A complement-based approach to studying nanomaterial biocompatibility : Studying kinetic trends and saturation behaviour *in vitro*

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INTRODUCTION AND OBJECTIVES

Nanomedicine is a rapidly growing area of research that promises drastic improvements in various diagnostic and therapeutic applications. In practice, this progress largely depends upon the selection of materials and delivery systems that are biocompatible with the human body. As such, lengthy and costly pre-screening studies and clinical trials may result in high rates of attrition in order to ensure that new nanomedical technologies do less harm than good.

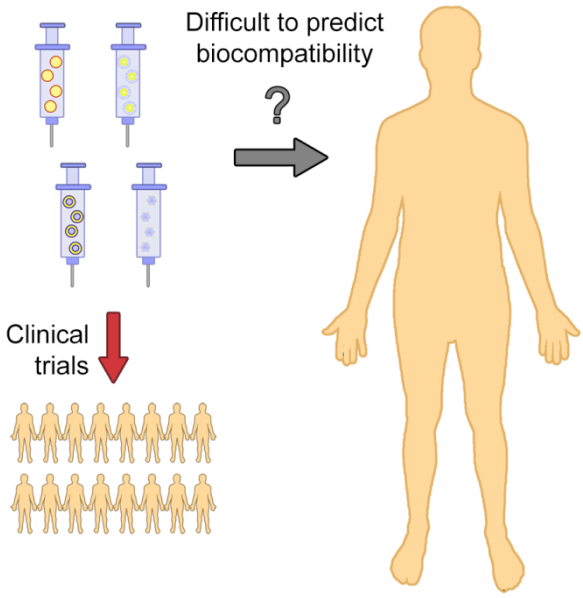

Figure 1 : Difficulties in the prediction of *in vivo* biocompatibility result in costly clinical trials.

Numerous methods have been discussed for the study of nanoparticle biocompatibility *in vitro*. Notably, approaches involving quantifying nanoparticle interactions with proteins and especially components of the immune system provide insight into how materials may behave *in vivo* (Karmali 2011). We propose that complement proteins hold particular significance in this approach, demonstrating the depth of information offered by studying complement system activity in the presence of nanoparticles.

MATERIALS AND METHODS

Materials

Whole sheep blood and rabbit polyclonal antibody to sheep red blood cell stroma were purchased from Cedarlane Laboratories (Burlington, ON, Canada). Pooled human complement serum was obtained from Innovative Research (Novi, MI, USA). Veronal-buffered saline (VBS^{2+}) was purchased from Boston BioProducts (Ashland, MA, USA). LUDOX colloidal silica was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polystyrene particles were purchased from PolySciences, Inc. (Warrington, PA, USA).

CH_{50} complement consumption assay

The CH_{50} complement consumption assay was performed as described elsewhere (Vonarbourg 2006). Briefly, nanoparticle (NP) suspensions were added to fixed volumes of human blood serum in VBS^{2+} and incubated. The addition of different amounts of NP-serum mixtures to sensitized sheep erythrocytes resulted in varying amounts of cell lysis as quantified by absorbance at 415 nm. CH_{50} values were calculated (based on regression analysis) as the volume of a given serum-NP mixture required to lyse 50% of antibody-sensitized cells.

RESULTS AND DISCUSSION

The study of complement activation by silica NPs in low lipid content and high lipid content human serum is summarized in Fig. 2. A difference in the surface area-dependent response highlights differences in the interactions of particles with proteins based on variation in serum composition, emphasizing the complexity of these interactions and the need for robust evaluation of biocompatibility *in vitro* before making confident predictions of *in vivo* behaviour.

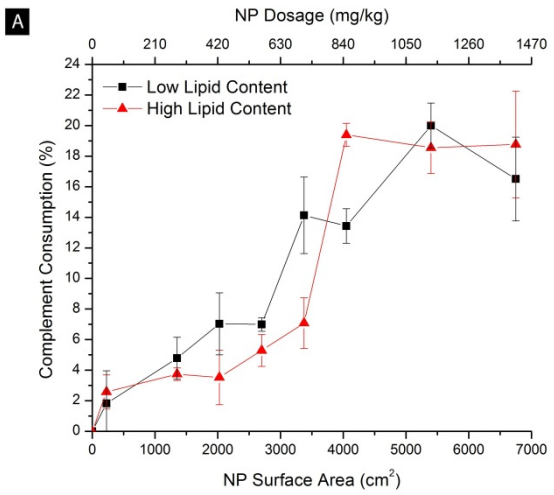

Figure 2 : Complement consumption by silica NPs under low lipid and high lipid human serum conditions (Meerasa 2013).

The biocompatibility of silica nanoparticles (20 nm) was evaluated using a kinetic CH_{50} approach, comparing the complement activation behaviour over 30, 60, 90, and 120 minutes as shown in Fig. 3. This kinetic study of silica NPs shows two clear trends: non-linearly increasing consumption with increasing particle concentrations (equivalently, surface areas) and moderately linear increases in consumption over time. The nature of this response with respect to time is particularly useful in comparing materials and predicting how rapidly they may interact with the body's immune system.

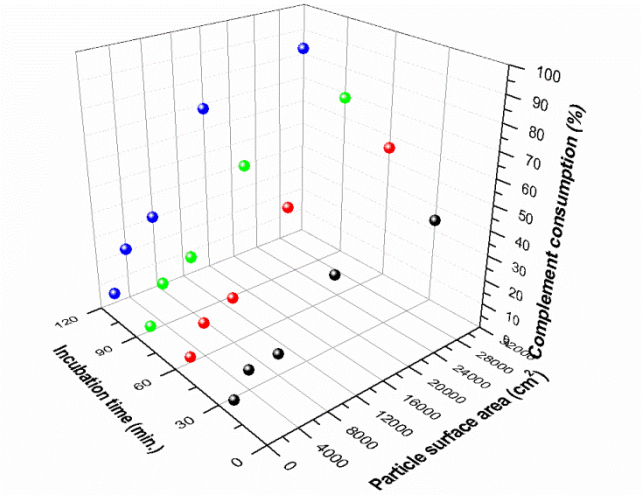

Figure 3 : Kinetic evaluation of complement consumption by silica NPs from 30-120 min.

In contrast, kinetic studies of polystyrene NPs reveal a different type of behaviour in Fig. 4. Two key differences are observed here: firstly, the complement consumption behaviour ranges from low (~10%) to saturation (~100%) over a much smaller range of surface areas compared to the silica NPs, suggesting that only small changes in concentration are required to cause rapid complement activation. Moreover, the complement consumption behaviour appears to saturate at higher surface areas from 60 min. and beyond.

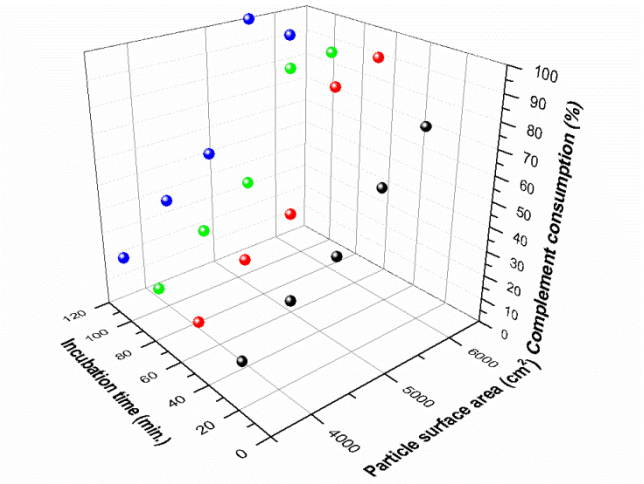

Figure 4 : Kinetic evaluation of complement consumption by polystyrene NPs from 30-120 min.

Additional studies involving polystyrene particles of different surface treatments (amino groups, untreated, carboxylic acid groups) were used as models for positive, uncharged, and negative NPs respectively. There was a distinct difference between the particles in the kinetic CH_{50} studies based on the previously discussed saturation behaviour, presented in Table 1.

Table 1 : Saturation behaviour and predicted biocompatibility of nominally positively charged (PS+), uncharged (PS), and negatively charged (PS-) polystyrene NPs.

	PS+	PS	PS-
Saturation time (min.)	N/A	60	90
Biocompatibility	High	Low	Medium

This contrast in behaviour should indicate the relative rates of immune response to different materials, as in the material comparison of Table 1. From a strict interpretation of lower saturation times indicating lack of biocompatibility and higher saturation times indicating relative biocompatibility, we are able to compare and make predictions on the behaviour of the polystyrene NPs.

CONCLUSIONS

A complement-based approach to evaluating biocompatibility has been developed and presented here in an effort to facilitate the bridging of *in vitro* and *in vivo* knowledge. Further studies will involve supplementary protein quantification techniques (ELISA), macrophage uptake studies, and investigations using animal models.

REFERENCES

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