Electrospun Natural Polysaccharide for Biomedical Application

Bor Shin Chee[1], Michael Nugent[1]

[1]Athlone Institute of Technology, Materials Research Institute, Athlone, Co. Westmeath, Ireland.

[NON-PRINT ITEMS]

Abstract

Electrospinning is a simple and versatile technique in nanotechnology for fabricating nanofibers scaffolds for tissue regenerations, drug delivery systems, wound healing, and cancer therapy. This chapter provides a synopsis and overview of using various type of natural polysaccharides from human, animals, plants, seaweeds and microbe origins for electrospinning. The electrospun natural polysaccharide nanofibers are widely used in the biomedical fields due to their biocompatibility, high porosity, large surface area-to-volume, improved drug encapsulation and excellent cell proliferation. The topics presented in this book chapter are focused on illustrating the chemical structures of several natural polysaccharides from different origins. In addition, an outline is present of previous research activities and the potential biomedical applications of these electrospun natural polysaccharide nanofibers. The electrospinning of solely polysaccharides present challenges because of chain entanglements and viscosity, so it is essential to blend with other natural and synthetic polymers to overcomes these aspects.

Key Words:

Electrospinning, Nanofibers, Nanotechnology, Natural polysaccharide, Biomaterials.

[Chapter Starts Here]

1.0 Introduction

Due to ongoing environmental concerns, there has been an increasing focus on using environmentally friendly biomaterials found in natural polymers. The preference of using natural polysaccharide is due to the high cell affinity, low immunogenicity, practicality, moldability, flexibility, lightness, durability, chemical and physicochemical stabilities, biodegradable, bio-adhesive, abundant in nature and cheap [1], [2]. Unfortunately, natural polysaccharides have relatively low mechanical strength. Blending natural polysaccharides with either natural or synthetic polymers can improve the physical properties and biological performances as well as delivering desired features of the resulting polymer composites [3], [4].

This chapter has demonstrated many types of research carried out for the advancement of electrospun nanofibers using selected natural polysaccharides for their potential in biomedical applications. Due to a wide range of natural polysaccharide, it is impossible to cover each natural polysaccharide comprehensively. Therefore, one to two substances were selected to discuss in each category (i.e. polysaccharide from the human, plant, seaweed, animal origins). It is expected that this chapter will serves as a good reference tool for nanomedicine researchers, especially in researching the natural polysaccharide for electrospinning. Moreover, this book chapter also highlights the factors that influence the formation of uniform and smooth nanofibers. This can give a better understanding to the researchers for future research activities.

2.0 Nanotechnology

“Nano” comes from the Greek Nanos, meaning extremely small. Nanotechnology is any science, engineering and technology conducted on a nanoscale. The measurement of nanoscale material is stated in nanometer [5]. Nanotechnology has enabled the researchers to explore the unique, enhanced or totally novel properties of a material in the Nano dimension, in which these properties cannot be discovered from either macro or microsystems.

The term ‘Nanomedicine’ indicates the integration of the knowledge of nanotechnology into biomedical applications. Nanomedicine involves several different types of applications and nanodevices: nanoparticles, nanofibers, nanorobots and nanoelectronics biosensors. In this chapter a focus is on reviewing the production of natural polysaccharide nanofibers using the technology of electrospinning for biomedical applications. The electrospun nanofibers are potentially used in tissue engineering sector due to biocompatible, high porosity and surface to volume ratio. These nanofibers can form scaffolds such as bone, cartilage, skin, blood vessel and cardiovascular stent with the ability to enhance cells attachment, migration and proliferation for tissue regeneration. In addition, electrospun nanofibers are also useful in drug delivery system. The high drug encapsulation efficacy of the nanofibers prevents drug degradation and controls the drug release rate into the human body either in a controlled way or fast dissolving rate. Moreover, in the medical diagnostic and therapeutic field, the surface area of electrospun nanofibers acts as an ultrasensitive biosensor to provide many highly specific binding sites for the detection of cancer cells [6], [7]. Besides the Nano-sized therapeutic agent encapsulated-polymer is able to circulate in the bloodstream and allow them to reach the target site [8].

3.0 Introduction of electrospinning

Electrospinning allows the processing of nanofibers using different polymers such as natural polymers, synthetic polymers and blends of synthetic and natural polymers according to the requirements of the specific applications [2]. A typical electrospinning apparatus requires a syringe, a syringe pump, high voltage supply and a collector. The electrospinning involves applying a high voltage at the metallic needle of the syringe and the collector plate. In addition, the syringe is attached to a pump to control the flow rate of the feed solution. When the polymer solution is pumped out from the syringe, it formed a conical shape called “Taylor cone” and accelerated toward the collector plate, forming a nanofibers mesh [9], [10]. Electrospinning is considered as an electro-fluid-dynamic (EFD) process because it is a progress of using an electrical charged polymer solution to form nanofibers [11].

There are two different electrospinning systems **(Table 1)** which included the solvent electrospinning and the solvent-free electrospinning that can be used to fabricate natural polysaccharide. The difference between both is the solvent electrospinning uses polymer solutions while the solvent-free electrospinning uses polymer melts. Occasionally, solvent-free polymer processing is desirable especially in industrial mass production owing to several problems such as the cost of solvents, the toxicity and inflammability, their storage, dispose and solvent recovery Therefore, the solvent-free electrospinning is used to produce nanofibers without any solvent residue [12].

3.1 The influence of parameters on nanofiber morphology

The polymer solution parameters, processing parameters and ambient parameters are three main groups of factors that can affect the nanofiber morphology such as the diameter of the nanofiber and the shape (i.e. bead, non-bead, flat) of the nanofiber [9]. The polymer solution parameters include solution viscosity, conductivity, chemical structure, concentration, molecular weight and volatility of solvent. While, the process parameters consist of the diameter of needle tip, solution flow rate and feed rate, the distance between capillary tip and collection plate. The ambient parameters include the solution temperature, relative humidity, surrounding air/gas and vacuum condition [9], [13]. The parameters mentioned above are closely related to the electrospinning behavior. For example, the formation of uniform electrospun polysaccharide nanofibers is dependent on the degree of chain entanglements, the viscosity of the solution and the most important requirement which is the weak shear thinning property to encourage the breakdown of liquid jet when dragged by the electric field [14]. The spinning solution is necessary to have a critical concentration as well as an appropriate viscoelasticity to cause polysaccharide chain entanglements. However, the viscoelasticity above the threshold will prevent the motion induced by the electric field. For instance, in the study of developing xanthan-based biomaterials, the xanthan solution concentration above 2.5 %w/v was too viscous to be electrospun at this high concentration. Whereas the solution concentration below 1 %w/v had a low viscosity and beaded nanofibers were formed. Hence the concentration between 1.5 and 2.5 %w/v had a suitable degree of viscoelasticity for electrospinning [15].

4.0 Natural polysaccharide

Four major classes of biopolymers including proteins (e.g. collagen, silk fibroin), polysaccharides **(Table 2)**, deoxyribonucleic acids (DNAs) (e.g. calf thymus Na-DNA) and lipids (e.g. lecithin) are possible to fabricate into nanofibers using electrospinning [4]. Polysaccharides can be categorized into synthetic polysaccharides and natural polysaccharides; the chapter is focused on natural polysaccharides. The term 'saccharide' is derived from the Greek word for sugar. There are four types of saccharides which includes monosaccharide, disaccharide, oligosaccharide and polysaccharide. A monosaccharide is a simple sugar with the smallest units of saccharide. Glucose, galactose and fructose are some of the examples of monosaccharide. D-glucose is the most abundant monosaccharide in animal cells. A disaccharide is made up of two monosaccharides. Maltose, lactose and sucrose are some of the examples of disaccharide. While an oligosaccharide consists of short chains of monosaccharides, typically less than 20 monosaccharides linked together. The example of oligosaccharide is maltotriose. When the oligosaccharide eventually exceeds 20 monosaccharides, it is called a polysaccharide or glycan. Additionally, polysaccharides can be sub-divided into two types: (a) homo-polysaccharide, which only contains a single type of monosaccharides, such as starch, dextran, cellulose and glycogen that made up of glucose and (b) hetero-polysaccharide, which contains two or more different monosaccharides. To make things a little bit more interesting a polysaccharide can also be branched or unbranched, this goes for both homo-polysaccharide and hetero-polysaccharide. For instance, starch has two forms, it can be branched (i.e. amylopectin) or unbranched (i.e. amylose). Moreover, when looking into their functionality, polysaccharides can also be sub-divided into two types: (a) storage polysaccharides (e.g. starch and glycogen) and (b) structural polysaccharides (e.g. cellulose, arabinoxylans, chitin, pectin).

4.1 Polysaccharide of human origin

The polysaccharide derived from human includes glycosaminoglycan (GAGs). GAGs are classified into four groups: (1) heparin/heparan sulfate (HSGAGs), (2) chondroitin

sulfate/dermatan sulfate (CSGAGs), (3) keratan sulfate and (4) hyaluronic acid. **Table 3** has listed some of the research studies carried out by the researchers and their potential biomedical applications of natural polysaccharide derived from humans. In particular, keratan sulfate has not been paid for many attentions because no papers were found regarding this specific polysaccharide in electrospinning. Hence, it has the potential to be electrospun in future.

4.1.1 Hyaluronic acid

Chemical structure and applications of hyaluronic acid

Hyaluronic acid (HA) is a natural polysaccharide found in human tissues such as skin, synovial fluids of joints, connective tissues. It is composed of repeated disaccharide units with the alternation of D-glucuronic acid and N-acetyl-D-glucosamine **(Figure 1)**. Each monosaccharide is connected by alternating β-(1→4) and β-(1→3) glycosidic bonds.

Its chains consist up to 30,000 repeating units with a high molecular weight range (1000 to 10,000,000 Da). HA is one of the most hydrophilic molecules because this large sugar molecule is capable to hold 500 times its own weight of water. HA is also knowns as a natural moisturizer. Hence, it is responsible for tissue hydration and lubrication. In the field of skin care, it is a powerful moisturizer that offers smoothness and softening effects to the skin. Furthermore, it can reduce the appearance of wrinkles.

Research in electrospun hyaluronic acid nanofibers

Due to the low mechanical strength of HA, HA is suggested to be either chemically modified or chemically crosslinked or blending with another polymer. Doğan *et.al.* (2015) have fabricated the uniform and bead-free coaxial nanofibers with silk fibroin (SF) as the shell while HA and olive leaf extract (OLE) as the core. The blend of 15% (w/v) SF, 0.5% (w/v) HA and 12% (w/v) OLE nanofibers have the diameter of 468.19±161.51 nm. When the concentration of OLE increased to 15% (w/v), the diameter of nanofibers

increased to 543.24±196.09 nm. It is explained that a higher voltage supply is needed to fabricate the nanofibers with the additional of OLE into SF/HA solution, resulted in an increase in average nanofibers diameter. The OLE is used because it has the antibacterial and antifungal properties that expose their potential to be used as novel products for scaffolding and drug release applications [22].

In addition, Wang *et. al.* (2015) have generated the core-shell polycaprolactone (PCL)/HA/epidermal growth factor (EGF) nanofibrous scaffolds for the use in wound healing using emulsion electrospinning. The PCL/HA/EGF nanofibers have the smaller diameter (149±4.5 nm and a pore size 0.17± 0.03 μm^2) when compared to the PCL nanofibers (272±38 nm and a pore size of 0.56 ±0.19 μm^2) and PCL/HA nanofibers (184±6 nm and a pore size of 0.16±0.02μm^2) [23]. Due to the viscosity of HA is very high, it is usually blended with other polymers for electrospinning. Furthermore, there are many studies used HA for electrospinning such as fabricating the PEO/HA core-shell electrospun nanofibers for tissue engineering scaffolds [24] and collagen/HA nanofibers for chronic wound healing [25].

4.2 Polysaccharide of plant and seaweed origin

The polysaccharide derived from plant includes cellulose, dextrin and its derivatives, amylose, xylan, starch, dextrin, guar gum, locust bean gum, inulin and pectin. Whereas, the polysaccharide derived from seaweed includes agarose and carrageenan extracted from red algae, alginate (alginic acid) found in the cell walls of brown algae, fucoidan extracted from brown algae, porphyrin extracted from red algae and ulvan extracted from green algae. **Table 4** have listed out some of the research activities performed by the researchers. However, locust bean gum, porphyrin and psyllium are not illustrated in the table because there are no studies found that demonstrate electrospinning of these three types of polysaccharide. Therefore, they have an extensive interesting area that can be developed using locust bean gum, porphyrin and psyllium in the future.

4.2.1 Guar gum

Chemical structure and applications of guar gum

Guar gum is a polysaccharide belonging to the group of galactomannans, being extracted from the endosperms of seeds of *Cyamposis tetragonolobus* (Leguminosae family) [42]. It consists of linear chains of β-(1-4)-D-mannose units (backbone) with side units of α-(1-6) linked galactose **(Figure 2)**.

The mannose backbone of guar gum with galactose side groups are suitable for human colon enzyme degradation, which can be developed to encapsulate a variety of bioactive drug ingredients for targeted drug delivery material [30]. For example, guar gum can be used as the colon-specific drug delivery application due to its resistance to human digestion and absorption as well as susceptibility to microbial degradation in the large intestine. Its drug release retarding behaviors in colon, guar gum is mainly used for the delivery of drug for the treatment of diseases associated with the colon which reduces the side effects and also the dose needed to reduce the dosing frequency [42]–[44]. In addition, guar gum not only widely used in biomedical applications, but it is also employed as food additives in food industries.

Research activities in electrospun guar gum nanofibers

Lubambo *et. al.* (2015) have investigated the guar gum/PVA electrospun membranes for better encapsulation of paramagnetic iron oxide (Fe_3O_4) nanoparticles in alkaline and non-alkaline condition. It is believed that the dispersion of Fe_3O_4 on the membranes can act as the bactericidal agent for the potential used as a biodegradable wound dressing. In non-alkaline condition, the nanofibers diameter of guar gum/PVA nanofibers is range from 200-250 nm. Whereas, the diameter of the nanofibers decreased to 190 nm in an alkaline condition. It is because the deprotonation of hydroxyl groups and the chelation of Fe^{2+} and Fe^{3+} ions which bring the molecules in the nanofibers closer together and resulted in the decrease in nanofibers diameter [29]. Shi *et. al.* (2016) have successfully fabricated the electrospun nanofibers membrane using guar gum, PVA and citric acid.

These nanofibers were proved to be insoluble in water by crosslinking them at high temperature (140 °C) for 2 h after electrospinning. Hence, they have the potential to be used for drug delivery application and tissue engineering. The average diameter of the non-cross-linked guar/PVA/citric acid nanofibers is between 194±23 nm and there is a slight increase in diameter after cross-linked to 204±18 nm [30].

4.2.2 Carrageenan

Chemical structure and applications of Carrageenan

Carrageenan is a family of linear sulfated polysaccharides that are obtained by alkaline extraction from some species of red seaweeds (e.g. Gigartinales, Rhodophyta) [45]. There are three major types of carrageenan which include Iota-carrageenan (i-carrageenan), Kappa-carrageenan (k-carrageenan) and Lambda-carrageenan (l-carrageenan). Carrageenan is formed by disaccharide repeating unit which consists of alternating 3-linked β-D-galactopyranose or 4-linked α-D-galactopyranose or 4-linked 3,6-anhydro-α-D-galactopyranose.

In the food industry, carrageenans are used as thickener, food stabilizer and gelling agents. Furthermore, over time, carrageenan as a microbiocidal compound was formulated in the form of wound dressings for successful wound healing [46].

Research activities in electrospun carrageenan nanofibers

Basilia *et. al.* (2008) has fabricated electrospun PCL)/i-Carrageenan nanofibrous scaffolds for *in-vitro* screening using simulated body fluids (SBF) and *in-vivo* screenings using mice for tissue regeneration. Both screenings showed increase bioactivity and no invoke adverse inflammation which proven to accelerate tissue healing. The nanofibers were not uniform and it is reported to be influenced by the concentration of carrageenan and the glass transition temperature of the blend solution. The hydrogen bonded complex and intermolecular co-adhesion might be the reason for having unstable electrospinning

[38]. Furthermore, Goono *et. al.* (2017) reported the research in blending κ–Carrageenan with PHB and PHBV to fabricate electrospun microfibers as scaffold materials in bone tissue engineering applications. Due to the higher molar weight of PHB exhibited quicker jet transformation, the PHBV/κ–carrageenan solution fabricated rough surface nanofibers whereas PHB/κ–carrageenan formed smooth surface nanofibers. The diameter of both fibers was at the range of 1.9 ± 0.6 μm and 1.6 ± 0.5 μm. κ–carrageenan was selected as it induced the higher production of the anti-inflammatory interleukin-10 (IL-10) when compared to λ-carrageenan. The results showed higher NIH 3T3 cell density and also determined an enhancement of the biomineralization using human osteosarcoma SaOS-2 cells as well as showed osteogenic differentiation potential in the blend nanofibers, especially on PHBV/κ- carrageenan nanofibers. [39].

4.3 Polysaccharide of animal origin

Apart from natural polysaccharide found in human, plants and seaweed, polysaccharides can be obtained from animals. It includes (a) the storage polysaccharide, glycogen which normally found in liver or muscle of animals and (b) the structural polysaccharide, chitin and its derivatives (e.g. chitosan) which found in the exoskeleton of arthropods as well as the cell wall of fungi. **Table 5** illustrated the studies of natural polysaccharide derived from animals by the researchers and their potential applications. Unfortunately, there were no studies about the fabrication of the glycogen nanofibers using electrospinning found in the literature, an only similar study was found to use glycogen from oyster to form nanofibers using freeze-drying method [47]. Hence, it is a potential field to investigate in the future. In this chapter, chitin and its derivatives derived from shell animals are mainly discussed.

4.3.1 Chitin

Chemical structure and applications of chitin

Chitin is a structural monosaccharide mainly found in the shell of arthropods such as insects, lobster, prawn, shrimp and crab. It is the second most abundant of nature

biopolymers after cellulose. Chitin is composed of a linear co-polymer chain of N-acetyl-D-glucosamine residues with peptidoglycan β-1-4 linkage **(Figure 4a)**.

Its physiochemical properties include odorless, white or creamy-white powder. Chitin is generally used in food, cosmetics, biomedical and pharmaceutical products. However, chitin has received little industrial based attention due to its poor solubility in water and common organic solvents which resulted in a complicated network formation consist of both inter- and intramolecular hydrogen bonding. Therefore, the deacetylation product of chitin called chitosan has been given an extensive interest to use in biomedical applications [49]. Because of its distinctive biological property of wound healing effect, chitin and its derivatives are widely used for the medical applications, specifically for wound management and tissue engineering.

Research activities in electrospun chitin nanofibers

Noh *et al*. (2006) reported relatively high human keratinocytes and fibroblasts cells attachment, migration and proliferation were observed on electrospun chitin nanofibers. Owing to chitin's poor solubility, halogenated compounds, HFIP was used to dissolve chitin. Other than that, strong acids such as methanesulfonic acid (MSA) or mixed solvents such as lithium chloride (LiCl)/dimethylacetamide (DMAc) can be used to dissolve chitin [48]. The result showed the chitin nanofibers have an average diameter of 163 nm. Additionally, the nanofibers coated with collagen significantly stimulated the cellular response. The overall results have indicated the chitin nanofibers could be potential candidates for wound healing and oral mucosa and skin regeneration [49]. Another research activity reported by Mina *et al*. (2004) showed the average diameter of chitin nanofibers of 110 nm. The study reported that certain chain entanglement in the chitin solution is essential to produce uniform nanofibers. The concentration of chitin solution higher or lower than the threshold can induce the formation of beaded nanofibers. The author also successfully performed the deacetylation of chitin nanofibers to chitosan nanofibers by chemically treated with a 40% aqueous NaOH solution at 60 °C and 100 °C. At high temperature (100 °C), 85% of deacetylation completed within 2

hours, while it took 1 day to complete 85% acetylation at low temperature (60 °C). Both chitin and chitosan nanofibers can be used as wound dressings. [14].

4.3.2 Chitosan

Chemical structure and applications of chitosan

Chitosan is a modified natural polysaccharide produced by partial deacetylation of chitin under a hot alkali condition with 40%-50% concentrated sodium hydroxide (NaOH) solution or via enzymatic conversion of N-acetyl-D-glucosamine residue of chitin into D-glucosamine residues of chitosan in the presence of chitin-deacetylase **(Figure 4b)**. Chitosan is obtained by eliminating enough number of acetyl groups, CH_3-CO and finally getting a linear polysaccharide consisting of randomly distributed β-1,4-D-glucosamine (deacetylated units) and N-acetyl-D-glucosamine (acetylated units). Basically, the actual difference between chitin and chitosan is the degree of acetylation (the ratio of N-acetyl-D-glucosamine to D-glucosamine structural units). The amount of N-acetyl-D-glucosamine greater than 50% represented as chitin whereas D-glucosamine level greater than 50% represented as chitosan [53].

Chitosan is extensively used for bone tissue engineering because of its structural similarity to the glycosaminoglycan in bone. In addition, its low toxicity and biodegradability with excellent biological activities such as good immunological, intrinsic antibacterial activity and low immunogenicity have provided many future perspective in development of biomaterials for wound dressing, drug delivery systems and cell culture [46], [54]. In detail, the deacetylated chitin derivative, chitosan is a cationic polysaccharide because it possesses positive ionic charges from its free amino group. So, this has given chitosan the opportunity to chemically bind strongly with negatively charged cell surface making it useful to formulate bio-adhesive dosage forms. Also, chitosan is able to bind strongly to anions on the bacterial cell wall and subsequently altered the mass transport across the cell wall, resulting in the suppression of biosynthesis and accelerating bacterial death [51], [55]. Furthermore, it is hard to

electrospin chitosan because the cationic charge increases the excessive surface tension of the solution, which resulted in a high electrical force demand to fabricate nanofibers. Therefore, another polymer is mixed with chitosan to overcome the problem.

Research activities in electrospun chitosan nanofibers

Agrawal and Pramanik (2016) reported a study related to the investigation of chitosan/ PVA nanofibers for tissue engineering. A series of chitosan/PVA blend ratios in 10:90, 20:80, 30:70, 35:65 and 40:60 were tested and concluding all the analysis of experiment, it was proved that the 35:65 ratio of Chitosan/PVA nanofibrous scaffold had the finest nanofibers in the average diameter of 260 nm with superior physicochemical analysis and *in vitro* study (i.e. high efficient for hMSCs proliferation). By figuring out the suitable ratio between chitosan and PVA, it is possible to get the appropriate morphology of nanofibers [51]. Another example of using chitosan for electrospinning is coming from the work of Venugopal *et. al.* (2011) for bone tissue regeneration. They have prepared the electrospun chitosan/hydroxyapatite nanofibrous scaffold with the average diameter of the chitosan/hydroxyapatite nanofibers around 510±198 nm. Besides, it is also proved to show these nanofibers were able to promote cell response and proliferation of human fetal osteoblast, hFOB cells which further enhance the bone forming ability [52], [56]. A similar study has been conducted by Zhang and colleagues (2008), they have fabricated chitosan/hydroxyapatite nanofibrous scaffolds together with ultra-high molecular weight poly (ethylene oxide) (UHMWPEO) as a fiber-forming facilitating additive. Uniform nanofibers with a diameter of 214 ± 25 nm were determined. Also, a significant level of bone cell formation ability was demonstrated at Day 15 with an increased level of hFOB cells proliferation [56], [57].

4.4 Polysaccharide of microbe origin

Microbial polysaccharide is also called exopolysaccharide as the polysaccharide is either obtained from the cell wall or secreted from the cell to form a layer over the surface of the microorganism such as bacteria, fungi and yeast. Due to the absence of virtually no known toxic agents and very cheap to harvest in large quantities, these extracellularly

produced natural polysaccharides by microorganisms are in food, pharmaceutical, and biomedical industries [58].

Microbial polysaccharides can be produced from either fungi or bacterium, including schizophyllan from the fungi *Schizophyllan commue*, pullulan from the fungus *Aureobasidium pullulans*, alginates from the bacterium *Azotobacter vinelandii*, xanthan gum from the bacterium *Xanthomonas campestris*, dextran from the bacterium *Leuconostoc mesenteroides*, gellan from the bacterium *Pseudomonas elodea* and curdlan from the bacterium *Alcaligenes faecalis*. **Table 6** illustrated the research activities of the natural polysaccharide from the microbe origin mentioned above. In this chapter, xanthan gum, pullulan and dextran as the representative examples of microbial polysaccharides are mainly discussed.

4.4.1 Xanthan gum

Chemical structure and applications of xanthan gum

Xanthan gum is a hetero-, branched polysaccharide formed during the fermentation process of the bacterium, *Xanthomonas Compestris* originally isolated from the Rutabaga plant [81]. It has a chemical structure of 5 sugar repeating units in the molar ratio of 2:2:1 with two D-glucose units, two D-mannose units and one D-glucuronic acid unit **(Figure 5)**. The backbone consists of β-1,4-D-glucose repeating units with additional side chains of trisaccharide at every other glucose units at C-3. The sidechain is built up of (1,4)- β -D-glucuronic acid unit sandwiched between α-1,2-D-mannose with an acetyl group attached to C-6 and β -D-mannose [3]. The terminal mannose residues are usually pyruvated and the non-terminal residue may have an acetyl group at C-6 [82]. Xanthan gum is a highly electronegative or 'polyanionic' molecule because of the presence of anionic side chains (i.e. pyruvate and acetate).

Xanthan gum is an ideal agent as an emulsifier, stabilizer and thickener for many types of water-based products ranging from food, pharmaceutical, cosmetic, cleaner, agricultural,

textile, coating, ceramic and oil fields. Whereas in the biomedical point of view, xanthan gum in combination with other hydrophilic or hydrophobic polymers can form a more stable gel system [3], [81], [82].

Research activities in electrospun xanthan gum nanofibers

There is a new breakthrough in fabricating xanthan gum nanofibers using electrospinning. Shekarforoush and his research team (2017) have fabricated the xanthan gum nanofibers without using any additive polymers. By using formic acid as the solvent to dissolve xanthan gum, it is possible to be obtained the ultrafine nanofibers with the average diameters ranging from 128 ± 36.7 to 240 ± 80.7 nm at the concentration of 1.0-2.5 %w/w. Due to the unstable molecular conformation of xanthan solution in aqueous solutions, formic acid is discovered to have the ability to stabilize the helical conformation of xanthan by neutralizing the pyruvic charges in xanthan solution with formate groups in formic acid. The xanthan gum nanofibers have the potential to be used as a carrier for the encapsulation of bioactive compounds in drug delivery applications [15], [71].

In addition, the xanthan gum has been successfully electrospun with a cationic natural polysaccharide, chitosan as drug delivery carrier by Shekarforoush et.al. (2018). The hydrophobic bioactive compound, curcumin is encapsulated in the xanthan gum/chitosan (X-Ch) nanofibers to overcome its low solubility and instability in a body fluid as well as to study its drug release in different pH buffers (i.e. pH 2.2, 6.5 and 7.6). The diameter of X-Ch nanofibers (750 ± 250 nm) increased approximately 160 nm with the addition of curcumin in xanthan solution (910 ± 440 nm). Moreover, the study showed a good curcumin encapsulation efficiency of 69.4 ± 4.1% in X-Ch nanofibers with no burst release effect in all three pH buffers. There is a low curcumin release of 20% in the acidic condition, pH 2.2 after 5 Days. However, the amount of curcumin released from the X-Ch nanofibers was increased as the pH value increased. 45% and 50% of curcumin released in pH 6.5 and pH 7.6, respectively. Hence, it is suggested that these X-Ch

nanofibers encapsulated curcumin is suitable for use as a long-term pH-stimulated drug release carrier [54].

4.4.2 Pullulan

Chemical structure and applications of pullulan

Pullulan is extracted from the fungus-like yeast, *Aureobasidium pullulans* [60]. As shown in **Figure 6**, it is formed by repeating maltotriose units. A maltotriose unit consists of three glucose units (i.e. α-1,4-glucan and α-1,6-glucan) connected by α-1,4 glycosidic bonds. In addition, the consecutive maltotriose units are bonded to each other via an α-1,6 glycosidic bond [46], [83].

The consistent alternation of α-1,4 and α-1,6 glycosidic linkages give rise to two unique properties of structural flexibility and improved solubility which have huge benefits to the pharmaceutical industries [84]. Pullulan is potentially used as pharmaceutical coatings for tablets, pills and granules due to its high water solubility and low moisture resistance. It also attracts great interest for the uses as pre-dosed formulations wrapping materials for soft and hard capsule because it is colorless, transparent and biodegradable [85]. Moreover, the non-immunogenic, non-mutagenic and non-carcinogenic nature of pullulan has been discovered as vaccine adjuvant in which was conjugated to various toxoids and showed very promising immunogenic results. A nasal vaccination research study conducted by Cevher *et. al.* is using pullulan incorporated with chitosan derivatives to form a nanocomposite for the encapsulation of a model antigen, bovine serum albumin (BSA). The result showed an efficient nanoparticles uptake by macrophage [86], [87]. Owing to its non-toxic, edible, and resistance to mammalian amylases characteristics, pullulan is also widely used in food industry as a low-calorie ingredient in foods. In addition, pullulan has a relatively low viscosity as compared to other natural polysaccharides, it is suitable to be used as low-viscosity filler in beverages and sauces [88].

Research activities in electrospun pullulan nanofibers

The electrospun pullulan nanofibers have been successfully fabricated by Sun *et. al.* (2012). In the study, by varying the pullulan solution concentration, applied voltage, flow rate and needle–collector plate distance to 22 %w/w solution concentration, 31 kV voltage, 0.5 ml/h flow rate and 20 cm needle–collector plate distance during electrospinning, the finest and more uniform diameter of nanofibers can be produced between 100-700 nm. Additionally, the diameter of pullulan nanofibers can be reduced by decreasing the concentration of pullulan solution and flow rate while increasing the applied voltage and needle–collector plate distance, It is proposed that these pullulan nanofibers are potential to be used to manufacture bandages or act as drug carriers [60]. In addition, Qian and King (2016) have investigated the gelatin/pullulan electrospun nanofibers to serve as a tissue engineering scaffold, particularly to mimic the extracellular matrix (ECM). The result showed an average nanofibers diameter of 152 nm. It was also discovered that not only the concentration and viscosity of the spinning solution but also the weight ratio of gelatin and pullulan influence the morphology and diameter of the nanofibers [63]. Besides, Li *et.al*. (2017) have fabricated the electrospun pullulan nanofiber membrane that has the potential utilized as a water-resistant biomaterial. The prepared pullulan nanofibers were crosslinked by ethylene glycol diglycidyl ether (EGDE) and ethanol absolute (1:7 ratio) to enhance its stability in water with lower water absorption. It is crucial to manage the crosslinking time. As the crosslinking time increased, the porosity of the nanofibers decreased [61].

Moreover, several types of pullulan nanofibers were prepared using electrospinning technique which includes pullulan-whey protein electrospun nanofibers [64] and curcumin-loaded amaranth-pullulan electrospun nanofibers [65] have a huge potential for biomedical applications.

4.4.3 Dextran

Chemical structure and applications of dextran

Dextran is a natural polysaccharide that sucrose is extracted from specific lactic acid bacteria such as *Leuconostoc mesenteroides* and *Streptococcus mutans*. It has a complex branched glucan structure as seen in **Figure 7**. Its main chain composed of glucose monomers linked via α-1,6 glycosidic bond with branches from either α-(1-2), α-(1-3), or α-(1-4) linkages.

The biocompatibility and biodegradability characteristics of dextran nanofibers are very important properties in biomedical applications for the use as drug carriers, plasma expander and tissue scaffolds [89]. Furthermore, dextran is soluble in both water and organic solvents include tetrahydrofuran, dimethyl sulfoxide, acetone. Owing to its unique solubility characteristic, dextran can be used to prepare different types of formulations by blending with either hydrophilic bioactive agents (e.g. penicillin) or hydrophobic bioactive agents (e.g. curcumin) or hydrophilic biodegradable polymers (e.g. PVA) or hydrophobic biodegradable polymers (e.g. PLGA). The hydrophilic polymers such as dextran are said to be soft and flexible in a swollen state with high affinity to cells, yet due to its low mechanical strength, it needs a co-polymer like hydrophobic polymers which have the high mechanical strength to assist them to improve the overall properties of electrospun nanofibers [90]. In addition, electrospinning has an advantage over other types of technology in allowing the use of blend polymers for nanofibers production.

Research activities in electrospun dextran nanofibers

Dextran encourages neovascularization and re-epithelialization in chronic wounds. It is a suitable natural polysaccharide used as wound dressings. Unnithan *et. al.* (2012) have conducted a study about developing polyurethane (PU)/dextran nanofibers loaded with a fluoroquinolone antibiotic, ciprofloxacin HCl for post-menopausal wound dressing. Ciprofloxacin HCl is chosen as the model drug due to its low minimal inhibitory

concentration (MIC) for both Gram-positive bacteria such as *S. aureus* and *B. subtilis* as well as Gram-negative bacteria such as *E. coli*, *S. typhimurium* and *V. vulnificus* that normally cause wound infections. The result presented a good antibacterial activity with the zone of inhibition around 15-20 mm for both types of bacteria. The diameter of the PU nanofibers also dramatically decreased from 401-100 nm to 101-300 nm with the addition of dextran and ciprofloxacin HCl [72]. Again in 2015, Unnithan led another research team to continue investigating the electrospun PU/dextran nanofibers for wound dressing mainly to treat the post-menopausal wounds. Thus, an estrogen, estradiol with potent anti-inflammatory and good blood clotting properties was loaded in the PU/dextran solution for electrospinning to accelerate the wound healing process. It was further proven in the study that the re-epithelialization property of dextran with the anti-inflammatory property of estradiol has shortened the healing time with animal testing. The result also showed the continuous uniform nanofibers with a diameter range from 500-600 nm when blending the PU, dextran and estradiol together [73].

Apart from using dextran for wound dressing materials, it is also potentially used in the drug delivery system. A study about oral fast-dissolving drug delivery was conducted by Maslakci *et. al*. (2017) by loading two different drugs, ibuprofen and acetylsalicylic acid separately into the electrospun PVP/dextran nanofiber mats. The diameter of the PVP/dextran and PVP/acetylsalicylic acid nanofibers were not stable with beaded morphology which mainly due to the viscosity of the solutions. However, by adding the drugs into the PVP/dextran solutions, the PVP/Dextran/Ibuprofen and PVP/Dextran/acetylsalicylic acid nanofibers showed the diameter range from 300-650 nm using scanning electron microscopy (SEM) with a uniform diameter and ultrafine surface. Therefore, to optimize the morphology, the ratio of PVP, Dextran, ibuprofen and acetylsalicylic acid is highly modulated [74]. In another study, Moydeen *et al.* (2018) reported the fabrication of electrospun PVA/dextran nanofibers loaded with ciprofloxacin and ciprofloxacin-HCl drugs separately for *in vitro* drug release. The coaxial electrospinning and emulsion electrospinning were employed and the transmission electron microscopy (TEM) result indicated that uniform core-shell nanofibers were observed by using the emulsion electrospinning. Owing to the uniformity, it showed a

slow release kinetics compared to the blended PVA/Dextran/ciprofloxacin using conventional co-axial electrospinning. Furthermore, by introducing the drug into the PVA/Dextran solution, the diameter of the nanofibers has decreased from 400-600 nm to 200-300 nm which further proven the flawlessly loaded of ciproflaxacin into the core [75].

5.0 Conclusion

As detailed in this book chapter, electrospun natural polysaccharide nanofibers derived from human, animals, plants, seaweed and microbes have promising biomedical applications in different fields such as drug delivery, wound dressing and tissue engineering. Some of the natural polysaccharides such as curdlan has proven to be very difficult for electrospinning [80]. Thus, different types of polysaccharide or polymer were chosen as a co-spinning system to exhibit the formation of continuous nanofibers. In addition, the diameter of the nanofibers depends on solution concentration, applied voltage, surface tension of solution, spinning flow rate etc. These nanofibers can be customized by modulating the electrospinning parameters.

It is also important to draw some attention to the production of the natural polysaccharide nanofibers. There is a limited number of biomedical products have commercially implemented electrospun nanofibers. Thus, there will be even less or none natural polysaccharide based electrospun nanofibers. The most relevant commercialized nanofibers product that used natural polysaccharide is the “HealSmart Personalized Wound Care System” from the provider, PolyRemedy, Inc in 2013. The company has announced an electrospun wound dressing with the addition of hyaluronic acid [91]. As majority of these nanofibers are still in the research progress, a lot of efforts have been made by the researchers to transform the nanofibers from laboratory to commercial production.

Other than that, the development of scale-up electrospun nanofibers production method is also a very important research area for the growth in the biomedical field. One approach

for employing the electrospinning technology to the natural polysaccharide is to utilize the model systems or formulations that have already been established in other fields such as the food, textiles, cosmetics sectors and adapting it to the requirements of the biomedical applications.

6.0 References

[1] S. Mohammadzadehmoghadam *et al.*, "Electrospinning: Current Status and Future Trends," in *Nano-Size Polymers: Preparation, Properties, Applications*, no. September, 2016, pp. 80–154.

[2] M. Zanin, N. P. Cerize, and A. Oliveira, "Production of Nanofibers by Electrospinning Technology: Overview and Application in Cosmetics," *Nanocosmetics and Nanomedicines*, pp. 311–332, 2011.

[3] C. Brunchi, M. Bercea, S. Morariu, and M. Avadanei, "Investigations on the interactions between xanthan gum and poly (vinyl alcohol) in solid state and aqueous solutions," *Eur. Polym. J.*, vol. 84, pp. 161–172, 2016.

[4] D. Liang, B. S. Hsiao, and B. Chu, "Functional electrospun nanofibrous scaffolds for biomedical applications," *Adv. Drug Deliv. Rev.*, vol. 59, no. 14, pp. 1392–1412, 2007.

[5] I. Z. Awan, S. B. Hussain, A. ul Haq, and A. Q. Khan, *Wondrous nanotechnology*, vol. 38, no. 6. 2016.

[6] E. Sapountzi, M. Braiek, J. F. Chateaux, N. Jaffrezic-Renault, and F. Lagarde, "Recent advances in electrospun nanofiber interfaces for biosensing devices," *Sensors (Switzerland)*, vol. 17, no. 8, 2017.

[7] N. Zhang *et al.*, "Electrospun TiO 2 nanofiber-based cell capture assay for detecting circulating tumor cells from colorectal and gastric cancer patients," *Adv. Mater.*, vol. 24, no. 20, pp. 2756–2760, 2012.

[8] J. H. Park, S. Lee, J. H. Kim, K. Park, K. Kim, and I. C. Kwon, "Polymeric nanomedicine for cancer therapy," *Prog. Polym. Sci.*, vol. 33, no. 1, pp. 113–137, 2008.

[9] B. S. Chee, G. G. De Lima, D. Devine, and M. J.D.Nugent, "Electrospun hydrogels composites for bone tissue engineering," in *Applications of Nanocomposite Materials in Orthopedics*, Elsevier Inc., 2018, pp. 39–70.

[10] G. G. de Lima, S. Lyons, D. M. Devine, and M. J. D. Nugent, "Electrospinning of Hydrogels for Biomedical Applications," in *Hydrogels*, Springer Singapore, 2018, pp. 219–258.

[11] O. Husain, W. Lau, M. Edirisinghe, and M. Parhizkar, "Investigating the particle to fibre transition threshold during electrohydrodynamic atomization of a polymer solution," *Mater. Sci. Eng. C*, vol. 65, pp. 240–250, 2016.

[12] H. W. He *et al.*, "Solvent-free electrospinning of UV curable polymer microfibers," *RSC Adv.*, vol. 6, no. 35, pp. 29423–29427, 2016.

[13] T. Subbiah, G. S. Bhat, R. W. Tock, S. Parameswaran, and S. S. Ramkumar, "Electrospinning of nanofibers," *J. Appl. Polym. Sci.*, vol. 96, no. 2, pp. 557–569, 2005.

[14] B. M. Min *et al.*, "Chitin and chitosan nanofibers: Electrospinning of chitin and deacetylation of chitin nanofibers," *Polymer (Guildf).*, vol. 45, no. 21, pp. 7137–7142, 2004.

[15] E. Shekarforoush, A. Faralli, S. Ndoni, A. C. Mendes, and I. S. Chronakis, "Electrospinning of Xanthan Polysaccharide," *Macromol. Mater. Eng.*, vol. 302, no. 8, pp. 1–11, 2017.

[16] H. Wang, Y. Feng, Z. Fang, R. Xiao, W. Yuan, and M. Khan, "Fabrication and characterization of electrospun gelatin-heparin nanofibers as vascular tissue engineering," *Macromol. Res.*, vol. 21, no. 8, pp. 860–869, 2013.

[17] Y. J. Ye, Y. Q. Zhou, Z. Y. Jing, Y. Y. Liu, and D. C. Yin, "Electrospun Heparin-Loaded Core–Shell Nanofiber Sutures for Achilles Tendon Regeneration In Vivo," *Macromol. Biosci.*, vol. 18, no. 7, pp. 1–12, 2018.

[18] Y. Su, X. Li, Y. Liu, Q. Su, M. L. W. Qiang, and X. Mo, "Encapsulation and controlled release of heparin from electrospun poly(L-lactide-co-ε-caprolactone) nanofibers," *J. Biomater. Sci. Polym. Ed.*, vol. 22, no. 1–3, pp. 165–177, 2011.

[19] A. Sadeghi, M. Pezeshki-Modaress, and M. Zandi, "Electrospun polyvinyl alcohol/gelatin/chondroitin sulfate nanofibrous scaffold: Fabrication and in vitro evaluation," *Int. J. Biol. Macromol.*, vol. 114, pp. 1248–1256, 2018.

[20] M. Pezeshki-Modaress, H. Mirzadeh, and M. Zandi, "Gelatin-GAG electrospun nanofibrous scaffold for skin tissue engineering: Fabrication and modeling of process parameters," *Mater. Sci. Eng. C*, vol. 48, pp. 704–712, 2015.

[21] J. Guo, H. Zhou, M. Y. Akram, X. Mu, J. Nie, and G. Ma, "Characterization and application of chondroitin sulfate/polyvinyl alcohol nanofibres prepared by electrospinning," *Carbohydr. Polym.*, vol. 143, pp. 239–245, 2016.

[22] G. Doğan, G. Başal, O. Bayraktar, F. Ozyildiz, A. Uzel, and I. Erdoğan, "Bioactive Sheath/Core nanofibers containing olive leaf extract," *Microsc. Res. Tech.*, vol. 79, no. 1, pp. 38–49, 2016.

[23] L. Dong *et al.*, "Evaluation of emulsion electrospun polycaprolactone/hyaluronan/epidermal growth factor nanofibrous scaffolds for wound healing," *J. Biomater. Appl.*, vol. 30, no. 6, pp. 686–698, 2015.

[24] G. Chen, J. Guo, J. Nie, and G. Ma, "Preparation, characterization, and application of PEO/HA core shell nanofibers based on electric field induced phase separation during electrospinning," *Polymer (Guildf).*, vol. 83, pp. 12–19, 2016.

[25] H. J. Lai *et al.*, "Tailor design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing," *Acta Biomater.*, vol. 10, no. 10, pp. 4156–4166, 2014.

[26] N. Patra, M. Salerno, and M. Cernik, *Electrospun polyvinyl alcohol/pectin composite nanofibers*. Elsevier Ltd., 2016.

[27] D. McCune *et al.*, "Electrospinning pectin-based nanofibers: a parametric and cross-linker study," *Appl. Nanosci.*, vol. 8, no. 1–2, pp. 33–40, 2018.

[28] E. G. Walaa Mohamed Ali Wahbi, E. G. Wael Mamdouh Sayed Sayed Ahmed, and E. G. Rania Siam, "Inulin nanofibers," WO2016086225A1, 2016.

[29] A. F. Lubambo *et al.*, "Tuning Fe3O4 nanoparticle dispersion through pH in PVA/ Guar Gum Electrospun Membranes," *Carbohydr. Polym.*, vol. 134, pp. 775–783, 2015.

[30] J. Shi and E. Yang, "Post-Electrospinning Crosslinking of Guar / Polyvinyl alcohol membrane," *Therm. Sci.*, vol. 20, no. 1, pp. 1–5, 2016.
[31] A. M. M. Sousa, H. K. S. Souza, J. Uknalis, S. C. Liu, M. P. Gonçalves, and L. Liu, "Electrospinning of agar/PVA aqueous solutions and its relation with rheological properties," *Carbohydr. Polym.*, vol. 115, pp. 348–355, 2015.
[32] W. Xu, R. Shen, Y. Yan, and J. Gao, "Preparation and characterization of electrospun alginate/PLA nanofibers as tissue engineering material by emulsion eletrospinning," *J. Mech. Behav. Biomed. Mater.*, vol. 65, pp. 428–438, 2017.
[33] N. A. Burns *et al.*, "Cyclodextrin facilitated electrospun chitosan nanofibers," *RSC Adv.*, vol. 5, no. 10, pp. 7131–7137, 2015.
[34] L. Kong and G. R. Ziegler, "Fabrication of pure starch fibers by electrospinning," *Food Hydrocoll.*, vol. 36, pp. 20–25, 2014.
[35] J. Venugopal *et al.*, "Xylan polysaccharides fabricated into nanofibrous substrate for myocardial infarction," *Mater. Sci. Eng. C*, vol. 33, no. 3, pp. 1325–1331, 2013.
[36] S. Kikionis, E. Ioannou, G. Toskas, and V. Roussis, "Electrospun biocomposite nanofibers of ulvan/PCL and ulvan/PEO," *J. Appl. Polym. Sci.*, vol. 132, no. 26, pp. 1–5, 2015.
[37] G. Toskas, R. D. Hund, E. Laourine, C. Cherif, V. Smyrniotopoulos, and V. Roussis, "Nanofibers based on polysaccharides from the green seaweed Ulva Rigida," *Carbohydr. Polym.*, vol. 84, no. 3, pp. 1093–1102, 2011.
[38] B. A. Basilia, A. P. Robles, K. A. Ledda, K. B. Dagbay, and M. St, "In-vitro and In-vivo Screenings of Electrospun Polycaprolactone-Carrageenan Nanofibrous Scaffolds for Tissue Engineering," *TechConnect Briefs*, vol. 2, pp. 306–309, 2008.
[39] N. Goonoo *et al.*, "κ-Carrageenan Enhances the Biomineralization and Osteogenic Differentiation of Electrospun Polyhydroxybutyrate and Polyhydroxybutyrate Valerate Fibers," *Biomacromolecules*, vol. 18, no. 5, pp. 1563–1573, 2017.
[40] W. Zhang *et al.*, "Electrospinning of fucoidan/chitosan/poly(vinyl alcohol) scaffolds for vascular tissue engineering," *Fibers Polym.*, vol. 18, no. 5, pp. 922–932, 2017.
[41] S. Chahala, F. S. J. Hussain, and M. M. Yusoff, "Characterization of modified cellulose (MC)/poly (vinyl alcohol) electrospun nanofibers for bone tissue engineering," *Procedia Engineering*, vol. 53. pp. 683–688, 2013.
[42] M. Manjunath *et al.*, "Guar Gum and Its Pharmaceutical and Biomedical Applications," *Adv. Sci. Eng. Med.*, vol. 8, no. 8, pp. 589–602, 2016.
[43] Y. S. R. Krishnaiah, S. Satyanarayana, Y. V. Rama Prasad, and S. Narasimha Rao, "Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers," *J. Control. Release*, vol. 55, no. 2–3, pp. 245–252, 1998.
[44] W. Yang, M. Zhang, X. Li, J. Jiang, A. M. M. Sousa, and Q. Zhao, "Incorporation of Tannic Acid in Food-Grade Guar Gum Fibrous Mats by Electrospinning Technique," *Polymers (Basel).*, vol. 11, no. 1, p. 141, 2019.
[45] L. Pereira and F. Van De Velde, "Portuguese carrageenophytes: Carrageenan composition and geographic distribution of eight species (Gigartinales, Rhodophyta)," *Carbohydr. Polym.*, vol. 84, no. 1, pp. 614–623, 2011.

[46] O. M. Dragostin *et al.*, "Polymeric materials as nanofibers used in the treatment of burns," *Rev. Med. Chir. Soc. Med. Nat.*, vol. 119, no. 3, pp. 903–909, 2015.
[47] M. Vetrik *et al.*, "Biopolymer-based degradable nanofibres from renewable resources produced by freeze-drying," *RSC Adv.*, vol. 3, no. 35, pp. 15282–15289, 2013.
[48] P. S. Barber, C. S. Griggs, J. R. Bonner, and R. D. Rogers, "Electrospinning of chitin nanofibers directly from an ionic liquid extract of shrimp shells," *Green Chem.*, vol. 15, no. 3, pp. 601–607, 2013.
[49] H. K. Noh *et al.*, "Electrospinning of chitin nanofibers: Degradation behavior and cellular response to normal human keratinocytes and fibroblasts," *Biomaterials*, vol. 27, no. 21, pp. 3934–3944, 2006.
[50] B. Dhandayuthapani, U. M. Krishnan, and S. Sethuraman, "Fabrication and characterization of chitosan-gelatin blend nanofibers for skin tissue engineering," *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, vol. 94, no. 1, pp. 264–272, 2010.
[51] P. Agrawal and K. Pramanik, "Chitosan-poly(vinyl alcohol) nanofibers by free surface electrospinning for tissue engineering applications," *Tissue Eng. Regen. Med.*, vol. 13, no. 5, pp. 485–497, 2016.
[52] V. Jayarama Reddy, G. D. V.R, S. T, S. D, D. G, and R. S, "Osteoblasts mineralization with Composite nanofibrous substrate for Bone tissue regeneration," *Cell Biol. Int.*, vol. 35, pp. 73–80, 2010.
[53] N. Shaari and S. K. Kamarudin, "Chitosan and alginate types of bio-membrane in fuel cell application: An overview," *J. Power Sources*, vol. 289, pp. 71–80, 2015.
[54] E. Shekarforoush, F. Ajalloueian, G. Zeng, A. C. Mendes, and I. S. Chronakis, "Electrospun xanthan gum-chitosan nanofibers as delivery carrier of hydrophobic bioactives," *Mater. Lett.*, vol. 228, pp. 322–326, 2018.
[55] S. O. Majekodunmi, "Current Development of Extraction , Characterization and Evaluation of Properties of Chitosan and Its Use in Medicine and Pharmaceutical Industry," *Am. J. Polym. Sci.*, vol. 6, no. 3, pp. 86–91, 2016.
[56] G. Chiara *et al.*, "Nanostructured biomaterials for tissue engineered bone tissue reconstruction," *Int. J. Mol. Sci.*, vol. 13, no. 1, pp. 737–757, 2012.
[57] Y. Zhang, J. R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, and C. T. Lim, "Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering," *Biomaterials*, vol. 29, no. 32, pp. 4314–4322, 2008.
[58] S. Lei and T. Feng Edmund, "Polysaccharides, Microbial," *Ref. Modul. Life Sci.*, pp. 482–494, 2017.
[59] M. R. Safaee-Ardakani *et al.*, "Electrospun Schizophyllan/polyvinyl alcohol blend nanofibrous scaffold as potential wound healing," *Int. J. Biol. Macromol.*, vol. 127, no. December, pp. 27–38, 2019.
[60] X. Bin Sun, D. Jia, W. M. Kang, B. W. Cheng, and Y. Bin Li, "Research on Electrospinning Process of Pullulan Nanofibers," *Appl. Mech. Mater.*, vol. 268–270, pp. 198–201, 2012.
[61] Y. Li, X. Ma, J. Ju, X. Sun, N. Deng, and Z. Li, "Preparation and characterization of crosslinked electrospun pullulan nanofiber membrane as a potential for biomaterials," *J. Text. Inst.*, vol. 5000, no. October, pp. 1–7, 2017.

[62] T. Pan, Y. Liu, W. Kang, Y. Wu, and Z. Li, "Preparation and characterization of long-term stable pullulan nanofibers via in situ cross-linking electrospinning," *Adsorpt. Sci. Technol.*, p. 026361741881301, 2018.
[63] Y. Qian and M. King, "Green electrospinning of gelatin and pullulan nanofibers to mimic the extracellular matrix," in *10th World Biomaterials Congress*, 2016.
[64] C. Drosou, M. Krokida, and C. G. Biliaderis, "Composite pullulan-whey protein nanofibers made by electrospinning: Impact of process parameters on fiber morphology and physical properties," *Food Hydrocoll.*, vol. 77, pp. 726–735, 2018.
[65] A. Blanco-Padilla, A. López-Rubio, G. Loarca-Piña, L. G. Gómez-Mascaraque, and S. Mendoza, "Characterization, release and antioxidant activity of curcumin-loaded amaranth-pullulan electrospun fibers," *LWT - Food Sci. Technol.*, vol. 63, no. 2, pp. 1137–1144, 2015.
[66] L. Zheng, F. Ye, M. Qi, L. Lv, M. King, and Y. Qian, "Incorporation of Rutin in Electrospun Pullulan/PVA Nanofibers for Novel UV-Resistant Properties," *Materials (Basel).*, vol. 9, no. 7, p. 504, 2016.
[67] S. I. Jeong, M. D. Krebs, C. A. Bonino, J. E. Samorezov, S. A. Khan, and E. Alsberg, "Electrospun Chitosan–Alginate Nanofibers with *In Situ* Polyelectrolyte Complexation for Use as Tissue Engineering Scaffolds," *Tissue Eng. Part A*, vol. 17, no. 1–2, pp. 59–70, 2011.
[68] S. I. Jeong, M. D. Krebs, C. A. Bonino, S. A. Khan, and E. Alsberg, "Electrospun alginate nanofibers with controlled cell adhesion for tissue engineering," *Macromol. Biosci.*, vol. 10, no. 8, pp. 934–943, 2010.
[69] A. Kyzioł, J. Michna, I. Moreno, E. Gamez, and S. Irusta, "Preparation and characterization of electrospun alginate nanofibers loaded with ciprofloxacin hydrochloride," *Eur. Polym. J.*, vol. 96, no. June, pp. 350–360, 2017.
[70] C. A. Bonino *et al.*, "Electrospinning alginate-based nanofibers: From blends to crosslinked low molecular weight alginate-only systems," *Carbohydr. Polym.*, vol. 85, no. 1, pp. 111–119, 2011.
[71] E. Shekarforoush, A. C. L. Mendes, and I. S. Chronakis, "The electrospinning of xanthan gum: from solution to nanofiber formation," in *Applied Nanotechnology and Nanoscience International Conference*, 2017, p. 235.
[72] A. R. Unnithan *et al.*, "Wound-dressing materials with antibacterial activity from electrospun polyurethane – dextran nanofiber mats containing ciprofloxacin HCl," *Carbohydr. Polym.*, vol. 90, no. 4, pp. 1786–1793, 2012.
[73] A. R. Unnithan *et al.*, "Electrospun polyurethane-dextran nanofiber mats loaded with Estradiol for post-menopausal wound dressing," *Int. J. Biol. Macromol.*, vol. 77, pp. 1–8, 2015.
[74] N. N. Maslakci *et al.*, "Ibuprofen and acetylsalicylic acid loaded electrospun PVP-dextran nanofiber mats for biomedical applications," *Polym. Bull.*, vol. 74, no. 8, pp. 3283–3299, 2017.
[75] A. M. Moydeen, M. S. Ali Padusha, E. F. Aboelfetoh, S. S. Al-Deyab, and M. H. El-Newehy, "Fabrication of electrospun poly(vinyl alcohol)/dextran nanofibers via emulsion process as drug delivery system: Kinetics and in vitro release study," *Int. J. Biol. Macromol.*, vol. 116, pp. 1250–1259, 2018.

[76] P. Vashisth, K. Nikhil, P. Roy, P. A. Pruthi, R. P. Singh, and V. Pruthi, "A novel gellan-PVA nanofibrous scaffold for skin tissue regeneration: Fabrication and characterization," *Carbohydr. Polym.*, vol. 136, pp. 851–859, 2016.

[77] P. Vashisth, P. A. Pruthi, R. P. Singh, and V. Pruthi, "Process optimization for fabrication of gellan based electrospun nanofibers," *Carbohydr. Polym.*, vol. 109, pp. 16–21, 2014.

[78] A. Thorvaldsson, J. Silva-Correia, J. M. Oliveira, R. L. Reis, P. Gatenholm, and P. Walkenström, "Development of nanofiber-reinforced hydrogel scaffolds for nucleus pulposus regeneration by a combination of electrospinning and spraying technique," *J. Appl. Polym. Sci.*, vol. 128, no. 2, pp. 1158–1163, 2013.

[79] R. Y. Basha, T. S. Sampath Kumar, and M. Doble, "Electrospun Nanofibers of Curdlan (β-1,3 Glucan) Blend as a Potential Skin Scaffold Material," *Macromol. Mater. Eng.*, vol. 302, no. 4, pp. 1–9, 2017.

[80] M. E. El-Naggar, A. M. Abdelgawad, C. Salas, and O. J. Rojas, "Curdlan in fibers as carriers of tetracycline hydrochloride: Controlled release and antibacterial activity," *Carbohydr. Polym.*, vol. 154, pp. 194–203, 2016.

[81] A. Eman, W. I. . Saber, M. M. . El-Sawah, F. I. A. Hauka, and M. M. Kassem, "Optimization of Fermentation Conditions for Xanthan Production by Xanthomonas Campestris NRRL B-1459," *J. Union Arab Biol.*, vol. 9B, pp. 211–228, 2000.

[82] A. Palaniraj and V. Jayaraman, "Production , recovery and applications of xanthan gum by Xanthomonas campestris," *J. Food Eng.*, vol. 106, no. 1, pp. 1–12, 2011.

[83] R. Li *et al.*, "Electrospinning pullulan fibers from salt solutions," *Polymers (Basel).*, vol. 9, no. 1, pp. 1–12, 2017.

[84] T. D. Leathers, "Substrate regulation and specificity of amylases from Aureobasidium strain NRRL Y-12 , 974," *FEMS Microbiol. Lett.*, vol. 110, pp. 217–221, 1993.

[85] R. S. Singh and G. K. Saini, "Biosynthesis of Pullulan and Its Applications in Food and Pharmaceutical Industry," in *Microorganisms in Sustainable Agriculture and Biotechnology*, A. P. Satyanarayana T., Johri B., Ed. Dordrecht: Springer, 2012, pp. 509–553.

[86] R. Yamaguchi *et al.*, "Conjugation of Sendai Virus with Pullulan and Immunopotency of the Conjugated Virus," *Microbiol. Immunol.*, vol. 29, no. 2, pp. 163–168, 1985.

[87] E. Cevher *et al.*, "Development of chitosan – pullulan composite nanoparticles for nasal delivery of vaccines : optimisation and cellular studies delivery of vaccines : optimisation and cellular studies," *J. Microencapsul.*, vol. 32, no. 8, pp. 755–768, 2015.

[88] S. Islam, J. Hyun, and A. Kumar, "Effect of pullulan / poly (vinyl alcohol) blend system on the montmorillonite structure with property characterization of electrospun pullulan / poly (vinyl alcohol)/ montmorillonite nanofibers," *J. Colloid Interface Sci.*, vol. 368, no. 1, pp. 273–281, 2012.

[89] F. Cengiz-Çallioğlu, "Dextran nanofiber production by needleless electrospinning process," *E-Polymers*, vol. 14, no. 1, pp. 5–13, 2014.

[90] H. Jiang, D. Fang, B. S. Hsiao, B. Chu, and W. Chen, "Optimization and characterization of dextran membranes prepared by electrospinning," *Biomacromolecules*, vol. 5, no. 2, pp. 326–333, 2004.

[91] West Corporation, "PolyRemedy Launches HealSmart™ Hyaluronic Acid Dressing Enhancement," 2013. [Online]. Available: http://www.marketwired.com/press-release/polyremedy-launches-healsmart-hyaluronic-acid-dressing-enhancement-1778690.htm. [Accessed: 08-Nov-2018].