Beta-glucans for developing multifunctional drug delivery platforms

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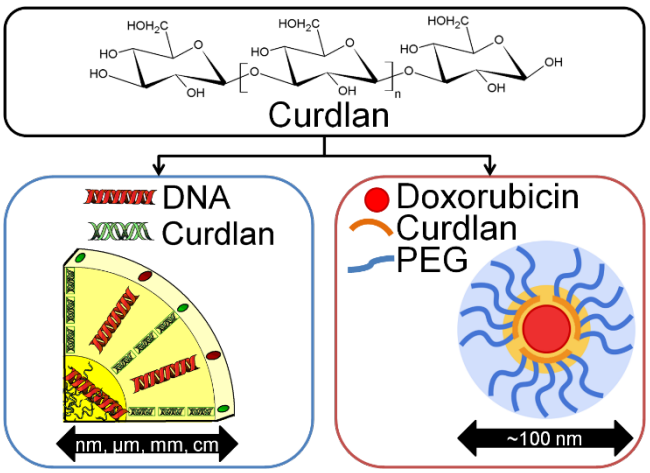
INTRODUCTION AND OBJECTIVE

Natural polysaccharides are used in a variety of applications due to their unique properties. These applications range from paper manufacturing to wound healing (Czaja 2006). One interesting class of polysaccharides comprises 1,3-β-glucans, which are glucopyranose polysaccharides with (1,3) glycosidic linkages and varying degree of (1,6) branches (Wasser 2002).

1,3-β-glucans are derived from microbial and fungal sources and hence have innate immunomodulatory properties. When these 1,3-β-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. When extracted 1,3-β-glucans are administered to animals or humans, they recruit macrophages and stimulate the immune system through a similar mechanism. This result has been utilized for various pharmacological applications including cancer inhibition (Le Mai Huong 2011), infection resistance (Stuyven 2010) and wound healing (Kofuji 2010).



Scheme 1: Curdlan used for two different drug delivery platforms (Lehtovaara 2012b)

Curdlan, a high molecular weight linear 1,3-β-glucan, is of particular interest because of its pharmacological properties (Na 2000). Curdlan is insoluble in water but it dissolves in basic solutions and forms liquid crystalline gels upon infusion of transition metal salts. These gels can be used to encapsulate deoxyribonucleic acid (DNA) in amorphous and crystalline regions (Lehtovaara 2012a). Curdlan can also be modified with poly(ethylene glycol) (PEG) to create an amphiphilic graft polymer for encapsulating hydrophobic drugs (Lehtovaara 2012b) as illustrated in Scheme 1.

Current research focuses on combining the structural properties of 1,3-β-glucans with the pharmacological ones to further enhance the efficacy of hybrid systems thus created.

MATERIALS AND METHODS

Curdlan (~90,000 Da) was obtained from Wako Pure Chemical Industries. Sodium hydroxide was purchased from Caledon Laboratory chemicals. Calcium chloride anhydrous salt, phosphotungstic acid (PTA) and dialysis membrane (Flat Width 45mm with12,000 to 14,000 Da MWCO) were purchased from Fisher Scientific. Monofunctional carboxylated PEG (~5,000 Da) was purchased from NanoCS. Dicyclohexylcarbodiimide (DCC), dimethylaminopyridine, DNA sodium salt from salmon testes and anhydrous dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich. Doxorubicin HCl was purchased from IntaTrade Chemicals GmbH, desalted using 2 molar equivalents triethylamine and extracted using dichloromethane to obtain a hydrophobic form of doxorubicin. Formvar coated transmission electron microscopy (TEM) grids were purchased from Canemco & Merivac (100 mesh copper grids). Linear polarizer sheets (2" x 2") were purchased from ThorLabs, Inc. and used to create the crossed nicols effect.

Liquid crystalline gels were synthesized by the infusion of a mixture of curdlan and DNA solution to a solution of calcium chloride using various methods. Large centimetre sized gels were obtained by using cylindrical dialysis membranes as templates. Micrometres to nanometres sized structures (Figure 1) were synthesized using nanoprecipitation, where solutions of DNA and curdlan were added in a drop-wise manner to an aqueous solution of calcium chloride under magnetic stirring.

A new core-shell nanoparticle containing the chemotherapeutic drug doxorubicin was formulated via amphiphilic graft copolymer self-assembly using a curdlan-graft-poly(ethylene glycol) (curdlan-g-PEG). The graft copolymer was synthesized through the DCC ester linkage of carboxylated PEG to the hydroxyl groups of the curdlan backbone. Nanoparticles were made using nanoprecipitation where the graft polymer and doxorubicin were dissolved in DMSO and added to water.

RESULTS AND DISCUSSION

Curdlan-DNA structures

Each of the synthesized gels displayed crystallinity as confirmed by the presence of perpendicular dark lines called isogyres, when viewed under crossed polarizers. Figure 1 demonstrates that control over the morphology of particles can be obtained by changing the concentration of curdlan and DNA. The core of these particles can be used to load nucleotides. CpG DNA is of particular interest for therapy because it can be delivered to enhance the immune response along with curdlan.

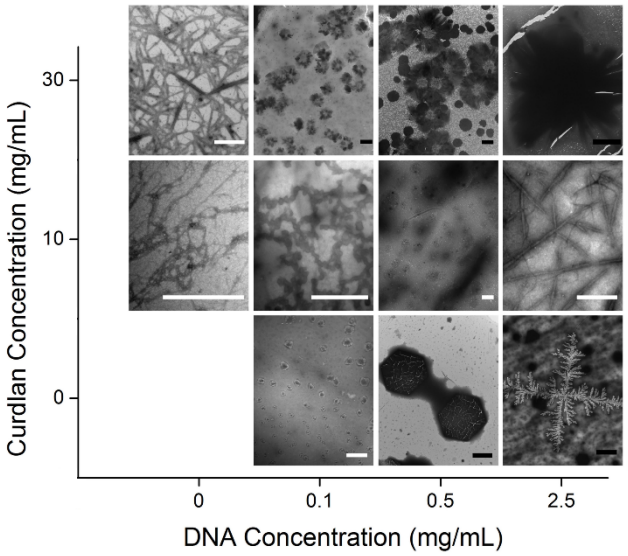


Figure 1: Curdlan and DNA microparticles and nanoparticles, white scale bars are 500 nm and black scale bars are 2000 nm. (Lehtovaara 2012a)

Curdlan-g-PEG doxorubicin nanoparticles

Nanoparticles synthesized using curdlan-g-PEG and doxorubicin were found to have an average particle size of 109.9 nm and encapsulate doxorubicin in high yield (4-5% wt/wt). This demonstrated the first nanoparticle formulation utilizing the hydrophobicity of curdlan to yield drug association while also concealing the immunomodulatory potential of curdlan within the core. These nanoparticles are seen in Figure 2, where the bright centre represents doxorubicin, the surrounding grey section represents curdlan and dark shell is composed of PEG.

CONCLUSIONS

1,3-β-glucans are extremely attractive natural polysaccharides for their structural and pharmacological properties. They can be used to deliver varying payloads ranging from small molecules to large polynucleotides. Their crystallinity can also be controlled easily. The next steps are to exploit the immunomodulatory effects of these polysaccharides and their derivative hybrid systems.

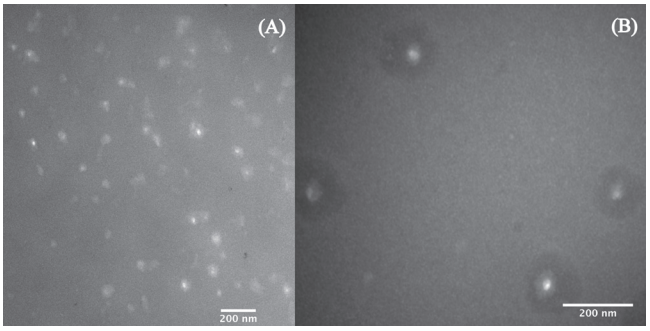


Figure 2: TEM images of Curdlan-*g*-PEG nanoparticles with encapsulated doxorubicin, PTA stain was used, scale bars are 200 nm. (Lehtovaara 2012b)

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