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# 1,3- $\beta$ -Glucans: Drug Delivery and Pharmacology

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## 1. Introduction

Natural polysaccharides are used in a variety of applications due to their unique properties. These applications range from paper manufacturing to wound healing [1]. One interesting class of polysaccharides comprises 1,3- $\beta$ -glucans, which are glucopyranose polysaccharides with (1,3) glycosidic linkages and varying degree of (1,6) branches [2]. 1,3- $\beta$ -glucans can form single or triple helical structures, which can be used to synthesize resilient gels by applying heat and humidity [3,4]. The properties of these gels are governed by the structure of the polysaccharide, which is determined by the degree of branching and the molecular weight. Thus, controlling the microscopic structure allows control over the macroscopic function. This is especially advantageous in the field of drug delivery because the encapsulation of different agents can be facilitated by the use of different polysaccharides. The properties of 1,3- $\beta$ -glucans can also be modified by covalently attaching functional units to the polysaccharide backbone [5].

1,3- $\beta$ -glucans are derived from microbial [6] and fungal [2] sources and hence have innate immunomodulatory properties. When these 1,3- $\beta$ -glucans are a component of the foreign pathogens, they can act as recognition sites for macrophages to facilitate the elimination and removal of these pathogens [7]. When extracted 1,3- $\beta$ -glucans are administered to animals or humans, they recruit macrophages and stimulate the immune system through a similar mechanism [8,9]. This result has been utilized for various pharmacological applications including cancer inhibition [10-17], infection resistance [18-21] and wound healing [22-24]. Current research is focusing on combining the structural properties of 1,3- $\beta$ -glucans with the pharmacological ones to further enhance the efficacy of hybrid systems thus created.

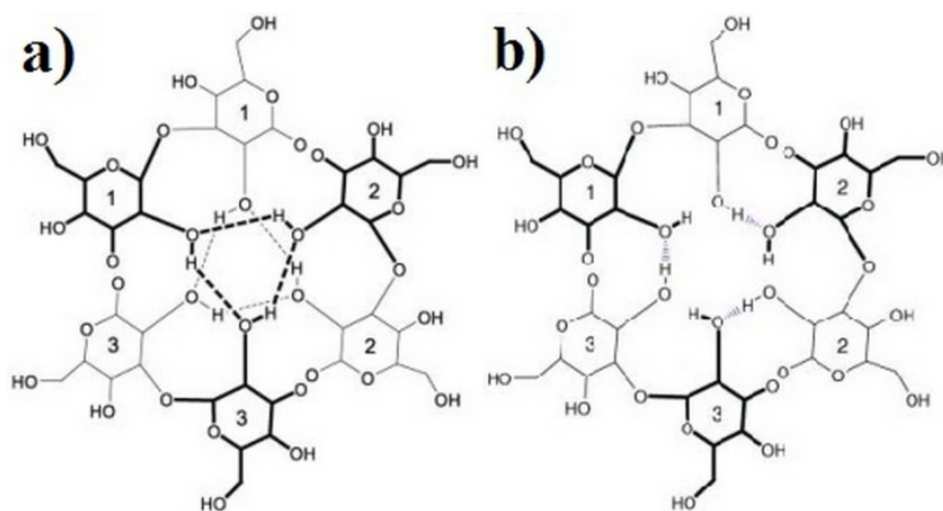
## 2. Crystallinity of 1,3- $\beta$ -glucans

### 2.1. Structure

1,3- $\beta$ -glucans are semi-crystalline polysaccharides comprising a combination of single helices, triple helices and random coils. The crystallinity of these polysaccharides has been

studied using X-ray diffraction (XRD). In this study, curdlan, which is a linear 1,3- $\beta$ -glucan, was used as a model polysaccharide [3]. Different forms and states of curdlan demonstrate different crystallinity. One example that was studied in detail is the annealed “dry” state, where the curdlan is dissolved in dimethyl sulfoxide, extracted in methanol and annealed in the presence of water at 145 °C. Curdlan is then dried *in vacuo* to obtain the sample for XRD experiments. The results from XRD measurements conclude that six-fold triple-helices are formed with an advance of 2.935 Å per monomer unit. The model of this structure confirms that the three strands of triple helices are held together by hydrogen bonding between O(2) hydroxyls while the helices are brought together by O(4) and O(6) hydrogen bonding [3].

An alternate structure of curdlan helices is presented based on semi-empirical modeling. It is proposed that hydrogen bonding of the strands occurs along the helix axis rather than perpendicular to it. The different structures are illustrated in Figure 1. It is demonstrated that this alternate structure provides a more stable structure of curdlan and hence is likely to have higher population [25].



**Figure 1.** Illustration of possible orientations of curdlan triple helices: a) hydrogen bonding perpendicular to helix axis; b) hydrogen bonding along helix axis. Figure adapted from [25].

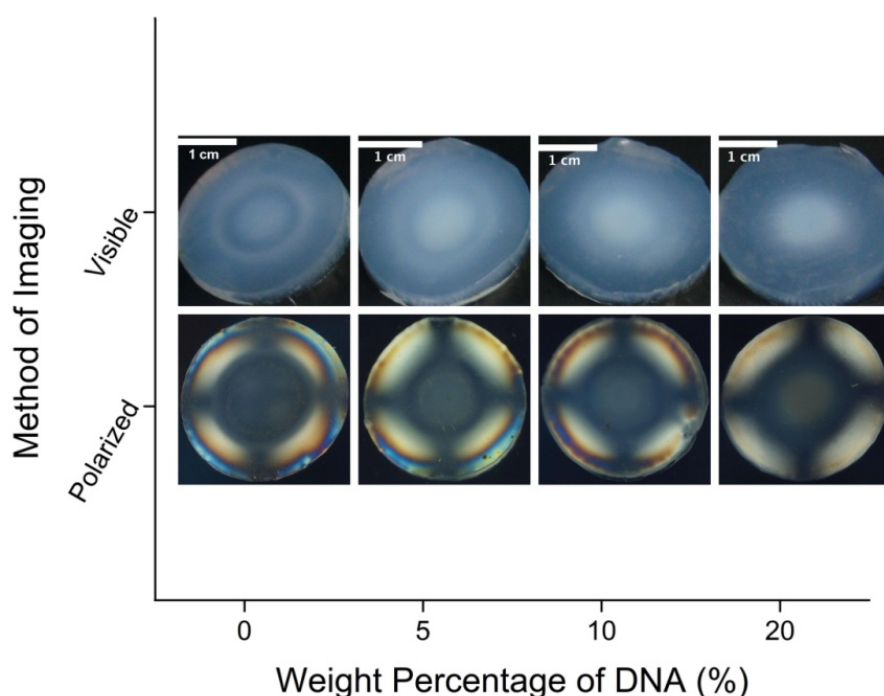
1,3- $\beta$ -glucans have also been complexed with nucleotides to form crystalline structures. In the example of curdlan and poly(cytidylic acid) complex, semi-empirical modeling suggests that two glucose units of different curdlan chains form hydrogen bonds with one base of the nucleotide chain [26]. This property of curdlan complexing with nucleotides has been exploited in forming liquid crystalline gels with deoxyribonucleic acid (DNA). Such structures could be synthesized at varying scales ranging from nanometers to centimeters [27].

## 2.2. Liquid crystalline gels

Curdlan can be used to form liquid crystalline gels when it is exposed to transition metal salts [28,29]. The crystallinity of these gels depends on the molecular weight of the gels [30]. DNA has also been used to synthesize gel beads [31]. When used together, DNA and curdlan

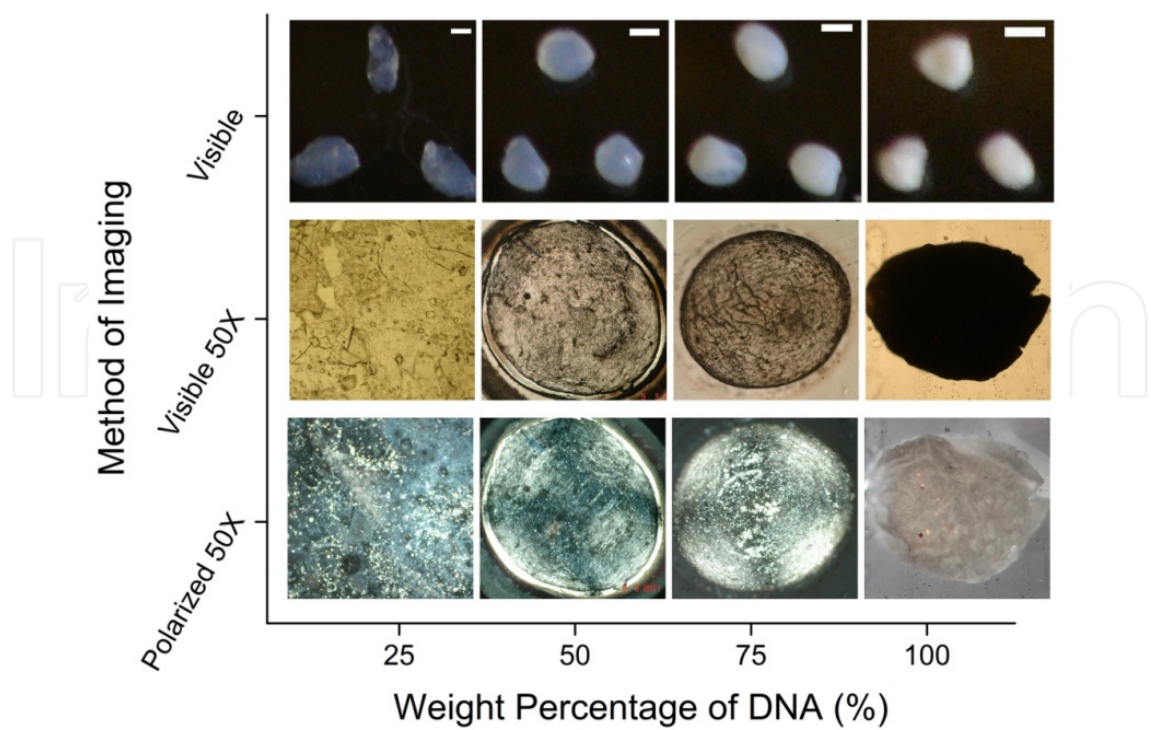
provide control over the size and morphology of the synthesized hybrid structures. Various structures can be obtained by modifying the concentration of curdlan and DNA [27].

Curdlan is insoluble in water but it dissolves in alkaline solutions. Thus, DNA and curdlan are mixed together in a basic solution and then this mixture is added to a solution of calcium chloride salt either directly or through a dialysis membrane. Direct addition leads to formation of structures at the nanometer and millimeter scales. Dialysis allows for the formation of centimeter sized gels. The macroscopic structures are assessed by using crossed nicols (Figure 2), while the nano- and micro-structures are characterized using transmission electron microscopy (TEM, Figure 4). When viewing the centimeter scale gels between two perpendicularly placed polarizers, orthogonal dark lines are observed on the gels. These lines are known as isogyres and indicate the anisotropy in liquid crystalline gels. It is observed that increasing the concentration of DNA decreases the crystallinity of the gel as the isogyres become less defined. This is illustrated in Figure 2 [27].

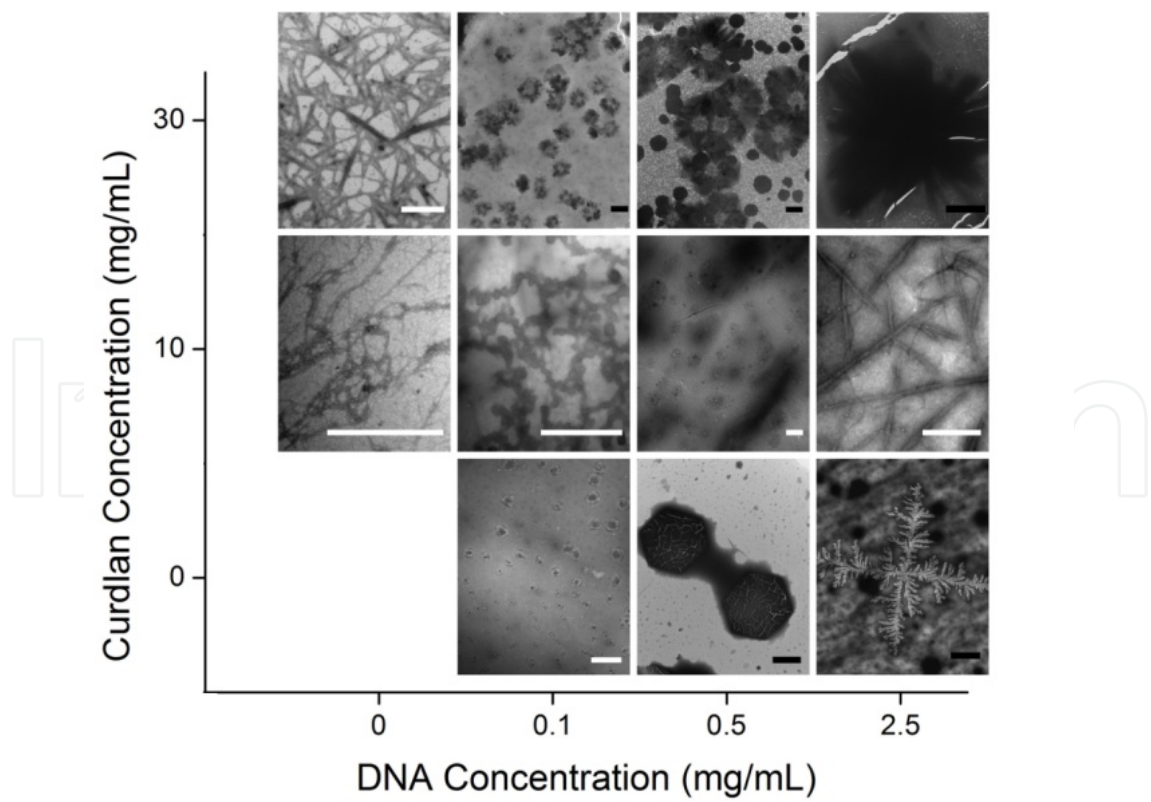


**Figure 2.** Liquid crystalline gels of curdlan and DNA: 100% curdlan, 5% DNA, 10% DNA and 20 % DNA as seen under visible light (top) and crossed nicols (bottom). Scale bars are 1 cm each. Figure obtained from [27].

A similar phenomenon was observed at the millimeter scale when the structure was observed under the microscope. It was seen that although DNA provided rigidity and well-defined shape to the structure, it reduced the crystallinity. This is likely because DNA forms a less crystalline structure compared to curdlan. It is possible that DNA might not be forming helices with curdlan, but instead forming a gel with microphase separation. The results from millimetre scale are highlighted in Figure 3. The opacity and lack of isogyres in DNA sample implies low crystallinity. Thus, a simple method is presented to determine degree of crystallinity of gels qualitatively [27].



**Figure 3.** Spherical gels of curdlan and DNA observed under visible light (top and middle) and polarized light (bottom). Scale bars are 1 mm each. Figures obtained from [27].



**Figure 4.** TEM images of micro- and nano-structures of curdlan and DNA. White scale bars are 500 nm and black scale bars are 2000 nm. Figure reproduced from [27].



At the micro- and nano-scale levels, the morphology of the gels could be changed between spheres and fibers by modulating the concentration of the DNA or curdlan. This is summarized in Figure 4. These hybrid liquid crystalline gel systems have the potential of creating advanced drug delivery vehicles where the crystalline regions of the system prevent degradation of the biomolecule and the amorphous regions maintain functionality of the encapsulated moiety. This hybrid system also serves as a tool for further studies of the molecular structure undertaken by 1,3- $\beta$ -glucan and DNA in various microenvironments [27].

### 3. Applications in drug delivery

The ability to control the structure of 1,3- $\beta$ -glucan based carriers has encouraged the application of these glucans in drug delivery. The glucans can be used as gels, nanoparticles, microparticles or complexes.

#### 3.1. Encapsulation within gels

Curdlan is the commonly used 1,3- $\beta$ -glucan for formation of gels. Curdlan gels can be prepared by heating the suspension of curdlan in aqueous solution and then cooling it down. If the suspension is heated to 60 °C, a low-set thermally reversible gel is formed whereas if the temperature is above 80 °C, a high-set thermally irreversible gel is formed. Drugs such as indomethacin, salbutamol sulfate and prednisolone have been encapsulated in curdlan gels. The gels are prepared by mixing curdlan in drug solutions at 5-10% curdlan concentration, adding the suspension to a glass test tube, heating the test tube in water bath at the desired temperature for 10 minutes and then unmolding the gel. The experiments conducted in [32] have demonstrated that a high-set gel can lower the rate of drug release. The curdlan based gels have been able to provide a sustained release of drugs for compared to commercially available formulations. These gels can be used as drug delivery suppositories for rectal administration, which bypasses hepatic first pass clearance [32].

Curdlan gels have also been used for developing protein delivery devices. Since proteins can denature at high temperatures, the temperature required for forming curdlan gels needs to be lowered. Curdlan can form aqueous gels in the presence of hydrogen bond disrupting agents such as dimethyl sulfoxide, urea and thiocyanates [33]. This property encourages the use of chaotropes for lowering the gelling temperature. It has been demonstrated that the presence of 8 M urea can decrease the gelling temperature of curdlan from 55 °C to 37 °C. This has been used for encapsulating bovine serum albumin (BSA) as a model protein. Although gels synthesized using urea are able to demonstrate sustained release of BSA over 100 hours, the toxicity of urea is a concern. Urea also has the possibility of disrupting the hydrogen bonds of BSA and hence denaturing the protein. Thus, an alternative method of reducing the gelling temperature has been developed by modifying the backbone of curdlan to form a hydroxyethyl derivative. This system was

also able to form gels at 37 °C but the BSA release was sustained for a shorter time period of 75 hours [34].

### 3.2. Microparticles and nanoparticles

Nanoparticles are typically used in drug delivery applications because of high drug encapsulation efficiency [35], controlled drug release and incorporation of diagnostic agents [36,37]. One of the major challenges with drug delivery is achieving specificity with cellular uptake. One method of overcoming this hurdle is by attaching targeting ligands on the surface of nanoparticles to allow enhanced uptake by specific cells [38]. Another strategy is the encapsulation of these drug loaded nanoparticle in glucan based microspheres. Glucan microspheres are derived from the cell walls of *Saccharomyces cerevisiae* (Baker's yeast) and are 2-4 µm in size. These are porous and hollow microparticles composed mainly of 1,3-β-D-glucan and small quantities of chitin [39]. The β-glucan on the microspheres serves as a specific target for uptake by immune cells such as macrophages and dendritic cells [40]. Glucan microspheres can be used for delivering various payloads such as proteins [19], DNA [40], siRNA [16,41] and small molecules [39]. Typically small molecules are not easily entrapped within these glucan microspheres and hence the use of nanoparticles is necessary. These nanoparticles can then be loaded in glucan microspheres for enhanced uptake. Recently, two types of nanoparticles have been encapsulated within glucan microspheres: fluorescent polystyrene nanoparticles and doxorubicin loaded mesoporous silica nanoparticles [39]. The polystyrene particles are encapsulated using capillary forces from the pores of microspheres, whereas the silica nanoparticles are loaded using electrostatic interactions. These particles are then used *in vitro* to demonstrate enhanced uptake by dectin-1 expressing fibroblast cells (NIH3T3-D1 cell line). It has been observed that doxorubicin loaded silica nanoparticles were more effective when encapsulated within glucan microspheres because of enhanced uptake. These results were consistent with fluorescently tagged polystyrene nanoparticle uptake as well [39].

Aside from being used as a targeting moiety, 1,3-β-glucans have also been used as structural units for encapsulating insoluble drugs. One example is the use of short chain curdlan with a molecular weight of 990 Da, derived from Vietnam medicinal mushroom *Hericium erinaceum*. This curdlan has been used to synthesize a nanoparticle formulation for encapsulating curcumin, which is a water-insoluble compound with promising anti-cancer activity. Curcumin is derived from the rhizomes of the herb *Curcuma longa* and has demonstrated cancer prevention and suppression through various signaling pathways [42]. The delivery of curcumin has been enhanced by encapsulating it within curdlan to form 50 nm nanoparticles. These nanoparticles are prepared by adding curcumin dissolved in ethanol to a solution of curdlan in water and stirring at room temperature. The solvents are then evaporated and nanoparticles are purified by centrifugation to remove excess curdlan and larger aggregates. These curcumin loaded curdlan nanoparticles have been able to inhibit tumor growth in Hep-G2 cell line *in vitro* [10].

Amphiphilic derivatives of 1,3- $\beta$ -glucans have also been extensively studied for formation of micellar nanoparticles with drug encapsulating capabilities. One example of an amphiphilic system is based on cholesterol-carboxymethylcurdlan. The hydrophilicity of curdlan is increased by modifying the backbone with carboxymethyl groups. The loading of hydrophobic drugs is increased by inclusion of cholesterol moieties in the curdlan backbone [17]. This system uses a remote loading method for encapsulating epirubicin, where a pH gradient between the interior and exterior of the nanoparticle is utilized to achieve high ratio of drug to carrier [43]. Remote loading method is implemented by preparing blank cholesterol-carboxymethylcurdlan nanoparticles using probe sonication, resuspending dried nanoparticles in ammonium sulfate, performing buffer exchange in sodium chloride and finally adding the desired amount of epirubicin to the solution. This method is able to achieve up to 39.6% drug loading, which is remarkable for polymeric systems. This curdlan based delivery device has been able to enhance the cytotoxicity of epirubicin *in vitro* when assessed using human cervical carcinoma (HeLa) cell lines as compared to free epirubicin. The delivery vehicle also increases the circulation half-life *in vivo* by 4.31 times as compared to free epirubicin when tested in Wistar rats. These results provide promising opportunities for utilizing a curdlan based drug delivery vehicle for enhanced efficacy of encapsulated drugs.

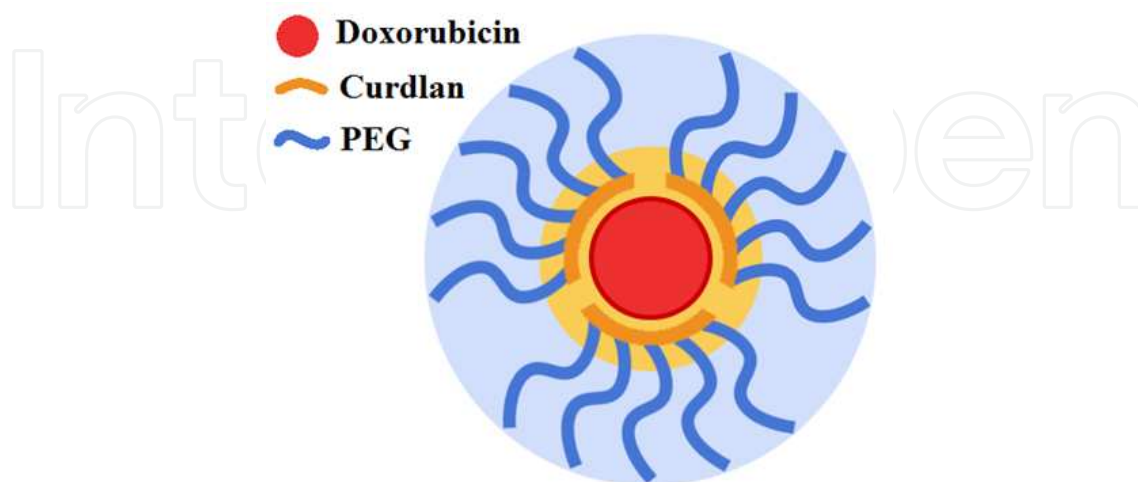
Alternatively, curdlan can also be used as the hydrophobic component in an amphiphilic drug delivery vehicle. To achieve this a graft copolymer of curdlan and poly(ethylene glycol) (PEG) has been synthesized [5]. PEG is a commonly used polymer for enhanced biocompatibility [44-48]. Doxorubicin can be incorporated in graft copolymer nanoparticle by nanoprecipitation method. In this technique, the copolymer and drug are dissolved in a common water miscible solvent and then added to magnetically stirred water in a drop-wise manner. The mixing causes self-assembly of polymers where hydrophobic components are at the core of the nanoparticle and hydrophilic components are at the surface. In the case of doxorubicin and curdlan-graft-PEG, dimethyl sulfoxide is used as the common solvent [5]. A schematic of the nanoparticle is presented in Figure 5.

The structure of curdlan-graft-PEG nanoparticles has been confirmed using TEM. The samples were stained using phosphotungstic acid, which acts as a negative stain. This makes the background appear dark and the samples of interest appear bright. Additionally, since phosphotungstic acid is hydrophilic, hydrophobic components will be excluded from the acid and hence appear brighter than hydrophilic components. As observed in Figure 6, the nanoparticles are about 109 nm in size. Doxorubicin is visible as the bright center of the nanoparticles. Figure 6 (B) shows the three distinct layers of the nanoparticle, which correspond well with the schematic presented in Figure 5 [5].

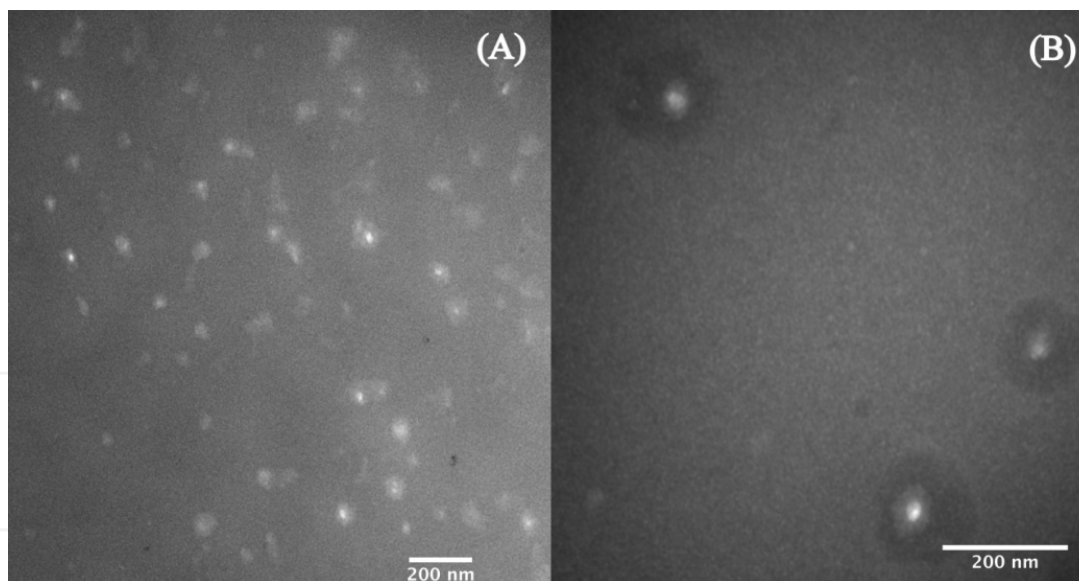
The doxorubicin formulation with curdlan-graft-PEG was tested *in vitro* to determine its drug release profile and it demonstrated sustained release over 24 hours following a Fickian diffusion release model. Since PEG graft is supposed to improve the biocompatibility of the nanoparticles, a hemolysis assay was implemented to assess this property. Sheep red blood cells (RBCs) were used for this assay and nanoparticles of interest were incubated with the



RBCs for one hour at 37 °C. The amount of lysis was compared to a negative control of veronal buffered saline and positive control of deionized water. Curdlan-graft-PEG nanoparticles showed hemolysis below 5% at clinically relevant concentrations [5], which is considered biocompatible [35].



**Figure 5.** Schematic of nanoparticle synthesized using curdlan-graft-poly(ethylene glycol) and doxorubicin. Doxorubicin is at the core, surrounded by curdlan and PEG forms the shell. Figure adapted from [5].



**Figure 6.** TEM images of doxorubicin encapsulated within curdlan-graft-PEG nanoparticles. (A) Bright center indicates doxorubicin and surroundings are darker because of higher electron density; (B) Three layers of the nanoparticle can be seen: bright doxorubicin core, dense curdlan layer in the middle and sparse PEG layer on the shell. Scale bars are 200 nm. Figure adapted from [5].

Therefore, nanoparticles and microparticles based on 1,3- $\beta$ -glucans provide promising opportunities for drug delivery as structural units and as targeting ligands. The combination of these properties can be exploited for developing a drug delivery vehicle with enhanced potency.

### 3.3. Glucan complexes with polynucleotides

Polynucleotides have found several applications in drug delivery as active agents due to their therapeutic effects [16,41,49]. One of the challenges faced by polynucleotide delivery is their rapid degradation *in vivo*. Encapsulation of polynucleotides thus becomes necessary for maintaining their function. 1,3- $\beta$ -glucans are a preferred choice for forming these complexes because of their helix forming capabilities. In the past, several polysaccharides have been tested for their ability to form complexes with the polynucleotide poly(C) and the complexation was assessed using circular dichroism (CD) where a change in the spectrum indicates complex formation. Only schizophyllan (1,3- $\beta$ -glucan with one 1,6 branch every three units) and lentinan (1,3- $\beta$ -glucan with two 1,6 branch every five units) have demonstrated changes in CD spectra, whereas curdlan, amylose (1,4- $\alpha$ -glucan), dextran (1,6- $\alpha$ -glucan) and pullulan (1,4- $\alpha$ -1,6- $\alpha$ -glucan) did not show any changes. This implies that only soluble 1,3- $\beta$ -glucans are able to form complexes with poly(C). Commercially available curdlan is unable to form a complex because of its low solubility in water, which leads to precipitation [50]. The solubility of curdlan can be improved by reducing the molecular weight of the polymer. At lower molecular weight, curdlan is able to form complexes with poly(C) but these complexes are not as stable as the ones formed with schizophyllan [51]. Complexes have also been formed with poly(A) using schizophyllan but poly(U) showed no complexation, which suggests that polysaccharides can bind to polynucleotides in a specific manner [52]. Curdlan backbone has also been modified with carbohydrate molecules using click chemistry to improve the solubility and hence induce complex formation with poly(C) [53].

Hitherto, only homo-sequence polynucleotides have been discussed. Often therapeutic polynucleotides are composed of heterogeneous base pairs. Drug delivery vehicles for hetero-sequence oligonucleotides have also been developed by synthesizing cationic curdlan chains. CpG DNA has demonstrated immune stimulating effects but it needs to be preserved from degradation [54]. Cationic curdlan, synthesized using click chemistry, has been able to form complexes with CpG DNA and increase the cellular uptake in macrophage-like cell line J774.A1. The complex also induces an increase in cytokine (IL-12) secretion, which suggests activation of the macrophage cells [54]. Another strategy of binding hetero-sequence oligonucleotides is by modification of one terminal to attach a homo-sequence such as poly(A). A schizophyllan derivative has been utilized for binding modified antisense oligonucleotides. Schizophyllan has been modified with galactose and PEG units to enhance cellular uptake. It has been observed that the antisense effect was maximized with the use of schizophyllan derivative when the complex is administered to hepatoblastoma HepG2 and melanoma A375 cell lines [55]. Thus, 1,3- $\beta$ -glucan based drug delivery devices serve as biocompatible carriers for a variety of nucleotides.

## 4. Applications in immunotherapy

1,3- $\beta$ -glucans have been known to generate an immune response including stimulation of cytokine production, oxidative burst, increased phagocyte and lymphocyte proliferation as well as phagocytosis of opsonized tissues. Various mechanisms are responsible for this

activation and this response has been utilized for varied applications including cancer resistance, disease immunity and wound healing [56].

#### 4.1. Biological pathways of activation

The complement system is responsible for innate immunity and can be activated by either classical, alternative or lectin pathways. Using lentinan, pachyman and pachymaran polysaccharides, it has been demonstrated that 1,3- $\beta$ -glucans use the alternative pathway of complement activation for generating an immune response since they show an increased consumption of C3 and C5 proteins from the complement cascade [57]. Additionally, opsonization is an important component of innate immune response. Typically complement protein C3b gets coated on pathogens and is later detected by complement receptor 1 or deactivated to form iC3b for regulation. In the presence of 1,3- $\beta$ -glucans, iC3b can be detected by complement receptor 3 (CR3) or dectin-1 and this mechanism can be exploited for attacking cancer cells coated with iC3b [8]. Although the effect of CR3 has been negligible in immune activation in murine models [9,58], functional CR3 is essential for phagocytosis in human neutrophils [59].

Most of the existing studies have focused on macrophages and neutrophils but some 1,3- $\beta$ -glucans such as lentinan are responsible for stimulation of T-cells and natural killer cells and hence affect the acquired immune response [60,61]. Curdlan has also been used to demonstrate increased proliferation of lymphocytes, which can in turn enhance the immune response [62]. Oxidative burst is yet another immunomodulatory effect that has been demonstrated by 1,3- $\beta$ -glucans. While curdlan has shown the induction of inducible nitric oxide synthase in rat macrophages [63], other 1,3- $\beta$ -glucans have shown an increase in the production of reactive oxygen species [62]. Some studies have demonstrated that immune activation is only possible by linear 1,3- $\beta$ -glucans [64] while others emphasize that complex branching is important for most effective stimulation of immune response [65]. Although, several aspects of the biological pathways of activation have been discovered, further studies are necessary to gain a better understanding of the intricate interactions between 1,3- $\beta$ -glucans and the immune system.

#### 4.2. Tumor suppression

The use of 1,3- $\beta$ -glucans in cancer therapy has been present in Japan since 1986, where they have been used for gastric, lung and cervical cancers [66]. Lentinan and pachymaran have demonstrated high tumor inhibition ratios of 99.6% and 96%, when tested against subcutaneous implantation of sarcoma 180 in mice [64]. It is hypothesized that the effect of lentinan and pachymaran is highly dependent on the activation of T-lymphocytes because the removal of the thymus from mice caused a suppression of antitumor effects from the 1,3- $\beta$ -glucans [60]. Additionally, it is also speculated that deactivation of protein helices might be important for antitumor effects because a study has demonstrated that only polysaccharides that deactivated bovine serum albumin showed antitumor activity [67]. Besides lentinan and pachymaran, other 1,3- $\beta$ -glucans have also exemplified tumor suppression. Some of the prominent examples are scleroglucan with an inhibition ratio of 90.4%, curdlan with inhibition

of 99-100% [68], grifolan with inhibition of 97.9% [69] and 1,3- $\beta$ -glucan from *Agaricus blazei* with inhibition of 99.3% [65]. These results have been encouraging and hence 1,3- $\beta$ -glucans have been used in combinatorial therapies with antibodies in implanted human tumor xenografts from melanoma, epidermoid carcinoma, breast carcinoma, metastatic lymphoma and daudi lymphoma. The mice had higher survival rates in the presence of 1,3- $\beta$ -glucans as compared to treatment with antibodies alone [12].

Subsequently, water soluble 1,3- $\beta$ -glucans have been derived to improve the usability of these polysaccharides. Some examples of these polysaccharides include carboxymethylpachymaran with tumor inhibition ratio of 99.6%, hydroxymethylpachymaran and hydroxypropylpachymaran with up to 100% tumor inhibition when assessed against solid sarcoma 180 at a dose of 5 mg/kg [70]. Several derivatives of curdlan including carboxymethyl, glucosyl, sulfoethyl and sulfopropyl attachments to the backbone have also retained antitumor activity [71,72]. These examples demonstrate the versatility of 1,3- $\beta$ -glucans in cancer inhibition and thus these polysaccharides can be modified to suit the desired application.

### 4.3. Infection prevention

Most common infections are caused by bacteria and fungi and since 1,3- $\beta$ -glucans can induce inflammatory response, these glucans can be used for providing infection resistance. It has been shown that when administering 1,3- $\beta$ -glucan to mice, their survival against *Staphylococcus aureus* infection increased from 70% to 97% [73]. Other 1,3- $\beta$ -glucans such as glucan phosphate, laminarin and scleroglucan have been studied in detail for assessing their pharmacokinetic profile following oral administration in rats. It has been observed that these glucans are able to translocate from the gastrointestinal tract to systemic circulation. The glucans were able to increase secretion of interleukins, increase expression of dectin-1 on macrophages and increase expression of toll-like receptor 2 on dendritic cells. Thus, these effects increase the long-term survival of rats from 0% to 40% when challenged with *Candida albicans* fungal infection and from 0% to 50% when challenged with *Staphylococcus aureus* bacterial infection [74].

When considering larger mammals, infection resistance in pigs, dogs and horses has also found applications of 1,3- $\beta$ -glucans. When piglets were fed with  $\beta$ -glucans after weaning, they demonstrated lower infection from enterotoxigenic *Escherichia coli*, which was highlighted by decreased diarrhoea and decreased content of inoculated *Escherichia coli* in the faeces as compared to control groups. These results present a significant advancement in veterinary medicine for pigs because the immunity of pigs usually suffers severely right after weaning and the current vaccines against *Escherichia coli* take a long period to be effective [20]. In the case of horses, the administration of 1,3- $\beta$ -glucan to pregnant mares has been able to increase the cellular immune response in foals. This becomes very useful for preventing premature deaths of neonates [75]. While most studies have focused on the effects of 1,3- $\beta$ -glucans on improving innate immunity, when these polysaccharides are administered to dogs, they showed enhancement in humoral immunity as indicated by changes in serum IgM and IgA levels [18].



1,3- $\beta$ -glucans have also been used in aquatic animals as exemplified by the use of schizophyllan with 60-80% survival rate and lentinan and scleroglucan with 55-75% survival rate when tested against *Edwardsiella tarda* bacteria attack on *Cyprinus carpio* L. carp. The survival rate was also increased when infected with *Aeromonas hydrophila* with survival of 60% with schizophyllan, 70% with lentinan and 80% with scleroglucan administration. In the absence of glucans, the carp underwent complete mortality upon any infection [76]. These results have been repeatable when tilapia and grass carp is exposed to *Aeromonas hydrophila* [77]. As more studies are conducted on the use of 1,3- $\beta$ -glucans, the quality of veterinary health care can be improved further and these results can eventually be transferred to human applications.

In addition to bacterial and fungal resistance, 1,3- $\beta$ -glucans have also demonstrated promising results against malaria [78], herpes simplex virus [79] and human immunodeficiency virus (HIV) [80-82]. Expanding on HIV research, various complexes have been synthesized with curdlan sulfate in order to enhance the efficacy of the polysaccharide. Some prominent examples include covalent conjugation of azidothymidine to curdlan sulfate for drug delivery to the lymph nodes and bone marrow [83] and conjugation of fullerene C<sub>60</sub> with curdlan for combining their anti-HIV effects [84].

#### 4.4. Wound healing

1,3- $\beta$ -glucans can have an impact on wound healing by recruiting macrophages to the wound site [85] and by increasing collagen deposition [24]. Beta glucan collagen matrix wound dressings have been used in children suffering from partial thickness burns and the dressings were able to simplify wound care by reducing analgesic requirements, improving cosmetic results and eliminating the need for repetitive dressing changes [86]. Other composites of  $\beta$ -glucan have been created with poly(vinyl alcohol) [23] and chitosan [22]. Poly(vinyl alcohol)/ $\beta$ -glucan composite was able to speed up the wound healing process when tested using rat models and hence decreased the healing time by 48% as compared to cotton gauze [23]. When using a composite of  $\beta$ -glucan and chitosan, a transparent dressing was obtained, which showed better results compared to commercially available chitosan based Beschitin® W. The synthesized chitosan composite did not dissolve during application period and was easy to remove because it did not adhere to wounds [22].

### 5. Conclusion

An assortment of 1,3- $\beta$ -glucans have been explored for their structural and pharmacological capabilities. The ability of 1,3- $\beta$ -glucans to form helical structures and gels has been advantageous for forming complexes with small molecules and macromolecules. The immunomodulatory effects of 1,3- $\beta$ -glucans have served to fight cancer and infections and to promote wound healing. Research is moving towards combining the ability of 1,3- $\beta$ -glucans to encapsulate bioactive agents with their own bioactivity for creating potent therapeutic devices against current challenges.



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