair than when using the materials separately or in the control

group (Snyders et al., 1993). Authors postulated that the addition of dextran increased porosity, allowing vascular and cellular ingrowth and promoting the calcium sulfate hemihydrate resorption, increasing the implant replacement by new tissue. When combined with nano-hydroxyapatite, dextran/pullulan hydrogels showed positive results both *in vitro* and *in vivo* (Fricain et al., 2013). When implanted subcutaneously in mice, the unloaded scaffold retained endogenous BMP and VEGF and supported tissue mineralization. It also induced highly mineralized tissue formation in a critical bone defect in rat femoral condyle, a goat transversal mandibular defect and a goat tibial osteotomy.

This literature shows that better bone healing can be achieved when using EPS with a particulate calcium phosphate. The main benefit of the EPS is likely coming from the creation of interstitial space between calcium phosphate particles, allowing better gas and nutrient diffusion and cell migration through the implant as the EPS is degraded. Other potential mechanisms include modulation of surface topochemistry, change in calcium availability, and modulation of the calcium phosphate solubility (Flautre et al., 2003).

3.3.2. Use as a carrier of bioglass particles

Bioglass is a group of glass materials composed of calcium and phosphorus with additional elements such as silicon. They have higher solubility and faster degradability than inorganic crystalline materials. They have been used extensively for bone regeneration and their combination with bacterial EPS has been used to overcome certain aspects such as their brittleness. For example, bioactive glass particles were added to gellan (Douglas, Piwowarczyk, et al., 2014; Gantar et al., 2014), which increased its mineralization *in vitro* and supported the growth of human adipose derived stem cells (Gantar et al., 2014) and rat MSCs (Douglas, Wlodarczyk, et al., 2014).

Similarly, in an *in vivo* model of bony defect in the lateral femoral condyles of adult New Zealand white rabbits, dextran increased cohesion and handling properties of bioglass without altering its bioactive properties, and leading to a complete healing after 6 weeks (Chan et al., 2002). Another study used crosslinked dextran scaffold, loaded with various types of bioglass nanoparticles and freeze-dried (Nikpour et al., 2018). Porous scaffolds were mineralized after immersion in body fluid and bioglass particles increased proliferation of human osteoblasts and alkaline phosphatase (ALP) levels *in vitro*.

3.3.3. Use as carrier for osteogenic molecules and ions

Osteogenic factors are commonly used additions to bone tissue engineering constructs as they stimulate cell differentiation and activities towards a bone phenotype and increase biomineralization. The most commonly used factors for bone applications include bone morphogenetic proteins (BMPs), platelet rich plasma, platelet derived growth factors and fibroblast growth factors (Nauth et al., 2011).

In one study an *in situ* gelling Hyaluronic acid/Alginate containing BMP-2 and vancomycin was prepared by simple mixing for osteomyelitis treatment (Jung et al., 2019). The gel had a gelation time of 4 min being sufficient for handling and injection in an osteomyelitis lesion and the BMP-2 and vancomycin both showed continuous release *in vitro* for 6 weeks. When used in a rat osteomyelitis femur model for 6 weeks, the vancomycin/BMP-2 loaded group showed higher bone density and bone biomechanical strength than the vancomycin loaded group and untreated osteomyelitis group, showing a specific positive effect from BMP-2 release in addition to the antibacterial effect of vancomycin.

Bacterial EPS matrices have been effectively used as carriers for osteogenic factors such as BMP-2 due to their binding capacities. For example, bacterial cellulose scaffolds showed increased biocompatibility and increased differentiation of precursor cells to osteoblasts when loaded with BMP-2 (Shi, Li, et al., 2012). When implanted subcutaneously *in vivo* in rats, both groups supported bone formation after 2 and 4 weeks, but new bone and calcium concentration increased with BMP-2 addition. In another study, rhBMP-2 loaded bacterial cellulose sheets were implanted *in vivo* in a sinus bone defect in rabbits (Koike et al., 2019). After both 4 and 8 weeks, the unloaded bacterial cellulose group showed a higher new bone formation than the control group, and this was increased when rhBMP-2 was loaded into the cellulose. The authors mentioned a sustained BMP-2 release from the bacterial cellulose but did not conclude if the increased bone formation of unloaded bacterial cellulose sheets compared to the control was due to bacterial cellulose itself or the presence of the porous structure given by the cellulose sheets. In another study, bacterial cellulose was pulverized to a fine paste to create emulsions that were later freeze dried to form scaffolds with a highly porous nanofibrous matrix, with the goal of facilitating further cell migration into the scaffold (Dubey et al., 2021). The scaffolds supported the adhesion, growth and infiltration of mesenchymal stem cells *in vitro*, which was attributed to the extra-cellular matrix mimicking architecture at both macro and micro level. When the cells were preconditioned with 50 ng/mL BMP-2 before seeding, an enhanced bone matrix secretion and maturation was observed. Another example is gellan being particularly effective for loading with alkaline phosphatase (ALP), an enzyme promoting mineralization (Orimo, 2010). Due to its carboxyl units, gellan has a high affinity with calcium (Douglas, Wlodarczyk, et al., 2014) allowing easily crosslinking and mineralization in body fluid. Several studies took advantage of this properties by mineralizing ALP loaded gellan hydrogels in calcium glycerosphosphate (Douglas, Wlodarczyk, et al., 2014) or media with various Ca/Zn proportions (Douglas et al., 2017), however *in vivo* data has not yet been published.

3.4. Modification of bacterial EPS used as carriers for calcium phosphates, bioglass and osteogenic compounds

While non-modified bacterial EPS have been used in a variety of studies, many others involve chemical modifications to the EPS to optimize mechanical properties, inclusion of cell binding sites, increased osteogenic binding capacities or adjust degradation profiles.

3.4.1. Modified bacterial EPS as calcium phosphate carriers

Several studies used modified bacterial EPS in combination with calcium phosphates. For example, modified hyaluronic acid-g-chitosan-g-poly(*N*-isopropylacrylamide) was combined with biphasic calcium phosphate (Chen et al., 2013). The use of an *N*-isopropylacrylamide functionalization was chosen to give thermoresponsive properties to the hydrogel for ease of CaP dispersion and handling. The addition of biphasic calcium phosphate increased human fetal osteoblast cells proliferation, calcium deposition, ECM mineralization and messenger ribonucleic acid (mRNA) osteoblastic markers. Another study used crosslinked urethacrylate dextran/polyacrylamide on which nano-hydroxyapatite was mineralized *in situ* (Fang et al., 2019).

A bacterial cellulose/β-glucan composite crosslinked by free radical polymerization was used to load hydroxyapatite nanoparticles with graphene oxide before freeze drying (Khan et al., 2021). Acrylic acid and N′ *N*-methylene-bis-acrylamide were respectively used as monomer and crosslinker, with potassium persulfate used as an initiator. The scaffolds showed a spongy-porous morphology with compressive strengths varying between 3.90 MPa and 12.13 MPa and also supported the adhesion and proliferation of the MC3T3-E1 osteoblast cell line *in vitro*. Moreover, when exposed to PBS for 30 days, the scaffolds decreased in weight by between 20% and 38%, with a decreasing degradation as graphene oxide increased. Another study used calcium chloride partially crosslinked TEMPO-oxidized bacterial cellulose/alginate hydrogels to create 3D-printed scaffolds (Abouzeid et al., 2018). The TEMPO-oxidization was used to introduce carboxyl groups, supporting the creation of a crosslinked network with alginate through calcium chloride crosslinking. High compressive strengths between 419 MPa and 455 MPa were reached for the printed composite scaffolds, and hydroxyapatite formation through biomineralization in simulated body fluid was confirmed. In another study, the effectiveness of two bacterial cellulose

modification methods on bone regeneration were compared in bacterial cellulose-based gelatin scaffolds (Wang et al., 2021). One chosen method was the TEMPO-oxidization (TEMPO-BC) due to its ability to give cellulose a sheer-thinning behavior which is a benefit for 3D printing, as well as its capacity to increase cellulose dispersibility in aqueous solutions due to the numerous carboxyl groups. The second chosen method used a maleic acid process (MA-BC), as it also allowed the introduction of carboxyl group but with a more environmentally friendly process than TEMPO-BC. Materials prepared by both methods maintained good osteoblast viability *in vitro*, but MA-BC increased the expression of osteogenic markers and mineralized nodule formation *in vitro* more than TEMPO-BC. Both methods stimulated bone regeneration in a rat calvaria bone defect *in vivo*, but MA-BC showed a higher bone mineral density and trabecular thickness of newly formed bone. Dextran was chosen to overcome the limitation of polyacrylamide, as dextran's high MW allows high stiffness after crosslinking and it has high affinity with cells. The hydroxyapatite mineralization increased compressive strength to 6.5 MPa, promoted osteoblast proliferation and differentiation *in vitro* and led to the formation of a highly mineralized bone tissue in an *in vivo* rabbit femoral condyle defect.

3.4.2. Modified bacterial EPS used as carrier of osteogenic compounds

Due to the variety of chemical groups polysaccharides can present, chemical reactions can be used to functionalize them and enhance their properties. For example, by covalently linking peptides containing the Arginine-Glycine-Aspartate (RGD) sequence to its carboxylic groups through carbodiimide chemistry, gellan can be modified to increase cell adhesion and enhance its biological performances (Bacelar et al., 2016). HA can be modified in many ways by targeting the primary and secondary hydroxyl groups, the glucuronic acid carboxylic acid group and the *N*-acetyl group after de-amidation. Each of these modifications have been reported extensively (Burdick & Prestwich, 2011), with several studies featuring modified HA carrying osteogenic compounds. In one example, 2-aminoethyl methacrylate HA loaded with BMP-2 and Growth differentiation factor 5 (GDF5) could successfully be coated on zirconium dioxide and crosslinked with UV-light (Bae et al., 2013), leading to a continuous release for 28 days. Moreover, the adsorption of the albumin protein was significantly increased, which was shown to influence the differentiation and proliferation of osteoblast-like cells (MG-63) (Huang et al., 2011). In another study, aldehyde modified HA with increased tissue adhesion was modified by incorporating an aminoglycerol side chain and then crosslinking (Martinez-Sanz et al., 2011). When loaded with BMP-2, a 28 days release was achieved *in vitro* and more new bone formation was achieved after 8 weeks in a rat calvarial defect. Another study used scaffolds composed of maleimide functionalized HA mixed with a cell adhesive peptide and gelled with an MMP sensitive peptide, which were then loaded with BMP-2 and Stromal Cell-Derived factor 1-alpha (SDF-1) chemokine (Holloway et al., 2015). Maleimide functionalization was chosen as the thiols contained in the cysteine residues from the peptides lead to fast crosslinking with the maleimide functional group through an addition reaction. The authors introduced mitochondrial processing peptidase (MPP) sensitivity to the hydrogel to create a dynamic growth factor release in response to proteases, which they confirmed by testing different collagenase concentrations. When tested in a rat calvarial defect model, it showed a synergistic action of BMP-2 and SDF-1 leading to a significantly higher bone formation. In another study, a hyaluronic acid tyramine/chondroitin sulfate tyramine hydrogel crosslinked through horseradish peroxidase loaded with BMP-2 supported BMSCs differentiation *in vitro*, as well as bone formation in a rat femur bone defect model (Zhang, Chen, et al., 2019). The polymers were chosen to combine the relatively high stiffness of HA and good energy dissipation of chondroitin sulfate. In another study, pyrogallol-conjugated hyaluronic acid was created by conjugating 5-hydroxydopamine hydrochloride through EDC/NHS chemistry before incorporating hydroxyapatite or whitlockite with or without BMP in the solution. This was frozen and lyophilized to create patches that were crosslinked by spraying a solution containing sodium periodate (Choi et al., 2020). The adhesive patch showed a prolonged release of BMP-2 for up to 28 days, enhanced osteogenic differentiation of human stem cells *in vitro*, and when implanted in rat calvarial model *in vivo*. The patches promoted bone regeneration, with significantly more bone formation with the addition of BMP-2.

Dextran can be functionalized to form many derivatives, however its most common modifications are esterification and the formation of ethers (Heinze et al., 2006). A study mixed dextran and functionalized derived dextran hydrogels to load rhBMP-2 (Maire et al., 2005). Dextran functionalization was achieved through substitution of native dextran with carboxylate, sulfate and benzylamide groups as the functionalized dextran showed binding capacity to heparin binding growth factors such as the transforming growth factor-beta-1 (TGF-B1) for example. The ratio of the dextran functionalization controlled the retention of growth factors and *in vitro* release kinetics, leading to a higher osteoinduction and calcification when implanted in an *in vivo* rat ectopic model. Another study used BMP-2 functionalized oxidized dextran coating on titanium alloys (Shi et al., 2009). The dextran was oxidized to create aldehyde groups and was then grafted to the titanium through a reductive amination reaction, and BMP-2 were immobilized on the dextran through a secondary reductive amination reaction. Compared to pristine substrates, the dextran coated groups showed decreased bacterial adhesion *in vitro*, and increased BMP-2 loading density, which promoted osteoblast spreading, ALP activity and calcium mineral deposition. In another study (Chen et al., 2007), glycidyl methacrylated dextran/gelatin hybrid hydrogels containing glycidyl methacrylated dextran/polyethylene glycol microspheres loaded with BMP-2 successfully supported bone formation when implanted for 8 weeks in a periodontal defect in dogs. The liquid/liquid immiscibility of the glycidyl methacrylated dextran and polyethylene during crosslinking was exploited to promote the development of an interconnected microporous structure. In another study, polyglucose-sorbitol-carboxymethyl (PSC) prepared from dextran was successfully used to create iron(III) oxide/PSC nanoparticles in the context of iron accumulation related osteoporosis (P. Yu et al., 2020). This condition can be a side effect of iron supplementation, when excessive iron ions reach the bone tissues, causing cells to create excessive reactive oxygen species (ROS) which in turn inhibit osteogenesis and increase osteoclast differentiation, thus disrupting the bone metabolism balance and favoring osteoporosis. Compared to the Fe2O3 group, the iron(III) oxide/PSC particles were effective *in vitro* in reducing ROS levels and osteoclasts differentiation, increasing osteogenic differentiation, and preventing iron accumulation-related osteoporosis in a mouse model.

Overall, the EPS used as an additive for bone applications modulate cell or protein adsorption, controlling the retention and release of osteogenic compounds or optimizing mechanical properties of the construct, rather than a direct biological mechanism of action. The functionalization of bacterial EPS reported thus far demonstrate the versatility of pure or modified EPS, however, there are a number of studies where EPS itself is the active agent.

3.5. Bacterial EPS showing a direct effect on bone biology

High molecular weight HA has been reported to enhance the osteogenic effect of BMP-2 (Kawano et al., 2011). Culturing MG63 cells with both BMP-2 and HA led to an increase in BMP-2-induced ALP activation, nuclear translocation and Smad 1/5/8 phosphorylation *via* the down regulation of BMP-2 antagonists and extracellular signal-regulated kinase (ERK) phosphorylation, as shown by mRNA expression and Western blot analysis (Kawano et al., 2011). In another study, the degradation products of HA have been shown to enhance angiogenesis *in vivo* on chick chorioallantoic membranes, especially for hyaluronate fragments containing 4 to 25 disaccharides (West et al., 1985). While not directly related to bone biology, this shows a potential benefit as pro-angiogenic factors will indirectly support bone regeneration. Some

studies have shown that sulfated and over-sulfated glycosaminoglycans, as well as the non-sulfated HA, could enhance the differentiation of osteoblasts precursors and early osteoblasts (Hempel et al., 2012; Hempel et al., 2014), although the precise mechanism of this is unknown. Nevertheless, the authors stated "The sulfate group in C-6 position of the N-acetyl-glucosamine seems to be mandatory for the pro-osteogenic effect of sulfated GAG derivatives" (Hempel et al., 2014), and hypothesized that the resemblance of these matrixes to the bone marrow environment was responsible for their influence on osteogenic differentiation (Hempel et al., 2014). Additionally, oversulfation of glycosaminoglycans leads to a better affinity with BMPs (Chen et al., 2021), which was for example demonstrated for oversulfated chondroitin sulfate with BMP4 (Miyazaki et al., 2008).

HE800 (Hyalurift®) is an EPS produced by *Vibrio Diabolicus*. It is a high MW heteropolysaccharide with a linear non-sulfated tetrasaccharidic repeating unit that includes the same amount of lucuronic acid and hexosamine (Senni et al., 2013). This composition can be considered as the association of the repetitive units of non-sulfated chondroitin and HA (Senni et al., 2013), which are major components of the human ECM. An *in vitro* study showed that its addition to a collagen matrix seeded with human fibroblast cells promoted both collagen structuring and ECM settling by the fibroblasts (Senni et al., 2013). The authors hypothesized that the increase in collagen structuring was due to the linear structure and anionic properties of HE800, allowing it to interact with the rod-like cationic structure of fibrillar collagen, which could encourage the deposition of tropocollagen fibrils. An *in vivo* study on a 5 mm rat parietal bone defect showed a significantly better bone defect closure when the defect was filled with HE800 (95.9%) compared to collagen (17.8%), as well as enhanced osteocyte inclusion and trabecular bone neovascularization, with living osteoblasts adhering to the external bone surface (Zanchetta et al., 2003a). The authors proposed that the improved bone closure was due to its high MW (800 kDa) that may enhance growth factor binding and protection from enzymatic degradation, in addition to its high calcium binding capacity. In another similar *in vivo* study, the bone defect closure was better on the empty contraleateral side (100%) compared to filled with HE800 (90.1%) (Zanchetta et al., 2003b). In the absence of any HE800, the healing in the empty defect was only 16% suggesting the HE800 was enzymatically cut and transported through the blood, increasing bone healing in the contralateral side. They further hypothesized the anti-inflammatory and angiogenic properties of HE800 were due to it mimicking the effect of the dextran derivative RTGA11 as has previously been shown for heparin.

GY785 is an EPS produced by *Alteromonas Infernus*. It is a negatively charged, branched nonasaccharide of high MW (Rederstorff et al., 2011) and mainly contains glucose, galactose, glucuronic and galacturonic acids and various amounts of rhamnose (Roger et al., 2004). An *in vitro* study showed that when added to a sylated hydroxypropylmethyl cellulose based hydrogel, it increased the cell viability of MC3T3-E1 incorporated into scaffolds after 48 h (Rederstorff et al., 2011). What is particularly interesting is that the viability was higher when adding GY785 compared to when adding HA. GY785 also favored the attachment of osteoblasts when cultured in 2D, which the authors attributed to the polysaccharide's negative charge interacting with the cationic amino-acid groups of proteins and thus increasing their adsorption. The effect of GY785 derivatives on osteosarcoma was also investigated (Heymann et al., 2016). After depolymerization and substitution reactions, oversulfated GY785 of different MW were used, which were found to inhibit murine and human osteosarcoma cell line invasiveness and migration *in vitro*. The EPS effectively inhibited the development of lung metastases from osteosarcoma in an *in vivo* mouse model, however they did not show a curative effect on the primary osteosarcoma tumor. In another study *in vitro*, the over sulfated EPS inhibited BMSC proliferation during osteoblastic differentiation through inhibition of mitosis, as well as mineralized nodule formation in pre-osteoblasts, and receptor activator of nuclear factor kappa-B ligand (RANKL) induced osteoclastogenesis and was shown to bind with immobilized RANKL (Velasco, Baud'huin, et al., 2011). The authors also showed that long-term administration of the over sulfated EPS in mice led to cancellous bone loss, which was partly attributed to an increase in the number of osteoclasts lined on the surface of the trabecular bone. In a mouse mice lung model of osteosarcoma metastasis (Velasco, Colliec-Jouault, et al., 2011), the groups treated with the original and over-sulfated EPS showed an increased survival rate as well as a decrease in metastatic nodules for the over-sulfated EPS, as well as a delayed growth of a direct tumor growth model.

MK1 EPS is produced by a bacteria isolated from the Neungee Mushrooms. It is a heteropolysaccharide composed of glucose, rhamnose, mannose, galactose, and glucosamine with an aproximate respective molar ratio of 1.0:0.8:0.7:0.29:0.21, and a MW of 10–55 Dka (Park et al., 2016). In an *in vivo* rat calvarial defect model filled with biphasic calcium phosphate granules, the addition of the MK1 EPS led to a significant increase in bone and blood vessel formation, showing a direct positive influence (Park et al., 2016). Based on their phosphorylation data, the authors suggested the underlying mechanism was related to the mitogen activated protein kinase (MAPK) signaling, a pathway promoting cell growth and proliferation activated by mitogens and growth factors, but not the serine/threonine protein kinase (AKT) signaling, promoting survival and growth in response of an external stimulus.

K5 EPS is produced by *Escherichia coli* O10:K5:H4. It is a heteropolysaccharide composed of *N*-acetylglucosamine and glucuronic acid with 1:1 M ratio with an average MW of 20KDa (Vann et al., 1981). K5 EPS was modified by *N*-deacetylation and *N*-sulfation (Casu et al., 1994) to form a heparin like polysaccharide K5-NSOS, which does not have the strong anticoagulant properties of heparin, which is a positive asset as anti-coagulants can have negative side effects (Pollari et al., 2012). *In vitro* experiments showed its ability to inhibit bone resorption activity of human osteoclasts through non-toxic effects, as well as TGF-β andIL11 production in highly aggressive and invasive breast cancer model cell lines (MDA-MB-231 (SA)) (Pollari et al., 2012). The authors hypothesized that highly sulfated exogenous heparan sulfates could interfere with the physiologic heparan sulfate/TGF-β interactions which in turn would impact TGF-β signaling. In an *in vivo* mouse breast cancer bone metastasis model, the daily inoculation of K5-NSOS was found to reduce osteolytic lesion area, tumor burden in bone and weight loss (Pollari et al., 2012). The authors suggested that the MW and degree of sulfation of the EPS played a major role in this effect, and that further optimization of these two parameters could reduce even more their anticoagulant activities while keeping their effect on bone biology.

EPS624 is an EPS produced by the probiotic strain *Bifidobacterium longum* subsp. *longum* 35,624. It is a negatively charged polysaccharide whose repeating unit is composed of two galactose, two glucose, one galacturonic acid and an unusual 6-dexy-L-talose (Altmann et al., 2016). Wallimann et al. showed prevention of osteoclast formation from murine bone marrow precursors by the EPS, through a toll-like receptor-2 (TLR-2) dependent pathway, which was observed both under normal and tumor necrosis factor alpha (TNF-α) induced inflammatory conditions (Wallimann et al., 2021). Other studies have also shown that stimulating TLR-2 through bacterial cell wall compounds, such as lipoteichoic acid (LTA) and peptidoglycans from *Staphylococcus aureus* or LTA and LPS from *Porphyromonas gingivalis* could prevent osteoclast formation by interfering with RANKL signaling (Souza & Lerner, 2019; Zhang, Liu, et al., 2011). Interlekin-10 (IL-10) was shown to inhibit osteoclast formation in bone marrow cultures and when co-culturing hematopoietic spleen cells with BMSCs, but has no effect on mature osteoclasts (Owens et al., 1996). This is potentially a very positive asset for bone regeneration, as temporarily reducing osteoclast formation could theoretically limit bone catabolism and lead to an increased bone formation. When used in osteogenic BMSCs cultures, EPS35624 also slightly increased mineralized matrix deposition (Wallimann et al., 2021).

4. Conclusions

In conclusion, Bacterial EPS are widely used in bone tissue repair as scaffold matrix, to deliver calcium phosphates, bioglass, osteoinductive compounds and mineralization factors. While fulfilling an important role for bone regeneration purpose, EPS in this basic form, has not necessarily be proven to outperform other polysaccharides and biopolymers, and can show the same limitations. These include limited mechanical properties, an internal structure and porosity differing the ones of the bone, improper degradation rate relative to the new bone formation, burst release when loading osteogenic factors and low cell attachment in some cases. Improved efficacy of bacterial EPS in the context of bone regeneration is often achieved through their modification, for example by increasing the cell or protein adhesion to the EPS, tuning its degradation profile, changing its mechanical properties, and modifying its binding capacity to osteogenic compounds.

More interestingly, a subset of bacterial EPS have been reported to have a direct biological effect in the context of bone or tissue engineering. Bacterial EPSs remain an underutilized source of structural and biofunctional polysaccharides, but the careful evaluation of bacteria glycocalyx has the ability to impact the tissue repair field, as recent scientific advances in the mammalian glycocalyx functions have shown its roles in cell morphology, membrane protein diffusion, cancer development and regulation of the immune system.

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References

Abouzeid, R. E., Khiari, R., Beneventi, D., & Dufresne, A. (2018). Biomimetic mineralization of three-dimensional printed alginate/TEMPO-oxidized cellulose nanofibril scaffolds for bone tissue engineering. *BioMacromolecules, 19*(11), 4442–4452.

Ahlfeld, T., Akkineni, A. R., Forster, Y., Kohler, T., Knaack, S., Gelinsky, M., & Lode, A. (2017). Design and fabrication of complex scaffolds for bone defect healing: Combined 3D plotting of a calcium phosphate cement and a growth factor-loaded hydrogel. *Annals of Biomedical Engineering, 45*(1), 224–236.

Ahn, S. J., Shin, Y. M., Kim, S. U., Jeong, S. I., Jeong, J. O., Park, J. S., & Lim, Y. M. (2015). Characterization of hydroxyapatite-coated bacterial cellulose scaffold for bone tissue engineering. *Biotechnology and Bioprocess Engineering, 20*(5), 948–955.

Altmann, F., Kosma, P., O'Callaghan, A., Leahy, S., Bottacini, F., Molloy, E., & O'Mahony, L. (2016). Genome analysis and characterisation of the exopolysaccharide produced by Bifidobacterium longum subsp. longum 35624. *PLoS One, 11*(9), Article e0162983.

Aman, A., Siddiqui, N. N., & Ul Qader, S. A. (2012). Characterization and potential applications of high molecular weight dextran produced by leuconostoc mesenteroides AA1. *Carbohydrate Polymers, 87*(1), 910–915.

Antunes, S., Freitas, F., Alves, V. D., Grandfils, C., & Reis, M. A. (2015). Conversion of cheese whey into a fucose- and glucuronic acid-rich extracellular polysaccharide by Enterobacter A47. *Journal of Biotechnology, 210*, 1–7.

Antunes, S., Freitas, F., Sevrin, C., Grandfils, C., & Reis, M. A. (2017). Production of FucoPol by enterobacter A47 using waste tomato paste by-product as sole carbon source. *Bioresource Technology, 227*, 66–73.

Bacakova, L., Novotna, K., & Parizek, M. (2014). Polysaccharides as cell carriers for tissue engineering: The use of cellulose in vascular wall reconstruction. *Physiological Research, 63*(Suppl. 1), S29–S47.

Bacelar, A. H., Silva-Correia, J., Oliveira, J. M., & Reis, R. L. (2016). Recent progress in gellan gum hydrogels provided by functionalization strategies. *Journal of Materials Chemistry B, 4*(37), 6164–6174.

Bae, M. S., Kim, J. E., Lee, J. B., Heo, D. N., Yang, D. H., Kim, J. H., & Kwon, I. K. (2013). ZrO(2) surface chemically coated with hyaluronic acid hydrogel loading GDF-5 for osteogenesis in dentistry. *Carbohydrate Polymers, 92*(1), 167–175.

Baptista, S., Torres, C. A. V., Sevrin, C., Grandfils, C., Reis, M. A. M., & Freitas, F. (2022). Extraction of the bacterial extracellular polysaccharide FucoPol by membrane-based methods: Efficiency and impact on biopolymer properties. *Polymers, 14*(3).

Benedini, L. J., & Santana, M. H. A. (2013). Effects of soy peptone on the inoculum preparation of streptococcus zooepidemicus for production of hyaluronic acid. *Bioresource Technology, 130*, 798–800.

Burdick, J. A., & Prestwich, G. D. (2011). Hyaluronic acid hydrogels for biomedical applications. *Advanced Materials, 23*(12), H41–H56.

Campana, V., Milano, G., Pagano, E., Barba, M., Cicione, C., Salonna, G., & Logroscino, G. (2014). Bone substitutes in orthopaedic surgery: From basic science to clinical practice. *Journal of Materials Science. Materials in Medicine, 25*(10), 2445–2461.

Casu, B., Grazioli, G., Razi, N., Guerrini, M., Naggi, A., Torri, G., & Lindahl, U. (1994). Heparin-like compounds prepared by chemical modification of capsular polysaccharide from E. coli K5. *Carbohydr Res, 263*(2), 271–284.

Celik, G. Y., Aslim, B., & Beyatli, Y. (2008). Characterization and production of the exopolysaccharide (EPS) from Pseudomonas aeruginosa G1 and pseudomonas putida G12 strains. *Carbohydrate Polymers, 73*(1), 178–182.

Cescutti, P. (2010). Chapter 6 - bacterial capsular polysaccharides and exopolysaccharides. In *Microbial Glycobiology* (pp. 93–108). Academic Press.

Chambi, D., Romero-Soto, L., Villca, R., Orozco-Gutierrez, F., Vega-Baudrit, J., Quillaguaman, J., & Carrasco, C. (2021). Exopolysaccharides production by cultivating a bacterial isolate from the hypersaline environment of Salar de uyuni (Bolivia) in pretreatment liquids of steam-exploded quinoa stalks and enzymatic hydrolysates of curupau sawdust. *Fermentation-Basel, 7*(1).

Chan, C., Thompson, I., Robinson, P., Wilson, J., & Hench, L. (2002). Evaluation of bioglass/dextran composite as a bone graft substitute. *International Journal of Oral and Maxillofacial Surgery, 31*(1), 73–77.

Chaplin, M. F., & Kennedy, J. F. (1994). *Carbohydrate analysis : A practical approach* (2nd ed.). Oxford University Press.

Chen, H. L., & Fang, J. N. (1997). *Structure and function of glycoconjugates* (2nd ed.). Shanghai Medical University Press.

Chen, J., Sun, T., You, Y., Wu, B., Wang, X., & Wu, J. (2021). Proteoglycans and glycosaminoglycans in stem cell homeostasis and bone tissue regeneration. *Front Cell Dev Biol, 9*, Article 760532.

Chen, J. P., Tsai, M. J., & Liao, H. T. (2013). Incorporation of biphasic calcium phosphate microparticles in injectable thermoresponsive hydrogel modulates bone cell proliferation and differentiation. *Colloids Surf B Biointerfaces, 110*, 120–129.

Chen, F. M., Zhao, Y. M., Zhang, R., Jin, T., Sun, H. H., Wu, Z. F., & Jin, Y. (2007). Periodontal regeneration using novel glycidyl methacrylated dextran (Dex-GMA)/gelatin scaffolds containing microspheres loaded with bone morphogenetic proteins. *J Control Release, 121*(1-2), 81–90.

Choi, S., Lee, J. S., Shin, J., Lee, M. S., Kang, D., Hwang, N. S., & Cho, S. W. (2020). Osteoconductive hybrid hyaluronic acid hydrogel patch for effective bone formation. *Journal of Controlled Release, 327*, 571–583.

Cimini, D., Rosa, M. D., & Schiraldi, C. (2012). Production of glucuronic acid-based polysaccharides by microbial fermentation for biomedical applications. *Biotechnology Journal, 7*(2), 237–250.

Costa, L., Silva-Correia, J., Oliveira, J. M., & Reis, R. L. (2018). Osteochondral tissue engineering - chapter13: gellan gum-based hydrogels for osteochondral repair. *Advances in Experimental Medicine and Biology, 1058*.

Cui, X., Huang, C., Chen, Z., Zhang, M., Liu, C., Su, K., & Pan, H. (2021). Hyaluronic acid facilitates bone repair effects of calcium phosphate cement by accelerating osteogenic expression. *Bioact Mater, 6*(11), 3801–3811.

Czaczyk, K., & Myszka, K. (2007). Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. *Polish Journal of Environmental Studies, 16*(6), 799–806.

Czaja, W. K., Young, D. J., Kawecki, M., & Brown, R. M., Jr. (2007). The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules, 8*(1), 1–12.

Delattre, C., Pierre, G., Laroche, C., & Michaud, P. (2016). Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnology Advances, 34*(7), 1159–1179.

DeVore, D. P., Hughes, E., & Scott, J. B. (1994). Effectiveness of injectable filler materials for smoothing wrinkle lines and depressed scars. *Med Prog Technol, 20*(3-4), 243–250.

Dols, M., Remaud-Simeon, M., Willemot, R. M., Vignon, M., & Monsan, P. (1998). Characterization of the different dextransucrase activities excreted in glucose, fructose, or sucrose medium by leuconostoc mesenteroides NRRL B-1299. *Applied and Environmental Microbiology, 64*(4), 1298–1302.

Donot, F., Fontana, A., Baccou, J. C., & Schorr-Galindo, S. (2012). Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers, 87*(2), 951–962.

Douglas, T. E. L., Pilarz, M., Lopez-Heredia, M., Brackman, G., Schaubroeck, D., Balcaen, L., & Pamula, E. (2017). Composites of gellan gum hydrogel enzymatically mineralized with calcium-zinc phosphate for bone regeneration with antibacterial activity. *J Tissue Eng Regen Med, 11*(5), 1610–1618.

Douglas, T. E. L., Piwowarczyk, W., Pamula, E., Liskova, J., Schaubroeck, D., Leeuwenburgh, S. C., & Dubruel, P. (2014). Injectable self-gelling composites for bone tissue engineering based on gellan gum hydrogel enriched with different bioglasses. *Biomed Mater, 9*(4), Article 045014.

Douglas, T. E. L., Schietse, J., Zima, A., Gorodzha, S., Parakhonskiy, B. V., KhaleNkow, D., & Skirtach, A. G. (2018). Novel self-gelling injectable hydrogel/alpha-tricalcium phosphate composites for bone regeneration: Physiochemical and microcomputer tomographical characterization. *J Biomed Mater Res A, 106*(3), 822–828.

Douglas, T. E. L., Wlodarczyk, M., Pamula, E., Declercq, H. A., de Mulder, E. L., Bucko, M. M., & Leeuwenburgh, S. C. (2014). Enzymatic mineralization of gellan gum hydrogel for bone tissue-engineering applications and its enhancement by polydopamine. *J Tissue Eng Regen Med, 8*(11), 906–918.

Dubey, S., Mishra, R., Roy, P., & Singh, R. P. (2021). 3-D macro/microporous-nanofibrous bacterial cellulose scaffolds seeded with BMP-2 preconditioned mesenchymal stem cells exhibit remarkable potential for bone tissue engineering. *International Journal of Biological Macromolecules, 167*, 934–946.

Esawy, M. A., Amer, H., Gamal-Eldeen, A. M., El Enshasy, H. A., Helmy, W. A., Abo-Zeid, M. A., & Awad, G. E. (2013). Scaling up, characterization of Levan and its inhibitory role in carcinogenesis initiation stage'. *Carbohydrate Polymers, 95*(1), 578–587.

Fang, J., & Wang, S. (1997). Recent advances in studying lentinan. *Chinese Pharmaceutical Journal-Beijing, 32*, 332–334.

Fang, J., Li, P., Lu, X., Fang, L., Lu, X., & Ren, F. (2019). A strong, tough, and osteoconductive hydroxyapatite mineralized polyacrylamide/dextran hydrogel for bone tissue regeneration. *Acta Biomater, 88*, 503–513.

Faour, O., Dimitriou, R., Cousins, C. A., & Giannoudis, P. V. (2011). The use of bone graft substitutes in large cancellous voids: Any specific needs? *Injury, 42*(Suppl. 2), S87–S90.

Favi, P. M., Ospina, S. P., Kachole, M., Gao, M., Atehortua, L., & Webster, T. J. (2016). Preparation and characterization of biodegradable nano hydroxyapatite–bacterial cellulose composites with well-defined honeycomb pore arrays for bone tissue engineering applications. *Cellulose, 23*(2), 1263–1282.

Fernandez de Grado, G., Keller, L., Idoux-Gillet, Y., Wagner, Q., Musset, A. M., Benkirane-Jessel, N., & Offner, D. (2018). Bone substitutes: A review of their characteristics, clinical use, and perspectives for large bone defects management. *Journal of Tissue Engineering, 9*, Article 2041731418776819.

Flautre, B., Lemaitre, J., Maynou, C., Van Landuyt, P., & Hardouin, P. (2003). Influence of polymeric additives on the biological properties of brushite cements: An experimental study in rabbit. *Journal of Biomedical Materials Research. Part A, 66*(2), 214–223.

Freitas, F., Alves, V. D., Pais, J., Costa, N., Oliveira, C., Mafra, L., & Reis, M. A. (2009). Characterization of an extracellular polysaccharide produced by a pseudomonas strain grown on glycerol. *Bioresource Technology, 100*(2), 859–865.

Freitas, F., Alves, V. D., Torres, C. A. V., Cruz, M., Sousa, I., Melo, M. J., & Reis, M. A. M. (2011). Fucose-containing exopolysaccharide produced by the newly isolated enterobacter strain A47 DSM 23139. *Carbohydrate Polymers, 83*(1), 159–165.

Freitas, F., Torres, C. A. V., & Reis, M. A. M. (2017). Engineering aspects of microbial exopolysaccharide production. *Bioresource Technology, 245*(Pt B), 1674–1683.

Fricain, J. C., Schlaubitz, S., Le Visage, C., Arnault, I., Derkaoui, S. M., Siadous, R., & Amedee, J. (2013). A nano-hydroxyapatite–pullulan/dextran polysaccharide composite macroporous material for bone tissue engineering. *Biomaterials, 34*(12), 2947–2959.

Galindo, E., Pena, C., Nunez, C., Segura, D., & Espin, G. (2007). Molecular and bioengineering strategies to improve alginate and polydydroxyalkanoate production by Azotobacter vinelandii. *Microbial Cell Factories, 6*, 7.

Gantar, A., da Silva, L. P., Oliveira, J. M., Marques, A. P., Correlo, V. M., Novak, S., & Reis, R. L. (2014). Nanoparticulate bioactive-glass-reinforced gellan-gum hydrogels for bone-tissue engineering. *Materials Science & Engineering. C, Materials for Biological Applications, 43*, 27–36.

Ghori, M. U., Mohammad, M. A., Rudrangi, S. R. S., Fleming, L. T., Merchant, H. A., Smith, A. M., & Conway, B. R. (2017). Impact of purification on physicochemical, surface and functional properties of okra biopolymer. *Food Hydrocolloids, 71*(2017), 311–320, 311e320, 71.

Gunzburg, R., Szpalski, M., Passuti, N., & Aebi, M. (2002). *The use of bone substitutes in spine surgery : A state of the art.* In (p. 129). Berlin, Heidelberg: Springer.

Haridas, N., & Rosemary, M. J. (2019). Effect of steam sterilization and biocompatibility studies of hyaluronic acid hydrogel for viscosupplementation. *Polymer Degradation and Stability, 163*, 220–227.

He, P. X., Geng, L. J., Wang, Z., Mao, D. B., Wang, J. Z., & Xu, C. P. (2012). Fermentation optimization, characterization and bioactivity of exopolysaccharides from Funalia trogii. *Carbohydrate Polymers, 89*(1), 17–23.

Heinze, T., Liebert, T., Heublein, B., & Hornig, S. (2006). Functional polymers based on dextran. *Advances in Polymer Science, 205*, 199–291.

Helenius, G., Backdahl, H., Bodin, A., Nannmark, U., Gatenholm, P., & Risberg, B. (2006). In vivo biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research. Part A, 76*(2), 431–438.

Hempel, U., Moller, S., Noack, C., Hintze, V., Scharnweber, D., Schnabelrauch, M., & Dieter, P. (2012). Sulfated hyaluronan/collagen I matrices enhance the osteogenic differentiation of human mesenchymal stromal cells in vitro even in the absence of dexamethasone. *Acta Biomaterialia, 8*(11), 4064–4072.

Hempel, U., Preissler, C., Vogel, S., Moller, S., Hintze, V., Becher, J., & Dieter, P. (2014). Artificial extracellular matrices with oversulfated glycosaminoglycan derivatives promote the differentiation of osteoblast-precursor cells and premature osteoblasts. *BioMed Research International, 2014*, Article 938368.

Hemshekhar, M., Thushara, R. M., Chandranayaka, S., Sherman, L. S., Kemparaju, K., & Girish, K. S. (2016). Emerging roles of hyaluronic acid bioscaffolds in tissue engineering and regenerative medicine. *International Journal of Biological Macromolecules, 86*, 917–928.

Heymann, D., Ruiz-Velasco, C., Chesneau, J., Ratiskol, J., Sinquin, C., & Colliec-Jouault, S. (2016). Anti-metastatic properties of a marine bacterial exopolysaccharide-based derivative designed to mimic glycosaminoglycans. *Molecules, 21*(3), 309.

Holland, N. B., Qiu, Y., Ruegsegger, M., & Marchant, R. E. (1998). Biomimetic engineering of non-adhesive glycocalyx-like surfaces using oligosaccharide surfactant polymers. *Nature, 392*(6678), 799–801.

Holloway, J. L., Ma, H., Rai, R., Hankenson, K. D., & Burdick, J. A. (2015). Synergistic effects of SDF-1alpha and BMP-2 delivery from proteolytically degradable hyaluronic acid hydrogels for bone repair. *Macromolecular Bioscience, 15*(9), 1218–1223.

Huang, H. M., Hsieh, S. C., Teng, N. C., Feng, S. W., Ou, K. L., & Chang, W. J. (2011). Biological surface modification of titanium surfaces using glow discharge plasma. *Medical & Biological Engineering & Computing, 49*(6), 701–706.

Hutchens, A. S., Benson, R. S., Evans, B. R., Rawn, C. J., & O'Neill, H. (2009). A resorbable calcium-deficient hydroxyapatite hydrogel composite for osseous regeneration. *Cellulose, 16*(5), 887–898.

Johnson, P. C. (1990). Platelet-mediated thrombosis in microvascular surgery: New knowledge and strategies. *Plastic and Reconstructive Surgery, 86*(2), 359–367.

Jung, S. W., Oh, S. H., Lee, I. S., Byun, J. H., & Lee, J. H. (2019). In situ gelling hydrogel with anti-bacterial activity and bone healing property for treatment of osteomyelitis. *Journal of Tissue Engineering and Regenerative Medicine, 16*(5), 479–490.

Kawano, M., Ariyoshi, W., Iwanaga, K., Okinaga, T., Habu, M., Yoshioka, I., & Nishihara, T. (2011). Mechanism involved in enhancement of osteoblast differentiation by hyaluronic acid. *Biochemical and Biophysical Research Communications, 405*(4), 575–580.

Khan, M. U. A., Haider, S., Haider, A., Razak, S. A., Kadir, M. R. A., Shah, S. A., & Al-Zahrani, A. A. (2021). Development of porous, antibacterial and biocompatible GO/n-HAp/bacterial cellulose/β-glucan biocomposite scaffold for bone tissue engineering. *Arabian Journal of Chemistry, 14*(2).

Koike, T., Sha, J., Bai, Y., Matsuda, Y., Hideshima, K., Yamada, T., & Kanno, T. (2019). Efficacy of bacterial cellulose as a carrier of BMP-2 for bone regeneration in a rabbit frontal sinus model. *Materials (Basel), 12*(15).

Kothari, V., Mishra, T., & Kushwah, P. (2014). Mutagenic effect of microwave radiation on EPS production in Xanthomonas campestris. *Current Trends in Biotechnology and Pharmacy, 8*, 29–37.

Kumar, M. A., Anandapandian, K. T. K., & Parthiban, K. (2011). Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. *brazArchives of Biology and Technology, 54*(2), 259–265.

Kushwah, P., Mishra, T., & Kothari, V. (2013). Effect of microwave radiation on growth, enzyme activity (Amylase and Pectinase), and/or exopolysaccharide production in Bacillus subtilis, Streptococcus mutans, xanthomonas campestris and pectobacterium carotovora. *British Microbiology Research Journal, 3*, 645–653.

Landeck, J. T., Walsh, W. R., Oliver, R. A., Wang, T., Gordon, M. R., Ahn, E., & White, C. D. (2021). Temporal response of an injectable calcium phosphate material in a critical size defect. *Journal of Orthopaedic Surgery and Research, 16*(1), 496.

Lavergne, M., Derkaoui, M., Delmau, C., Letourneur, D., Uzan, G., & Le Visage, C. (2012). Porous polysaccharide-based scaffolds for human endothelial progenitor cells. *Macromolecular Bioscience, 12*(7), 901–910.

Lee, Y. J., An, S. J., Bae, E. B., Gwon, H. J., Park, J. S., Jeong, S. I., & Huh, J. B. (2017). The effect of thickness of resorbable bacterial cellulose membrane on guided bone regeneration. *Materials (Basel), 10*(3).

Lee, J. H., Kim, J., Baek, H. R., Lee, K. M., Seo, J. H., Lee, H. K., & Lee, C. K. (2014). Fabrication of an rhBMP-2 loaded porous beta-TCP microsphere-hyaluronic acid-based powder gel composite and evaluation of implant osseointegration. *J Mater Sci Mater Med, 25*(9), 2141–2151.

Lee, S. H., Lim, Y. M., Jeong, S. I., An, S. J., Kang, S. S., Jeong, C. M., & Huh, J. B. (2015). The effect of bacterial cellulose membrane compared with collagen membrane on guided bone regeneration. *J Adv Prosthodont, 7*(6), 484–495.

Li, X., Xiong, F., Liu, Y., Liu, F., Hao, Z., & Chen, H. (2018). Total fractionation and characterization of the water-soluble polysaccharides isolated from Enteromorpha intestinalis. *International Journal of Biological Macromolecules, 111*, 319–325.

Limoli, D. H., Jones, C. J., & Wozniak, D. J. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiol Spectr, 3*(3).

Litwiniuk, M., Krejner, A., & Grzela, T. (2016). Hyaluronic acid in inflammation and tissue regeneration. *Wounds, 28*, 78–88.

Liu, L., Du, G., Chen, J., Wang, M., & Sun, J. (2008). Influence of hyaluronidase addition on the production of hyaluronic acid by batch culture of streptococcuszooepidemicus. *Food Chemistry, 110*(4), 923–926.

Liu, L., Lu, Y. M., Li, X. H., Zhou, L. Y., Yang, D., Wang, L. M., & Chen, Y. (2015). A novel process for isolation and purification of the bioactive polysaccharide TLH-3 ' from tricholoma lobayense. *Process Biochemistry, 50*(7), 1146–1151.

Maia, J., Evangelista, M. B., Gil, H., & Ferreira, L. (2014). Dextran-based materials for biomedical applications. *Carbohydrates Applications in Medicine, 2*, 31–53.

Maire, M., Chaubet, F., Mary, P., Blanchat, C., Meunier, A., & Logeart-Avramoglou, D. (2005). Bovine BMP osteoinductive potential enhanced by functionalized dextran-derived hydrogels. *Biomaterials, 26*(24), 5085–5092.

Manda, M. G., da Silva, L. P., Cerqueira, M. T., Pereira, D. R., Oliveira, M. B., Mano, J. F., & Reis, R. L. (2018). Gellan gum-hydroxyapatite composite spongy-like hydrogels for bone tissue engineering. *Journal of Biomedical Materials Research. Part A, 106*(2), 479–490.

Martinez-Sanz, E., Ossipov, D. A., Hilborn, J., Larsson, S., Jonsson, K. B., & Varghese, O. P. (2011). Bone reservoir: Injectable hyaluronic acid hydrogel for minimal invasive bone augmentation. *Journal of Controlled Release, 152*(2), 232–240.

Mazmanian, S. K., & Kasper, D. L. (2006). The love-hate relationship between bacterial polysaccharides and the host immune system. *Nature Reviews. Immunology, 6*(11), 849–858.

Mehta, A., Prasad, G. S., & Choudhury, A. R. (2014). Cost effective production of pullulan from agri-industrial residues using response surface methodology. *International Journal of Biological Macromolecules, 64*, 252–256.

Miyazaki, T., Miyauchi, S., Tawada, A., Anada, T., Matsuzaka, S., & Suzuki, O. (2008). Oversulfated chondroitin sulfate-E binds to BMP-4 and enhances osteoblast differentiation. *Journal of Cellular Physiology, 217*(3), 769–777.

Mockl, L. (2020). The emerging role of the mammalian glycocalyx in functional membrane organization and immune system regulation. *Frontiers in Cell and Development Biology, 8*, 253.
Mohd Nadzir, M., Nurhayati, R. W., Idris, F. N., & Nguyen, M. H. (2021). Biomedical applications of bacterial exopolysaccharides: A review. *Polymers (Basel), 13*(4).
Moreno, J., Vargas, M. A., Olivares, H., Rivas, J., & Guerrero, M. G. (1998). Exopolysaccharide production by the Cyanobacterium anabaena sp. ATCC 33047 in batch and continuous culture. *Journal of Biotechnology, 60*(3), 175–182.
Moscovici, M. (2015). Present and future medical applications of microbial exopolysaccharides. *Frontiers in Microbiology, 6*.
Nauth, A., Ristevski, B., Li, R., & Schemitsch, E. H. (2011). Growth factors and bone regeneration: How much bone can we expect? *Injury, 42*(6), 574–579.
Nauth, A., Schemitsch, E., Norris, B., Nollin, Z., & Watson, J. T. (2018). Critical-size bone defects: Is there a consensus for diagnosis and treatment? *Journal of Orthopaedic Trauma, 32*(Suppl. 1), S7–S11.
Nikpour, P., Salimi-Kenari, H., Fahimipour, F., Rabiee, S. M., Imani, M., Dashtimoghadam, E., & Tayebi, L. (2018). Dextran hydrogels incorporated with bioactive glass-ceramic: Nanocomposite scaffolds for bone tissue engineering. *Carbohydrate Polymers, 190*, 281–294.
Nwodo, U. U., Green, E., & Okoh, A. I. (2012). Bacterial exopolysaccharides: Functionality and prospects. *International Journal of Molecular Sciences, 13*(11), 14002–14015.
Oliveira, R., Marques, F., & Azeredo, J. (1999). Purification of polysaccharides from a biofilm matrix by selective precipitation of proteins. *Biotechnology Techniques, 13*(6), 391–393.
Oliveira, J. T, Martins, L., Picciochi, R., Malafaya, P. B., Sousa, R. A., Neves, N. M., & Reis, R. L. (2010). Gellan gum: a new biomaterial for cartilage tissue engineering applications. *J Biomed Mater Res A, 93*(3), 852–863.
Orimo, H. (2010). The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *Journal of Nippon Medical School, 77*(1), 4–12.
Owens, J. M., Gallagher, A. C., & Chambers, T. J. (1996). IL-10 modulates formation of osteoclasts in murine hemopoietic cultures. *Journal of Immunology, 157*(2), 936–940.
Ozcan, E., Sargin, S., & Goksungur, Y. (2014). Comparison of pullulan production performances of air-lift and bubble column bioreactors and optimization of process parameters in air-lift bioreactor. *Biochemical Engineering Journal, 92*, 9–15.
Palaniraj, A., & Jayaraman, V. (2011). Production, recovery and applications of xanthan gum by xanthomonas campestris. *Journal of Food Engineering, 106*, 1–12.
Papinutti, L. (2010). Effects of nutrients, pH and water potential on exopolysaccharides production by a fungal strain belonging to ganoderma lucidum complex. *Bioresource Technology, 101*(6), 1941–1946.
Park, J. Y., Kim, B. S., & Lee, J. (2016). Evaluation of the angiogenic potency of a novel exopolysaccharide produced by the MK1 bacterial strain. *Archives of Pharmacal Research, 39*(9), 1223–1231.
Pintado, A. I. E., Ferreira, J. A., Pintado, M. M. E., Gomes, A. M. P., Malcata, F. X., & Coimbra, M. A. (2020). Efficiency of purification methods on the recovery of exopolysaccharides from fermentation media. *Carbohydrate Polymers, 231*.
Pollari, S., Kakonen, R. S., Mohammad, K. S., Rissanen, J. P., Halleen, J. M., Warri, A., & Kakonen, S. M. (2012). Heparin-like polysaccharides reduce osteolytic bone destruction and tumor growth in a mouse model of breast cancer bone metastasis. *Molecular Cancer Research, 10*(5), 597–604.
Prajapati, V. D., Jani, G. K., Zala, B. S., & Khutliwala, T. A. (2013). An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydrate Polymers, 93*(2), 670–678.
Rana, S., & Upadhyay, L. S. B. (2020). Microbial exopolysaccharides: Synthesis pathways, types and their commercial applications. *International Journal of Biological Macromolecules, 157*, 577–583.
Rederstorff, E., Weiss, P., Sourice, S., Pilet, P., Xie, F., Sinquin, C., & Laib, S. (2011). An in vitro study of two GAG-like marine polysaccharides incorporated into injectable hydrogels for bone and cartilage tissue engineering. *Acta Biomaterialia, 7*(5), 2119–2130.
Rehm, B. H. A. (2010). Bacterial polymers: Biosynthesis, modifications and applications. *Nature Reviews Microbiology, 8*(8), 578–592.
Roger, O., Kervarec, N., Ratiskol, J., Colliec-Jouault, S., & Chevolot, L. (2004). Structural studies of the main exopolysaccharide produced by the deep-sea bacterium alteromonas infernus. *Carbohydrate Research, 339*(14), 2371–2380.
Ross, P., Mayer, R., & Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiological Reviews, 55*(1), 35–58.
Rudolph, K. W. E., Gross, M., Ebrahimnesbat, F., Nollenburg, M., Zomorodian, A., Wydra, K., & Z, K. (1994). The role of extracellular polysaccharides as virulence factors for phytopathogenic pseudomonads and xanthomonads. *Molecular Mechanisms of Bacterial Virulence*, 357–378.
Rutering, M., Schmid, J., Ruhmann, B., Schilling, M., & Sieber, V. (2016). Controlled production of polysaccharides-exploiting nutrient supply for Levan and heteropolysaccharide formation in paenibacillus sp. *Carbohydrate Polymers, 148*, 326–334.
Saikia, K. C., Bhattacharya, T. D., Bhuyan, S. K., Talukdar, D. J., Saikia, S. P., & Jitesh, P. (2008). Calcium phosphate ceramics as bone graft substitutes in filling bone tumor defects. *Indian J Orthop, 42*(2), 169–172.
Sarvaiya, N., & Kothari, V. (2015). Effect of audible sound in form of music on microbial growth and production of certain important metabolites. *Microbiology, 84*, 227–235.
Saska, S., Barud, H. S., Gaspar, A. M., Marchetto, R., Ribeiro, S. J., & Messaddeq, Y. (2011). Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration. *Int J Biomater, 2011*, Article 175362.
Schanté, C. E., Zuber, G., Herlin, C., & Vandamme, T. F. (2011). Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications. *Carbohydrate Polymers, 85*(3), 469–489.
Schiavi, E., Gleinser, M., Molloy, E., Groeger, D., Frei, R., Ferstl, R., & O'Mahony, L. (2016). The surface-associated exopolysaccharide of bifidobacterium longum 35624 plays an essential role in dampening host proinflammatory responses and repressing local TH17 responses. *Applied and Environmental Microbiology, 82*(24), 7185–7196.
Schmid, J., Sieber, V., & Rehm, B. (2015). Bacterial exopolysaccharides: Biosynthesis pathways and engineering strategies. *Frontiers in Microbiology, 6*, 496.
Sengupta, D., Datta, S., & Biswas, D. (2018). Towards a better production of bacterial exopolysaccharides by controlling genetic as well as physico-chemical parameters. *Applied Microbiology and Biotechnology, 102*(4), 1587–1598.
Senni, K., Gueniche, F., Changotade, S., Septier, D., Sinquin, C., Ratiskol, J., & Colliec-Jouault, S. (2013). Unusual glycosaminoglycans from a deep sea hydrothermal bacterium improve fibrillar collagen structuring and fibroblast activities in engineered connective tissues. *Marine Drugs, 11*(4), 1351–1369.
Seviour, R. J., McNeil, B., Fazenda, M. L., & Harvey, L. M. (2011). Operating bioreactors for microbial exopolysaccharide production. *Critical Reviews in Biotechnology, 31*(2), 170–185.
Shah, A., Raval, A., & Kothari, A. (2016). Sound stimulation can influence microbial growth and production of certain key metabolites. *Journal of Microbiology, Biotechnology and Food Science, 5*, 330–334.
Shi, L. (2016). Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. *Int J Biol Macromol, 92*, 37–48.
Shi, L., Aid, R., Le Visage, C., & Chew, S. Y. (2012). Biomimicking polysaccharide nanofibers promote vascular phenotypes: a potential application for vascular tissue engineering. *Macromol Biosci, 12*(3), 395–401.
Shi, Q., Li, Y., Sun, J., Zhang, H., Chen, L., Chen, B., & Wang, Z. (2012). The osteogenesis of bacterial cellulose scaffold loaded with bone morphogenetic protein-2. *Biomaterials, 33*(28), 6644–6649.
Shi, Z., Neoh, K. G., Kang, E. T., Poh, C., & Wang, W. (2009). Titanium with surface-grafted dextran and immobilized bone morphogenetic protein-2 for inhibition of bacterial adhesion and enhancement of osteoblast functions. *Tissue Eng Part A, 15*(2), 417–426.
Shih, I. L. (2010). Microbial exo-polysaccharides for biomedical applications. *Mini Rev Med Chem, 10*(14), 1345–1355.
Shih, I. L., Chen, L. D., & Wu, J. Y. (2010). Levan production using Bacillus subtilis natto cells immobilized on alginate. *Carbohydrate Polymers, 82*(1), 111–117.
Shih, I. L., Yu, J. Y., Hsieh, C., & Wu, J. Y. (2009). Production and characterization of curdlan by agrobacterium sp. *Biochemical Engineering Journal, 43*(1), 33–40.
Snyders, R. V., Jr., Eppley, B. L., Krukowski, M., & Delfino, J. J. (1993). Enhancement of repair in experimental calvarial bone defects using calcium sulfate and dextran beads. *Journal of Oral and Maxillofacial Surgery, 51*(5), 517–524.
Souza, P. P. C., & Lerner, U. H. (2019). Finding a toll on the route: The fate of osteoclast progenitors after toll-like receptor activation. *Frontiers in Immunology, 10*, 1663.
Steinmetz, I., Rohde, M., & Brenneke, B. (1995). Purification and characterization of an exopolysaccharide of burkholderia (Pseudomonas) pseudomallei. *Infection and Immunity, 63*(10), 3959–3965.
Stevens, L. R., Gilmore, K. J., Wallace, G. G., & Panhuis, I. H. M. (2016). Tissue engineering with gellan gum. *Biomaterials Science, 4*(9), 1276–1290.
Survase, S. A., Saudagar, P. S., & Singhal, R. S. (2007a). Enhanced production of scleroglucan by sclerotium rolfsii MTCC 2156 by use of metabolic precursors. *Bioresource Technology, 98*(2), 410–415.
Survase, S. A., Saudagar, P. S., & Singhal, R. S. (2007b). Use of complex media for the production of scleroglucan by sclerotium rolfsii MTCC 2156. *Bioresource Technology, 98*(7), 1509–1512.
Sutherland, I. (1990). Biotechnology of microbial exopolysaccharides. In *Cambridge Studies in Biotechnology*. Cambridge: Cambridge University Press.
Tazi, N., Zhang, Z., Messaddeq, Y., Almeida-Lopes, L., Zanardi, L. M., Levinson, D., & Rouabhia, M. (2012). Hydroxyapatite bioactivated bacterial cellulose promotes osteoblast growth and the formation of bone nodules. *AMB Express, 2*(1), 61.
Torgbo, S., & Sukyai, P. (2018). Bacterial cellulose-based scaffold materials for bone tissue engineering. *Applied Materials Today, 11*, 34–49.
Torres, C. A. V., Antunes, S., Ricardo, A. R., Grandfils, C., Alves, V. D., Freitas, F., & Reis, M. A. (2012). Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. *Bioresour Technol, 119*, 148–156.
Torres, C. A. V., Marques, R., Ferreira, A. R. V., Antunes, S., Grandfils, C., Freitas, F., & Reis, M. A. M. (2014). Impact of glycerol and nitrogen concentration on Enterobacter A47 growth and exopolysaccharide production. *International Journal of Biological Macromolecules, 71*, 81–86.
Vacheethasanee, K., & Marchant, R. E. (2000). Surfactant polymers designed to suppress bacterial (Staphylococcus epidermidis) adhesion on biomaterials. *Journal of Biomedical Materials Research, 50*(3), 302–312.
Valepyn, E., Berezina, N., & Paquot, M. (2012). Optimization of production and preliminary characterization of new exopolysaccharides from gluconacetobacter hansenii LMG1524. *Advances in Microbiology, 2*, 488–496.
Van Zyl, W. F., Deane, S. M., & Dicks, L. M. T. (2020). Molecular insights into probiotic mechanisms of action employed against intestinal pathogenic bacteria. *Gut Microbes, 12*(1), 1831339.
Vann, W. F., Schmidt, M. A., Jann, B., & Jann, K. (1981). The structure of the capsular polysaccharide (K5 antigen) of urinary-tract-infective Escherichia coli 010:K5:H4. A polymer similar to desulfo-heparin. *European Journal of Biochemistry, 116*(2), 359–364.
Vazquez, J. A., Montemayor, M. I., Fraguas, J., & Murado, M. A. (2009). High production of hyaluronic and lactic acids by streptococcus zooepidemicus in fed-batch culture using commercial and marine peptones from fishing by-products. *Biochemical Engineering Journal, 44*(2–3), 125–130.

Vejlens, L. (1971a). Glycosaminoglycans of human bone tissue. *Calcified Tissue Research, 7*, 175–190.
Vejlens, L. (1971b). Glycosaminoglycans of human bone tissue. I. Pattern of compact bone in relation to age. *Calcified Tissue Research, 7*(2), 175–190.
Velasco, C. R., Baud'huin, M., Sinquin, C., Maillasson, M., Heymann, D., Colliec-Jouault, S., & Padrines, M. (2011). Effects of a sulfated exopolysaccharide produced by altermonas infernus on bone biology. *Glycobiology, 21*(6), 781–795.
Velasco, C. R., Colliec-Jouault, S., Sinquin, C., Heymann, D., & Padrines, M. (2011). An exopolysaccharide produced by alteromonas infernus reduces lung metastasis and prolongs survival rate of osteosarcoma-bearing mice. *Abstracts/Bone, 48*(P64).
Volpi, N. (2004). Purification of the Escherichia coli K5 capsular polysaccharide and use of high-performance capillary electrophoresis to qualitative and quantitative monitor the process. *Electrophoresis, 25*(18–19), 3307–3312.
Vorhölter, F. J., Schneiker, S., Goesmann, A., Krause, L., Bekel, T., Kaiser, O., & Pühler, A. (2008). The genome of xanthomonas campestris pv. Campestris B100 and its use for the reconstruction of metabolic pathways involved in xanthan biosynthesis. *Journal of Biotechnology, 134*(1–2), 33–45.
Wallimann, A., Hildebrand, M., Groeger, D., Stanic, B., Akdis, C. A., Zeiter, S., & Thompson, K. (2021). An exopolysaccharide produced by bifidobacterium longum 35624(R) inhibits osteoclast formation via a TLR2-dependent mechanism. *Calcified Tissue International, 108*(5), 654–666.
Wang, X., Tang, S., Chai, S., Wang, P., Qin, J., Pei, W., & Huang, C. (2021). Preparing printable bacterial cellulose based gelatin gel to promote in vivo bone regeneration. *Carbohydrate Polymers, 270*, Article 118342.
West, D. C., Hampson, I. N., Arnold, F., & Kumar, S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. *Science, 228*(4705), 1324–1326.
Witzler, M., Buchner, D., Shoushrah, S. H., Babczyk, P., Baranova, J., Witzleben, S., & Schulze, M. (2019). Polysaccharide-based systems for targeted stem cell differentiation and bone regeneration. *Biomolecules, 9*(12).
Wugeditsch, T., Zachara, N. E., Puchberger, M., Kosma, P., Gooley, A. A., & Messner, P. (1999). Structural heterogeneity in the core oligosaccharide of the S-layer glycoprotein from aneurinibacillus thermoaerophilus DSM 10155. *Glycobiology, 9*(8), 787–795.
Xu, Y., Hou, M., Ruan, J., Qu, M., Sun, H., Xu, J., & Zhou, S. (2014). Effect of magnetic field on surface properties of Bacillus cereus CrA and its extracellular polymeric substances (EPS). *Journal of Adhesion Science and Technology, 28*, 2196–2208.
Yang, M., Zhu, Y., Li, Y. M., Bao, J., Fan, X. Y., Qu, Y. H., & Li, Q. (2016). Production and optimization of curdlan produced by Pseudomonas sp QL212. *International Journal of Biological Macromolecules, 89*, 25–34.
Yoshikawa, M., Tsuji, N., Kakigi, H., Yabuuchi, T., Shimomura, Y., Hayashi, H., & Ohgushi, H. (2010). Dextran coating on and among fibers of polymer sponge scaffold for osteogenesis by bone marrow cells in vivo. *J. Biomedical Science and Engineering, 3*, 751–757.
Yoshioka, K., Katayama, M., Nishiyama, T., Harada, K., Takeshita, S., & Kawamata, Y. (2019). Biocompatibility study of different hyaluronan products for intra-articular treatment of knee osteoarthritis. *BMC Musculoskeletal Disorders, 20*(1), 424.
Yu, K. W., Shin, K. S., Choi, Y. M., & Suh, H. J. (2004). Macrophage stimulating activity of exo-biopolymer from submerged culture of Lentinus edodes with rice bran. *Journal of Microbiology and Biotechnology, 14*(4), 658–664.
Yu, P., Zheng, L., Wang, P., Chai, S., Zhang, Y., Shi, T., & Jiang, Q. (2020). Development of a novel polysaccharide-based iron oxide nanoparticle to prevent iron accumulation-related osteoporosis by scavenging reactive oxygen species. *International Journal of Biological Macromolecules, 165*(Pt B), 1634–1645.
Zanchetta, P., Lagarde, N., & Guezennec, J. (2003a). A new bone-healing material: A hyaluronic acid-like bacterial exopolysaccharide. *Calcified Tissue International, 72*(1), 74–79.
Zanchetta, P., Lagarde, N., & Guezennec, J. (2003b). Systemic effects on bone healing of a new hyaluronic acid-like bacterial exopolysaccharide. *Calcified Tissue International, 73*(3), 232–236.
Zhai, P., Peng, X., Li, B., Liu, Y., Sun, H., & Li, X. (2020). The application of hyaluronic acid in bone regeneration. *International Journal of Biological Macromolecules, 151*, 1224–1239.
Zhang, Y., Chen, H., Zhang, T., Zan, Y., Ni, T., Cao, Y., & Pei, R. (2019). Injectable hydrogels from enzyme-catalyzed crosslinking as BMSCs-laden scaffold for bone repair and regeneration. *Mater Sci Eng C Mater Biol Appl, 96*, 841–849.
Zhang, L., Fan, C., Liu, S., Zang, Z., Jiao, L., & Zhang, L. (2011). Chemical composition and antitumor activity of polysaccharide from Inonotus obliquus. *J. Med. Plant. Res., 5*, 1251–1260.
Zhang, W., Huang, J., Wang, W., Li, Q., Chen, Y., Feng, W. W., & Wu, X. Y. (2016). Extraction, purification, characterization and antioxidant activities of polysaccharides from Cistanche tubulosa. *International Journal of Biological Macromolecules, 93*, 448–458.
Zhang, Q., Lei, Z., Peng, M., Zhong, M., Wan, Y., & Luo, H. (2019). Enhancement of mechanical and biological properties of calcium phosphate bone cement by incorporating bacterial cellulose. *Materials Technology, 34*(13), 800–806.
Zhang, T., Li, R. J., Qian, H. B., Mu, W. M., Miao, M., & Jiang, B. (2014). Biosynthesis of levan by levansucrase from Bacillus methylotrophicus SK 21.002. *Carbohydrate Polymers, 101*, 975–981.
Zhang, P., Liu, J., Xu, Q., Harber, G., Feng, X., Michalek, S. M., & Katz, J. (2011). TLR2-dependent modulation of osteoclastogenesis by Porphyromonas gingivalis through differential induction of NFATc1 and NF-kappaB. *J Biol Chem, 286*(27), 24159–24169.
Zhang, Y. J., Yan, X., Jiang, Y., & Cong, J. (2010). Development and characteristic of bacterial cellulose for antimicrobial wound dressing. *Advanced Materials Research, 152-153*, 978–987.
Zhu, S., Han, J., Yan, Z. C., Wu, Y. Z., Zhang, W. Q., Xia, W., & Feng, H. F. (2022). Structure elucidation and immunological activity of a novel exopolysaccharide from Paenibacillus bovis sp. nov BD3526. *Carbohydrate Polymers, 282*.
Zhu, Z. Y., Liu, X. C., Fang, X. N., Sun, H. Q., Yang, X. Y., & Zhang, Y. M. (2016). Structural characterization and anti-tumor activity of polysaccharide produced by Hirsutella sinensis. *International Journal of Biological Macromolecules, 82*, 959–966.
Ziadi, M., Bouzaiene, T., M'Hir, S., Zaafouri, K., Mokhtar, F., Hamdi, M., & Boisset-Helbert, C. (2018). Evaluation of the efficiency of ethanol precipitation and ultrafiltration on the purification and characteristics of exopolysaccharides produced by three lactic acid bacteria. *BioMed Research International, 2018*, 1896240.
Zisu, B., & Shah, N. P. (2003). Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysaccharides by Streptococcus thermophilus 1275. *Journal of Dairy Science, 86*(11), 3405–3415.
Zverlov, V. V., & Schwarz, W. H. (2008). Bacterial cellulose hydrolysis in anaerobic environmental subsystems–Clostridium thermocellum and clostridium stercorarium, thermophilic plant-fiber degraders. *Annals of the New York Academy of Sciences, 1125*, 298–307.