Beta-glucans as multifunctional drug delivery vehicles

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Introduction

Natural polysaccharides are used in a variety of applications due to their unique properties. These applications range from paper manufacturing to wound healing. One interesting class of polysaccharides comprises β-glucans, which are glucopyranose polysaccharides with β-glycosidic linkages and varying degree of branches (Lehtovaara 2012a).

β-glucans can be derived from microbial and fungal sources and hence have innate immunomodulatory properties. Curdlan, a high molecular weight linear 1,3-β-glucan, is of particular interest because of its pharmacological properties. Curdlan is insoluble in water but it dissolves in basic solutions and forms liquid crystalline gels upon infusion of transition metal salts as seen in Figure 1. The objective of this work is to exploit the gelation properties of β-glucans for the delivery of varying payloads ranging from small molecules to macromolecules.

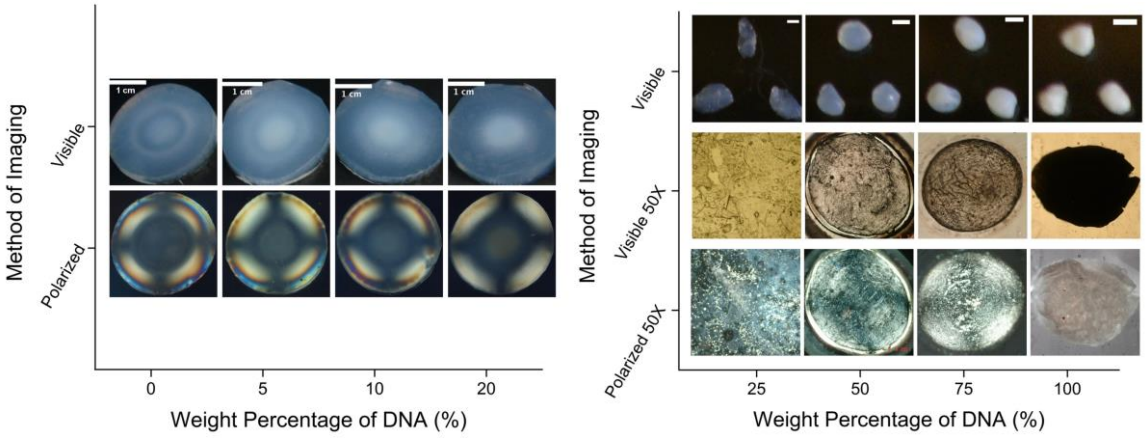

Figure 1: Curdlan and DNA cylindrical liquid crystalline gels: macroscopic gels, scale bar 1 cm (left); millispheres, scale bar 1 mm (right) (Lehtovaara 2012a)

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 and desalted.

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 and are seen in Figure 1. Millimetres to nanometres sized structures 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 when viewed under crossed polarizers (Figure 1). Areas of amorphous regions were also observed and these areas got bigger with increasing content of DNA (Figure 1). It is expected that these gels can provide an outer crystalline layer to protect the encapsulated amorphous payload in the core. Thus, the functionality of core can be protected in harsh conditions. CpG DNA is of particular interest for therapy because it can be delivered to enhance the immune response along with curdlan.

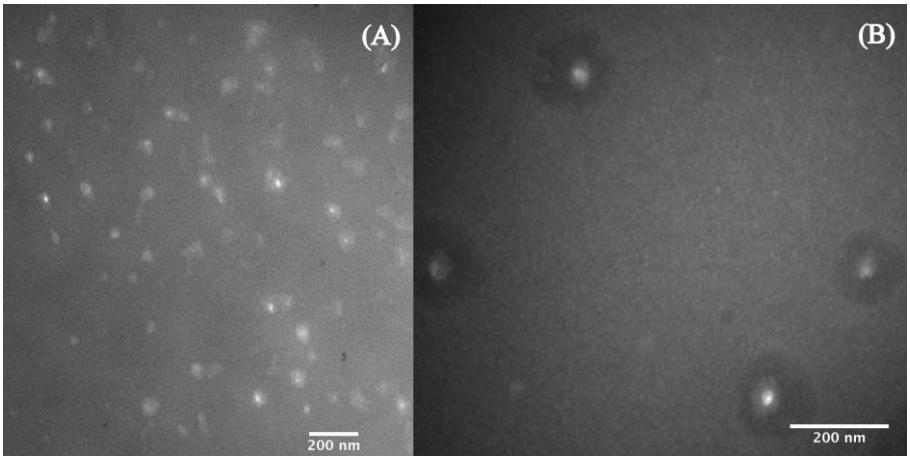

Figure 2: TEM images of Curdlan-*g*-PEG nanoparticles with encapsulated doxorubicin, Phosphotungstic acid stain was used, scale bars are 200 nm. (Lehtovaara 2012b)

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

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.

References

- Lehtovaara, B. C. et al. (2012a) *Multi-phase ionotropic liquid crystalline gels with controlled architecture by self-assembly of biopolymers.*Carbohydrate Polymers 87 (2) 1881-1885.
- Lehtovaara, B. C. et al. (2012b) *Synthesis of curdlan-graft-poly(ethylene glycol) and formulation of doxorubicin-loaded core-shell nanoparticles.*Journal of Bioactive and Compatible Polymers 27 (1) 3-17.