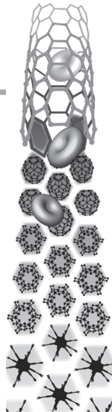

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Of mice, men and nanoparticle biocoronas: are *in vitro* to *in vivo* correlations and interspecies extrapolations realistic?

"*...how can one extrapolate between static* in vitro *cell conditions and the dynamic* in vivo *'milieu intérieur'?*"

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A great deal of research on the potential biological effects and biodistribution of nanomaterials in humans are inferred from *in vitro* cell culture studies or *in vivo* rodent models. A common theme emerging in predicting nanoparticle biological effects is the role of the protein corona. However, to link these data to what occurs in humans requires the use of extrapolation techniques that take into account species-specific physiology, such as the basal metabolic rate that drives most biodistribution and elimination processes. Formation and stabilization of nanoparticle protein coronas are independent of these physiological rates, making simple extrapolations theoretically problematic. Similarly, if nanoparticle biocorona complexes determine *in vivo* biodistribution, how does one extrapolate *in vitro* cell culture data, where coronas may be fundamentally different or even absent, to *in vivo* data, where they are deterministic. Currently, simple extrapolation strategies used in pharmacology and toxicology studies cannot compensate for these processes. The development of mechanistic physiologically based pharmacokinetic models could incorporate these processes if appropriate data were available. These issues have not been adequately considered by the nanomedicine or nanotoxicology community, and are even ignored in current risk assessment paradigms, yet are crucial for making biologically meaningful extrapolations.

Recent research has made the formation of nanoparticle protein coronas an essential component in describing nanoparticle biodistribution in the body [1–3]. This has long been recognized to occur with particle opsonization and has been a major factor driving pharmaceutical strategies to avoid this process (e.g., PEGylation) [4]. However, the latest studies have described a more complex nanoparticle protein corona composed of a dynamic structure where nanoparticles interact with hundreds of proteins and biomolecules to form biocoronas, which evolve over time to relatively stable metastructures that dictate cellular uptake and patterns of biodistribution [2,3,5]. Recent work has shown that even if nanoparticles are functionalized with target proteins (e.g., transferrin), once *in vivo* these proteins may then exchange with components of the blood proteome [6]. Recently, our group, and others, has demonstrated *in vitro* that patterns of cellular uptake are protein dependent and may completely change when preincubated with different plasma proteins [7,8]. Dynamic studies of this process suggest a thermodynamic-driven association between nanoparticles and available proteins, which is dependent upon the stoichiometry of the available molecules in the local environment [5,9,10]. A 'soft' biocorona forms and, over a longer period of time (minutes to hours), evolves to form a 'hard' corona that dictates subsequent biodistribution and, thus, activity.

This brings to question how can one extrapolate between static *in vitro* cell conditions and the dynamic *in vivo* 'milieu intérieur'? A more interesting question is, if the final state of the nanoparticle biocorona is dependent upon a kinetic process, how can one extrapolate between species such as mice (with very high basal metabolic rates [BMR], and, thus, short blood circulation times) to large mammals such as humans (with lower metabolic rates and much longer circulation times)? In larger animals there is more time to allow for further evolution of the nanoparticle protein corona complex, since it is the transit time in the systemic circulation that will determine the extent of evolution possible toward a stable nanoparticle biocorona. The short circulation

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time seen in mice would not allow sufficient time for the same stable corona to form as would be seen in humans, since by the time it would take for this to occur in the mouse, all nanoparticle complexes would have either been distributed to a tissue or eliminated.

A similar situation involving mismatching time scales occurs with oral administration of sustained-release dosage forms designed for humans when administered to small species. Intestinal transit times can be three- to four-times longer in humans compared with rats or even dogs [11]. In this case, the controlled release profile designed for longer human intestinal transit times results in a capsule being excreted by a rodent or dog before formulation, dissolution and release of the active drug have occurred, resulting in greatly reduced systemic bioavailability. This is conceptually equivalent to the time needed to form a stable nanoparticle protein corona compared with systemic residence times involved in distribution and elimination.

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Allometric scaling allows drug and small molecule extrapolations to be made between species of vastly different body sizes (e.g., mice to elephants), since most processes affecting biodistribution (e.g., distribution, elimination and metabolism) are dependent upon BMR and, thus, uniformly scaled using allometry [12,13]. By contrast, nanoparticle protein corona formation is not driven by BMR but rather by thermodynamics and the local environment, which would be improperly scaled using the type of uniform allometric approach that is standard in current pharmacology and toxicology. Put in a different context, nanoparticle corona formation occurs in astronomical time (linear time scale), while its subsequent biodistribution occurs in physiological time (time scale corrected for bodyweight raised to an exponent) [14]. To further complicate this, different blood proteomes between species could significantly alter the nature of the final corona complex, as would simple species differences in body temperature. These are difficult if not impossible to compensate for with mathematical algorithms.

There are computational methods to determine how protein corona formation could affect biodistribution, but more complex mechanistic pharmacokinetic models would be required to link biology with nanoparticle protein corona formation. One example would be to use physiologically based pharmacokinetic (PBPK) models as described for some nanomaterials. PBPK models compensate for altered BMR by including species-specific blood flows and tissue volumes. Models constructed to date have largely ignored the issue of protein corona formation [15,16]. However, the PBPK modeling framework would allow incorporation of equations describing the rate of nanoparticle protein corona formation in the blood circulation modules, similar to that recently described [10]. In addition, membrane-limited tissue uptake pathways, whose rate and extent is a function of the specific protein corona present in the blood circulation at that timepoint, could account for the specific cellular uptake pathway associated for that nanoparticle protein corona and cell type. Of course, it is readily apparent that formation of such models is mathematically complex but tractable. The major limitation is the lack of both appropriate *in vivo* data of sufficient granularity to both define the nature and dynamics of nanoparticle protein corona complex evolution, and appropriate *in vitro* cellular uptake data that link a specific nanoparticle corona complex to a cellular uptake pathway.

These issues affecting both *in vitro* to *in vivo* correlations, as well as interspecies extrapolations, two activities central to both nanomedicine development and nanomaterial safety risk assessments, have not been considered by workers in the field and deserve attention if a realistic paradigm to evaluate true *in vivo* safety and efficacy of nanomaterials is to be developed. These well-established biological processes must be taken into consideration when integrating both *in vitro* assays, as well as when extrapolating small rodent laboratory animal data to humans.

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