P-051	**Complement activation by nanoparticles as an indicator of biocompatibility**	

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INTRODUCTION AND OBJECTIVES

A wide range of nanocarriers has been used in the development of clinically available pharmaceutical products. Their applicability to systemic administration largely depends upon complex interactions with biomolecules and immune cells present in the bloodstream. Given the widely varying surface characteristics, such as charge, hydrophilicity, curvature and density, of these nanocarriers, further progress in the continued development and optimization of nanocarriers requires a more fundamental understanding of how these material properties influence nanoparticle behaviour in the body.

Achieving a long circulatory lifetime is typically an important and highly desirable objective in the design of nanoparticles for intravenous administration. Specifically, the adsorption of blood proteins to the surfaces of nanoparticles in circulation has been noted as a key process in the recognition and clearance of nanocarriers from the bloodstream. Focusing on this particular aspect of nanoparticle behaviour may thus enable a relatively simple approach to assessing biocompatibility and – most importantly – predicting nanoparticle behaviour in complex biological environments.

We have investigated the biocompatibility of a range of nanoparticles through the use of *in vitro* techniques assessing the extent of their interactions with blood components. A standard hemolytic assay was employed to study the hemocompatibility of these nanoparticles, while the use of an adapted complement activation assay, studying the propensity of nanoparticles to interact with a particular class of immune proteins called complement proteins, facilitated a more in-depth evaluation of nanoparticle biocompatibility.

MATERIALS AND METHODS

Materials

PLA and poly(lactic-*co*-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) were purchased from Lakeshore Biomaterials (Birmingham, AL, USA). LUDOX colloidal silica and zymosan were obtained from Sigma-Aldrich (St. Louis, MO, 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). Pooled human complement serum was obtained from Innovative Research (Novi, MI, USA).

Nanoparticle preparation

Polymeric nanoparticles (NPs) were prepared using a nanoprecipitation method, as described elsewhere (Cheng 2007), to form NPs comprised of three different types of polymers: PLA, PLGA-PEG, and PLA-Dextran. Zymosan microparticles were prepared through boiling of a zymosan suspension and subsequent purification via centrifugation as reported previously (Vonarbourg 2006). Silica nanoparticles were used as obtained from the supplier.

Hemolysis assay

Polymeric 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 areas obtained through calculations and incubated for 60 minutes at 37 °C. 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 as in the following equation.

$$\text{Complement consumption } (\%) = 100\% \times \frac{CH_{50(Sample)} - CH_{50(Control)}}{CH_{50(Sample)}} (\%) = 100\% >$$

RESULTS AND DISCUSSION

Hemolysis experiments were performed using NPs formulated from PLA-Dextran and PLGA-PEG block

copolymers in addition to the uncoated PLA NPs. Fig. 1 shows the differences in hemolytic tendencies between NPs formulated using the three different types of polymers.

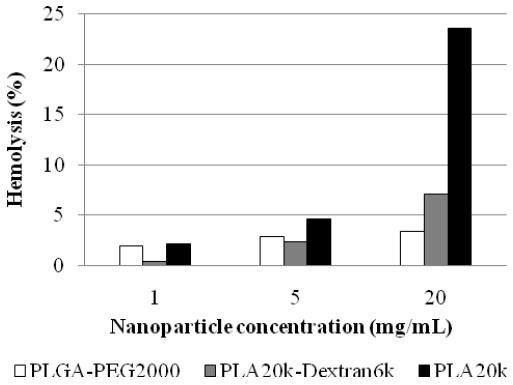

Figure 1 : Comparison of the extent of hemolysis (%) for three different types of NPs.

It is clear that the two block copolymer-based NP formulations outperform the uncoated PLA NPs, and the result supports the selection of hydrophilic polymeric blocks (PEG, dextran) to provide a more biocompatible surface.

The CH_{50} complement consumption assay was used to explore the biocompatibility of different NP formulations in more depth. As a validation of this approach, zymosan microparticles, which are known to specifically activate complement, were used as a reference for measurements on the relatively hydrophobic silica NPs and hydrophilic PLA-dextran NPs. The results are shown in Fig. 2, demonstrating the effectiveness of the assay in distinguishing between different types of materials based on the slopes of their consumption plots.

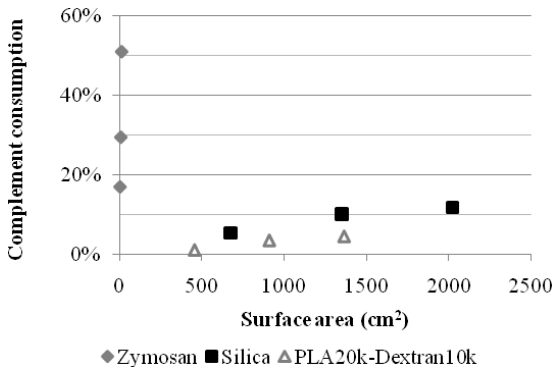

Figure 2 : Complement consumption of various materials.

The expected behaviour is that hydrophilic NPs will exhibit reduced protein adsorption and interaction compared to more hydrophobic NPs, and the results appear to support this trend. This methodology is also applicable, for example, to comparisons between polymeric NP formulations based on varying molecular weights of the same block copolymer. Moreover, the technique can be utilized to explore more novel aspects of nanoparticle-blood interactions, such as the effect of blood lipid concentrations in human serum on NP complement consumption. Turbid pooled serum samples were centrifuged in order to remove excess lipids, resulting in clear samples with relatively low blood lipid concentrations (enzymatically determined lipid levels not shown). Complement interactions between these serum samples and silica particles are reported in Fig. 3.

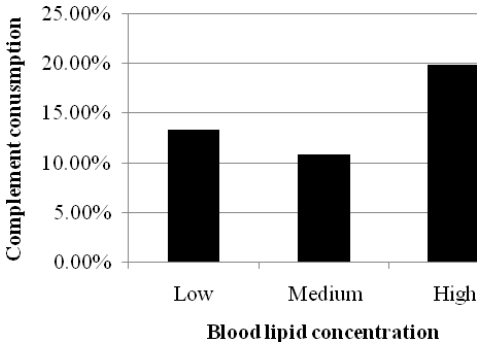

Figure 3 : Effect of blood lipid concentration on complement consumption by silica NPs.

Initial indications are that lipid content in the blood may significantly influence complement consumption, and this result is noteworthy in light of recent reports that lipids comprise a major portion of adsorbed biomolecules on NP surfaces (Hellstrand, 2009).

CONCLUSIONS

Increased interest in the development of nanoparticles for various applications, particularly in biomedical research, necessitates effective methods of assessing NP biocompatibility and predicting *in vivo* performance. We find that the CH_{50} complement consumption assay is highly applicable to studies varying from relatively straightforward comparisons between different NP formulations to more complex investigations of surface characteristics and factors such as blood lipid levels. Further work in this area will focus on developing a fundamental understanding of how NP surface characteristics affect biocompatibility, in addition to establishing correlation with to *in vivo* studies.

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