Kinetic evaluation of nanoparticle biocompatibility for predicting *in vivo* behaviour

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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, advances in the development of nanocarriers require 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. As such, 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. This approach is given further depth through the incorporation of kinetic elements to an adapted complement activation assay, providing insight into the propensity of nanoparticles to interact with a particular class of immune proteins called complement proteins and facilitating 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 and rabbit polyclonal antibody to sheep red blood cell stroma was purchased through Cedarlane Laboratories (Burlington, ON, Canada). Pooled human complement serum was obtained from Innovative Research (Novi, MI, USA).

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 VBS2+ 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.

CH50 complement consumption assay

The CH50 complement consumption assay was performed as described elsewhere (Vonarbourg 2006). Briefly, NP suspensions were added to human blood serum in VBS2+ 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 CH50 value is obtained as the amount of NP-serum mixture required to cause the lysis of 50% of the sensitized sheep erythrocytes.

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. 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.

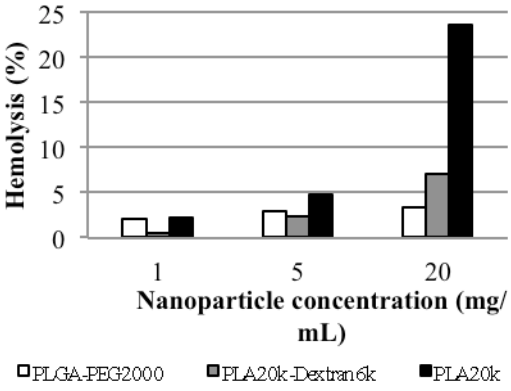

Figure 1 : Comparison of the extent of hemolysis (%) for three different types of NPs.

The CH50 complement consumption assay was used to explore the biocompatibility of different NP formulations in greater depth. 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 in Fig. 2 demonstrate the effectiveness of the assay in distinguishing between different types of materials based on the slopes of their consumption plots.

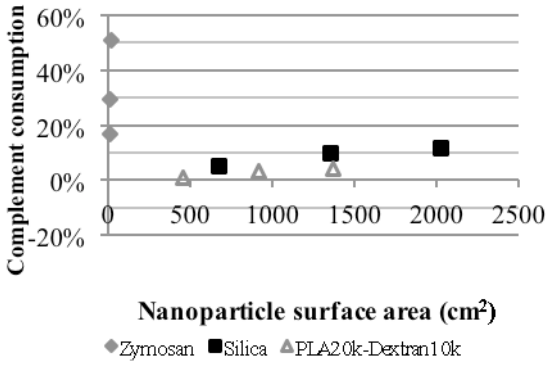

Figure 2 : Complement consumption of various materials.

This methodology is also applicable for comparing samples with more subtle differences in composition or surface characteristics. 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, as shown in Fig. 3.

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). 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).

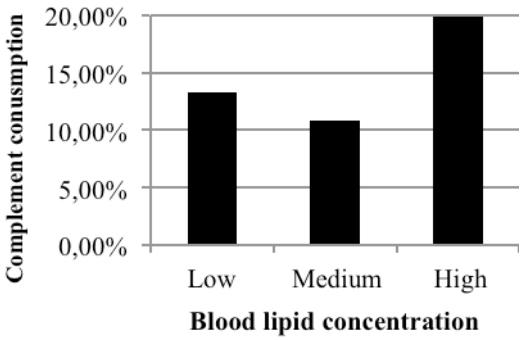

Figure 3 : Effect of blood lipid concentration on complement consumption by silica NPs.

Another key aspect of these interactions can be elucidated through kinetic assessment of complement activation. Silica particles were incubated with human serum for 30, 60, 90, and 120 minutes and evaluated using the CH50 complement assay, as in Fig. 4.

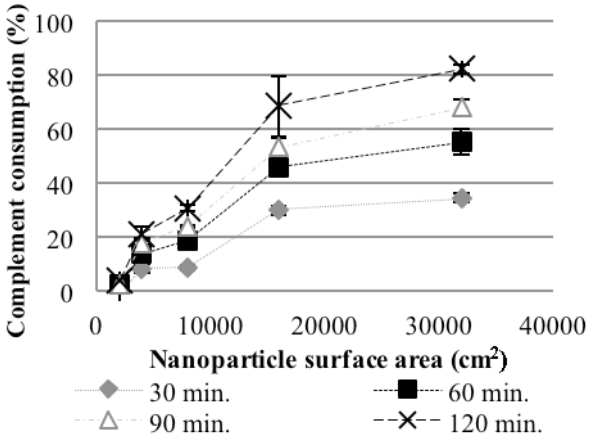

Figure 4 : Kinetic evaluation of complement consumption by silica from 30-120 min.

Based on this kinetic study, complement activation by silica NPs was seen to increase with moderate linearity over time. It is conceivable that less biocompatible nanoparticles with greater propensities to activate complement would more rapidly approach the 100% complement consumption limit, effectively saturating at some measurable time point. Future experiments will further explore this behaviour.

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 CH50 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 *in vivo* studies.

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