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In vivo biomolecule corona and the transformation of a foe into an ally for nanomedicine

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Nanoparticles (NPs) administered in the human body will undergo rapid surface modification upon contact with biological fluids driven by their interfacial interaction with a diverse range of biomolecules. Such spontaneous self-assembly and adsorption of proteins and other biomolecules onto the NP surface constitute what is commonly known as the protein or biomolecule corona. This surface biotransformation of the NPs modulates their biological interactions and impact on physiological systems and can influence their overall pharmacological profile. Here, we comment on how the initially considered 'nuisance' of the in vivo corona formation can now be considered a nanoparticle engineering tool for biomedical use, such as in endogenous tissue targeting, personalized biomarker discovery and immunomodulation.

What have we learnt about the in vivo biomolecule corona

There is widespread consensus today that proteins, along with their multi-molecular complexes, spontaneously adsorb onto nanoparticle (NP) surfaces, forming a 'biomolecule corona'[1]. Even though the NP 'corona' concept emerged from previous efforts in the 1980s and 1990s to understand the opsonization of NPs, the direct demonstration and proteomic characterization of the in vivo biomolecule corona was only reported a decade ago[2–4]. Since then, we have learnt that a molecularly complex and dynamic biomolecule corona is rapidly formed on the surface of different types of intravenously administered NPs. The NP corona composition, spatial orientation and exchange kinetics with biofluid molecules all depend on the NP physicochemical properties (such as its size, surface charge and shape) together with the type of biofluid (serum, urine or cerebrospinal fluid) and the biophysical environment parameters governing this interaction (temperature, pressure and flow).

A substantial knowledge gap persists regarding the underlying mechanisms governing the formation of the in vivo corona on NP surfaces within complex physiological environments. Nevertheless, there is wide acceptance today that the in vivo-assembled corona plays a pivotal role in facilitating the communication between intravenously administered NPs and immune components (cellular or acellular)[5]. This communication, in turn, critically influences the biodistribution and pharmacokinetic profile of the NPs. The nanomedicine field has been broadening its focus beyond the extensively studied protein corona as a 'nuisance' or 'handicap' to be overcome and is currently exploring the interaction of NPs with various biomolecule species, including lipids, metabolites and cell-free DNA, collectively forming the in vivo biomolecule corona[6], with the ultimate goal of unlocking numerous innovative applications for medical and clinical use.

Fundamentals of in vivo biomolecule corona formation and its limitations

Investigating the biomolecule corona within in vivo settings, spanning both preclinical and clinical scenarios, presents notable advantages over in vitro or ex vivo studies (Box 1) that form the majority of current knowledge. Although research conducted in vitro and ex vivo environments provides informative insights, the in vivo NP corona yields a deeper, more reliable and precise understanding of their significance and impact. Also, the in vivo approach is essential for addressing various critical challenges encountered in both in vitro and ex vivo studies, such as the incapability of accurately replicating immune system responses or the immunogenic character of NPs, thereby minimizing the likelihood of misinterpretations[7]. In vivo investigations ensure a more authentic representation of how the biomolecule corona behaves and influences biological processes in living organisms.

The conventional method for creating a biomolecule corona involves a series of steps: collecting biological fluids, mixing NPs with these fluids, incubating them for a set period at a specific temperature with continuous agitation, isolating the corona-coated NPs, purifying to remove loosely bound and excess biomolecules, and finally characterizing the biomolecule corona using proteomics techniques. By contrast, in vivo analysis of the biomolecule corona eliminates experimental variables that may lead to inaccuracies in data obtained through pre-determined in vitro or ex vivo conditions. For example, inconsistencies during plasma collection to be incubated with NPs ex vivo can arise during the selection and application of anticoagulant factors and from variations in storage conditions, including temperature and duration. Additionally, the in vivo NP corona can address potential discrepancies in incubation parameters, like temperature and agitation levels, which can substantially influence the results.

Although probing the biomolecule corona in vivo enables the design of more accurate investigations, in vivo parameters can also present several limitations, different to those critical for in vitro and

Box 1

Advantages, constraints and potential biomedical use of in vivo biomolecule corona

Key advantages of analysing the biomolecule corona

Reduced errors. Minimizes errors common in in vitro or ex vivo methods, such as plasma collection variations and incubation discrepancies.

Personalized insights. Provides in-depth, individualized insights into biomolecule corona formation.

Dynamic environmental analysis. Offers real-time insights into the evolution of the biomolecule corona.

Disease impact assessment. Explores how diseases and co-morbidities affect biomolecule corona composition.

Tissue interaction insights. Reveals complex tissue-specific interactions.

Immune response monitoring. Allows for the detection of immune signals within the biomolecule corona.

Clinical translation. Facilitates the transfer of nanomedicine technologies from lab to clinical use.

Regulatory approval support. Provides essential data for the safety and efficacy of nanomedicine.

Capturing real-world variability. Accounts for factors like diet, lifestyle and environment.

Constraints in investigating the biomolecule corona

Ethical and regulatory hurdles. Requires stringent ethical and regulatory compliance, especially for humans.

Sample size limitations. Finding suitable animal or human models with specific conditions is challenging.

Invasiveness. Requires invasive methods to retrieve corona-coated nanoparticles.

Biological complexity. Isolating and analysing tissue-specific coronas is difficult due to complex biological interactions.

Medication influences. Patient medications can alter biomolecule corona composition.

Individual variability. Genetic and immune response differences add complexity to data analysis.

Accessibility issues. Access to specific tissues or organs for analysis is limited, especially in certain diseases.

Biomedical use and exploitation of the biomolecule corona

Modulation of the immune system. The nanoparticle biomolecule corona can be tuned to evade or target the immune system (see the figure and Supplementary Information). Next steps include the following.

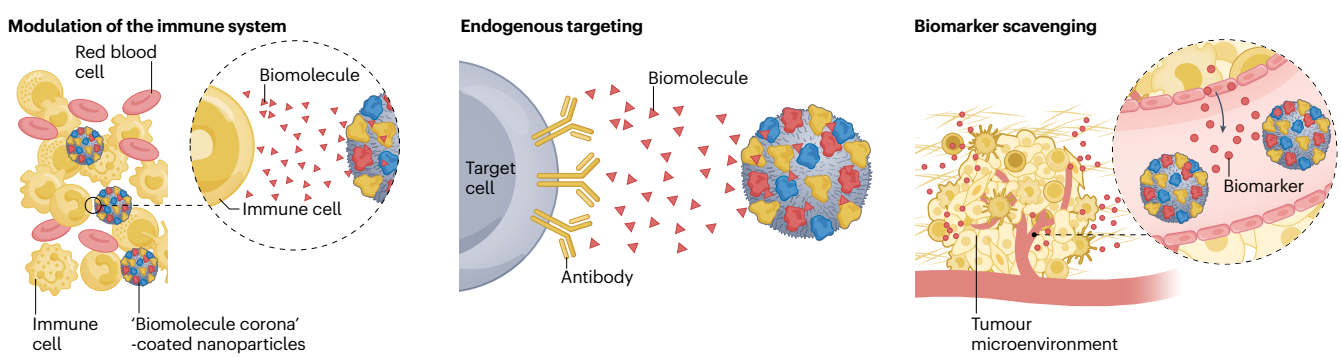
- Understanding the impact of the personalized protein corona on the interaction of nanoparticles with the immune system
- Learning how viral nanoparticle vectors modulate their interaction with blood proteins to evade or target the immune system

Endogenous targeting. The nanoparticle surface can be engineered to interact with specific proteins in the blood that can act as targeting ligands for specific organs or cells (see the figure and Supplementary Information). Next steps include the following.

- Deciphering the structural organization and receptor binding function of the biomolecule corona
- Focusing on the interaction of low-abundance corona proteins with target receptors
- Molecular profiling of the in vivo biomolecule corona at the target tissue

Biomarker scavenging. The nanoparticle biomolecule corona formation can be exploited to enrich disease-specific biomolecules in complex biological fluids (see the figure and Supplementary Information). Next steps include the following.

- Multiomics analysis of the in vivo biomolecule corona (nano-omics)
- Longitudinal analysis of the in vivo biomolecule corona across the disease continuum
- Understanding the impact of comorbidity and multiple drug treatments used (polypharmacy) on the composition of the biomolecule corona



ex vivo studies (Box 1). Limitations mostly result from the use of animal models and include ethical and logistical issues, constraints in administration routes and biological variability. Consequently, overcoming these limitations is imperative for all stakeholders (nanomedicine researchers, manufacturers and regulatory authorities) to facilitate the consistent and expanded implementation of biomolecule corona studies in vivo. In human cases, for instance, considerations could include incorporating plasma collection from participants involved in ongoing clinical trials of different nanomedicines in development, for secondary use in biomolecule corona analysis. This approach could enable utilization of banked plasma samples that may contain corona-formed NPs, whose in vivo biomolecule coronas could be subsequently analysed.

Exploitation of the in vivo protein corona

Alongside mechanistic and molecular investigations of the in vivo biomolecule corona, paradigm-shifting studies have emerged with the central aim of exploiting corona layering as a versatile NP functionalization strategy with a modular engineering capacity. Promising applications and strategies currently under exploration include the modulation of the immune system, endogenous tissue or cell targeting, and the discovery of disease biomarkers (Box 1 and Supplementary Information).

For immune system modulation. Depending on the NP target site, various strategies have been developed to leverage the in vivo biomolecule corona for both evading and triggering immune responses. For instance, the interaction of opsonin proteins (complement factors, immunoglobulins and coagulation factors) with the NP surface has been correlated with recondition and clearance of the NPs by blood-circulating macrophages and monocytes. By contrast, the presence of albumin and apolipoproteins in the NP biomolecule corona has been shown to extend the NP blood circulation life[8].

Nano-enabled immunomodulation approaches are advancing rapidly and have led to the development of mRNA vaccine formulations against SARS-CoV-2, reinvigorating the use of lipid NPs (LNPs) for the delivery of nucleic acid drugs across a diverse range of applications such as cancer immunotherapy and protein replacement therapy. With over a billion doses of mRNA–LNP vaccines administered globally, it becomes imperative to comprehend the modulatory impact of the in vivo biomolecule corona on the interaction between nanovaccines and the immune system. The inclusion of polyethylene glycol (PEG) in clinically used mRNA vaccines has been linked to the production of anti-PEG IgG and IgM antibodies, with varying degrees observed in different individuals based on age, sex and underlying comorbidities[9]. Further in vivo investigations are warranted to elucidate how the composition of a 'personalized' corona may influence either PEG-based or overall LNP immunogenicity. The currently suspected alterations in organ accumulation and accelerated clearance of administered PEG-containing nanoformulations underscore the necessity for understanding of the in vivo biomolecule corona upon the repeated administration of NPs.

Although our knowledge of how the in vivo biomolecule corona modulates the immune system continues to advance, we urge for rigorous investigations of the 'surface-camouflage' strategies that nano-sized viruses and other pathogens use to evade or trigger the immune system under physiologically relevant conditions. Mimicking how viruses engage with the immune system by in vivo NP corona engineering can, in turn, contribute to the development of NP corona-mediated immunostimulant or immunosuppressive strategies[10].

For endogenous targeting. Endogenous targeting is defined as the engineering of a NP surface to interact with specific proteins in the bloodstream, directing them to specific organs and/or cells[8]. For instance, coronas containing apolipoprotein E act as targeting moieties for low-density lipoprotein receptors, resulting in an increased accumulation of NPs in hepatocytes. Utilizing the in vivo protein corona to reduce the accumulation of lipid NP–mRNA complexes in the liver has already been proposed[11]. Given the multi-layered structure of the biomolecule corona, it is unlikely that a single protein is solely responsible for the organ and/or cell targeting[12]. Hence, attention should also be directed towards the structural organization and receptor binding function of the complex in vivo biomolecule corona, encompassing also corona proteins of low abundance.

When exploiting the in vivo biomolecule corona for endogenous targeting, it is also essential to consider the underlying comorbidities that may influence the composition of blood and, consequently, the biomoleculr corona composition. For example, increased levels of cholesterol (observed in hypercholesterolaemic mice) enhanced the interaction of gold and silver NPs with apolipoproteins while diminishing their interaction with complement proteins. Biodistribution data indicated that NPs in hypercholesterolaemic mice were more likely to be delivered to the liver, spleen and brain, and less likely to accumulate in the lungs, and therefore achieve some degree of endogenous targeting[13].

Finally, the molecular characterization of the in vivo biomolecule corona following NP accumulation at the target tissue poses several experimental challenges. Nevertheless, it should be regarded as the next frontier in the field. Machine learning is increasingly used to investigate the formation of NP biomolecule coronas, specifically aiming to understand the mechanisms behind NP organ accumulation. Through the analysis of diverse datasets that include NP physicochemical properties and in vivo environmental conditions, machine learning approaches seek to facilitate the design of tailored biomolecule coronas leading to anticipated NP pharmacokinetic profiles[14].

For biomarker scavenging. The emerging field of nano-omics utilizes the biomolecule corona for enriching and analysing molecular biomarkers in complex biological fluids[15]. Specifically, nano-omics entails the use of biofluid-incubated NPs as scavenging platforms to enrich and isolate disease-specific analytes before subjecting them to omics analysis, with the ultimate aim of identifying novel panels of 'liquid biopsy' biomarkers. Although the ex vivo corona has been used for the analysis of human clinical samples, the molecularly richer in vivo corona has demonstrated its potential in facilitating the discovery of biomarkers in preclinical models. The molecular complexity of the biomolecule corona offers a compelling integrated solution for conducting multi-omics biomarker discovery to meet a wide range of unmet clinical needs. Critical next steps involve the longitudinal analysis of the in vivo biomolecule corona throughout the disease continuum, while concurrently exploring the impact of comorbidities and polypharmacy in patient-relevant preclinical models.

Conclusion

Initially seen as a hindrance, the in vivo biomolecule corona is increasingly acknowledged as a means to innovatively refine NP properties with potential applications spanning immune system modulation, targeted drug delivery and biomarker discovery. The dynamic interaction between NPs and biological systems paves a new way for revolutionary therapeutic and diagnostic advancements. Focusing on drawing insights from the interaction of viral NPs with the immune system, implementing machine learning approaches to analyse the composition of the biomolecule corona at the target tissue and incorporating in vivo biomolecule corona investigations into human clinical trials are key milestones for the development of corona-mediated immunomodulation and targeting approaches. Leveraging the in vivo biomolecule corona for multi-omics biomarker discovery holds significant promise in advancing early disease detection and stratified medicine. To unlock its full potential, it is essential to conduct longitudinal studies of the in vivo biomolecule corona at various disease progression stages, taking into consideration also the impact that comorbidities and the administration of multiple medicines (polypharmacy) will have on its composition.

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Competing interests

M.M. discloses that (i) he is a co-founder and director of the Academic Parity Movement, a non-profit organization dedicated to addressing academic discrimination, violence and incivility; (ii) he is a co-founder of Targets' Tip; and (iii) he receives royalties/honoraria for his published books, plenary lectures and licensed patent. M.H. and K.K. are inventors of granted patents describing the use of nanoparticle corona.

Additional information

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Comment

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Supplementary information for

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