P-027	**Studying nanocarrier biocompatibility through interactions with blood components**	

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INTRODUCTION AND OBJECTIVES

A wide range of nanocarriers has been used in the development of clinically available pharmaceutical products (Torchilin 2006). Among these classes of nanovehicles for the transportation of therapeutic agents *in vivo*, delivery systems composed of polymeric materials are said to possess numerous advantages including reduced toxicity and controlled drug release (Nishiyama 2006). However, further progress in this area of research may require significant improvements with regards to the biocompatibility of these materials.

The behaviour of polymeric nanocarriers in the complex biological environment of the human body is of great relevance to the optimal design of such a system. It is often desirable for these nanostructures to be capable of long circulatory lifetimes upon intravenous injection (Moghimi 2001); as such, the ability to produce polymeric nanoparticles (NPs) that are able to avoid interaction with biological components and escape removal by the immune system is highly advantageous. Moreover, minimizing or eliminating altogether the toxic effects of these biomaterials on the human body is a necessity for novel drug delivery systems to become approved and available for clinical use.

Considering the rigorous and resource-intensive process of conducting the preclinical studies and clinical trials necessary to demonstrate both safety and efficacy, an extremely attractive approach to the design and development of nanocarriers for biomedical applications is to incorporate *in vitro* and *in vivo* experimental techniques evaluating biological behaviour, with particular emphasis on establishing the effectiveness of *in vitro* methods at predicting *in vivo* performance.

We have investigated the biocompatibility of polymeric nanocarriers by employing *in vitro* techniques to assess the extent of their interactions with components of blood. Experimental techniques for quantifying the toxic effects of nanoparticles on erythrocytes and evaluating their propensity to interact with immune proteins have been adapted in the study of a novel polysaccharide-coated nanocarrier formulated using block copolymers consisting of poly(lactic acid) (PLA) and dextran.

MATERIALS AND METHODS

Materials

PLA and poly(lactic-*co*-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) were purchased from Lakeshore Biomaterials (Birmingham, AL, USA). VBS^{2+} was obtained from Boston BioProducts (Ashland, MA, USA). Whole sheep blood in anticoagulant was purchased through Cedarlane Laboratories (Burlington, ON, Canada). Rabbit polyclonal antibody to sheep red blood cell stroma was obtained from Abcam (Cambridge, MA, USA). Human blood serum was obtained and pooled from volunteers at the University of Waterloo.

Nanoparticle preparation via nanoprecipitation

Nanoparticles were prepared using a nanoprecipitation method, as described elsewhere (Cheng 2007), to form NPs comprised of three different types of polymers. Briefly, the newly developed PLA-dextran block copolymer was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL and added dropwise to 10 parts of stirring distilled water. NPs were purified by ultrafiltration at 4000 *g* for 15 minutes using Amicon ultrafiltration tubes (10k MW filtration membrane) and resuspended to 1 mg/mL in distilled water. The same procedure was applied in formulating NPs using PLA dissolved in DMSO or (PLGA-PEG) dissolved in acetone. The formulated NPs were subsequently characterized using dynamic light scattering to determine the mean diameter for each type of NP.

Hemolysis assay

NPs were purified and resuspended in VBS^{2+} at 40 mg/mL. Sheep erythrocytes were prepared at a concentration of $1x10^8$ cells/mL. Varying amounts of the concentrated NP suspension were added to 200 µL of suspended sheep erythrocytes in volumes of VBS^{2+} necessary to obtain a total volume of 1 mL and final NP concentrations ranging from 1 to 20 mg/mL. After 60 minutes of incubation at 37 °C, absorbance measurements were recorded at 415 nm to determine the extent of hemolysis relative to negative and positive controls (erythrocytes in VBS^{2+} and deionized water, respectively).

CH_{50} complement consumption assay

The CH_{50} complement consumption assay was performed as described elsewhere (Vonarbourg 2006). Briefly, NP suspensions were added to human blood serum in VBS^{2+} in volumes corresponding to a range of NP surface areasobtained through calculations and incubated for 60

minutes at 37 °C. Antibody-sensitized sheep erythrocytes were prepared using rabbit polyclonal antibody to sheep red blood cell stroma and concentrated at $1x10^8$ cells/mL. The addition of different amounts of NP-serum mixture to sensitized sheep erythrocytes resulted in varying amounts of cell lysis, quantifiable using a microplate reader at 415 nm. The CH_{50} value is obtained as the amount of NP-serum mixture required to cause the lysis of 50% of the sensitized sheep erythrocytes; these values are compared across different surface areas for each type of NP as a quantitative assessment of complement protein consumption.

RESULTS AND DISCUSSION

Hemolysis experiments were performed using NPs formulated from polysaccharide-coated block copolymers in addition to the uncoated PLA NPs. These copolymers were selected at a range of molecular weights suitable for comparison between the uncoated and coated NPs. Given the prevalence of PEG as a surface coating for developing nanocarriers (Vonarbourg 2006), the hemolytic behaviour of PLGA-PEG NPs has also been evaluated. Fig. 1 shows the differences in hemolytic tendencies between NPs formulated using the three different types of polymers.

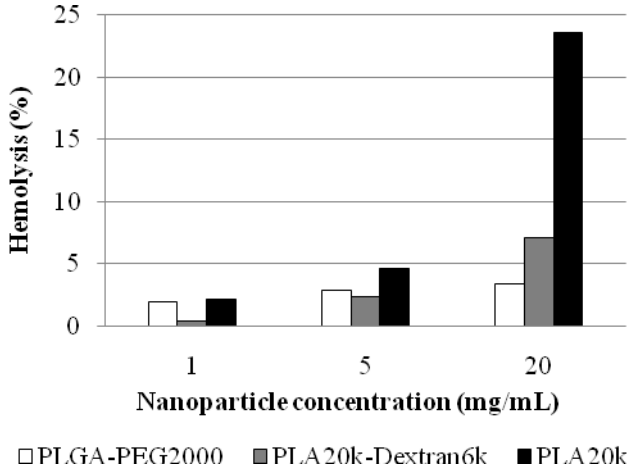

Figure 1 : Comparison of the extent of hemolysis (%) for three different types of NPs.

It is clear that the 20k MW PLA NPs cause hemolysis to an extent that greatly exceeds that observed in the case of 6k MW dextran-coated NPs. This is to be expected, as the surface coating was selected in order to impart biocompatibility, as well as colloidal stability, to the prepared NPs. PLGA-PEG NPs appear to perform favourably at higher concentrations in comparison to the dextran-coated NPs; however, the extent of hemolysis for the PEG-coated NPs does not increase as drastically with NP concentration as for the other polymers. A possible explanation for this is that the PEG-coated NPs may be less stable at higher concentrations, resulting in the aggregation of NPs and, thus, reduced surface area with which to interact with erythrocytes and induce hemolysis. Still, given that hemolysis values above 5% are considered to indicate significant toxicity (Wang 2010), further purification or improved design of the polymers may be necessary in order to obtain less toxic NPs.

The results obtained from a CH_{50} complement consumption assay indicate the propensity of a material to interact with complement proteins that play a key role in the immune response. This experimental technique has been reported in the literature for the evaluation of both PEG-coated and dextran-coated polymeric nanoparticles (Lemarchand 2006). Furthermore, it can be applied in the evaluation of NPs with a vast number of different properties (e.g. polymer composition, polymer chain molecular weight, surface modification) to obtain a better understanding of these parameters in facilitating optimal design schemes.

CONCLUSIONS

Novel polysaccharide-coated nanoparticles have been developed as drug delivery vehicles in working toward improved biocompatibility and predictable *in vivo* behaviour. Initial experiments have demonstrated the reduced toxicity of the coated NPs compared to uncoated NPs, based on hemolytic behaviour. Further work will expand on these results and incorporate the study of immune protein interactions in order to aid future polymer/NP design, with the intention of demonstrating some correlation or predictive ability of these *in vitro* assays with regards to *in vivo* biological behaviour.

REFERENCES

• Cheng J. et al. (2007) *Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery*. Biomaterials 28(5) 869-876.

• Lemarchand C. et al. (2006) *Influence of polysaccharide coating on the interactions of nanoparticles with biological systems*. Biomaterials 27(1) 108-118.

• Moghimi S. M. et al. (2001) *Long-circulating and target-specific nanoparticles: Theory to practice*. Pharmacological Reviews 53(2) 283-318.

• Nishiyama N. et al. (2006) *Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery*. Pharmacology & Therapeutics 112(3) 630-648.

• Torchilin V. P. (2006) *Multifunctional nanocarriers*. Advanced Drug Delivery Reviews 58(14) 1532-1555.

• Vonarbourg A. et al. (2006) *Evaluation of pegylated lipid nanocapsules versus complement system activation and macrophage uptake*. Journal of Biomedical Materials Research Part A 78A(3) 620-628.

• Wang W. et al. (2010) *Protective effect of PEGylation against poly(amidoamine) dendrimer-induced hemolysis of human red blood cells*. Journal of Biomedical Materials Research Part B 93B(1) 59-64.