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EDITORIAL

The biomolecular corona of extracellular nanoparticles holds new promises for advancing clinical molecular diagnostics

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1. Classic corona

In the last decade, the impasse in effective clinical translation of engineered nanomaterials triggered an unprecedented effort in understanding the biological responses to synthetic nanoparticles (SNPs), which is still underway. The main result of such an effort is the concept of biomolecular corona (BC), that captures the key role played by the complex dynamic interaction occurring between the surface of SNPs immersed in a biological fluid and the biomolecules (proteins, lipids, metabolites, and nucleic acids) populating that fluid, in determining the SNP biological identity and physiological fate [1,2].

The BC changes the physicochemical features of the SNPs, such as surface energy, surface charge, hydrodynamic radius, and aggregation/stability properties. On the flip side, SNPs can induce structural and conformational changes of the proteins confined in the BC.

It has been observed that fast diffusive proteins initially adsorb on SNP surface, gradually replaced by slower diffusive ones bearing higher affinity for SNP surface (the so-called Vroman effect). Following this mechanism, the widely accepted hypothesis of BC formation depicts a stable inner layer of biomolecules irreversibly physiosorbed onto the SNP surface (the 'hard corona') interfacing an outer layer of loosely bound molecules, which dynamically exchanges with the medium (the 'soft corona') [2].

Sticking to 'mere' BC composition, irrefutable evidence points to the fact that the BC may enrich (concentrate) specific biomolecules or outline-specific biomolecular patterns hidden in the biological fluid [1,3,4]. This striking feature makes the BC extraordinarily appealing for diagnostics, opening the possibility to single out low abundant biomarkers, which would be otherwise missed, especially in complex biofluids (e.g. blood) and to follow their evolution over time and space.

In view of this, explorative studies, which started appearing from 2014, have leveraged the scavenging features of BC to search for novel diagnostic and prognostic biomarkers: thus, unique BC fingerprints could be related to specific diseases [5,6]. For example, different hydrophobic and hydrophilic SNPs have been incubated with plasma of patients with different diseases (i.e. breast cancer, thalassemia, diabetes) or conditions (i.e. pregnancy, smoke, hypercholesterolemia), revealing alteration of the BC (namely, of the hard corona) composition in respect to healthy samples. Notably, the BC resulted also sensitive to individual heterogeneity, unspecified disorders, and gene–gene interactions (i.e. 'personalized' corona [7]). Others have used BC arrays to identify cancer at various stages. Moreover, BC is also influenced by the metabolite composition of the biofluid. Molecular dynamics studies, later corroborated by *in vitro* studies, have demonstrated that glucose and cholesterol, respectively, model metabolites of diabetes and hypercholesterolemia, could substantially affect the interaction, the amount, and the conformation of fibrinogen protein in the BC of NPs [6].

Most of the studies have so far focused on BC proteomics, even though the analysis of protein post-translational modifications, or the analysis of biomolecules, which may also constitute the BC, such as metabolites and nucleic acids, is gaining considerable interest [4].

Despite the exciting promises, the full potentiality of BC of SNPs for diagnostic studies still remains hindered by important drawbacks, mainly ascribable to the experimental hurdles in (i) design and synthesis of nontoxic SNPs capable of collecting a 'biomarker BC' as well as in (ii) high-throughput BC separation and characterization [8].

2. The corona grips extracellular nanoparticles

In the recent years, we started to realize that nature has been using NPs much longer than we have in our laboratories. Biological fluids are indeed nanostructured, featuring populations of extracellular nanoparticles (ENPs) secreted by cells in the extracellular space that count exomeres, lipoproteins (LPs), extracellular vesicles (EVs), and midbody remnants, to cite any [9]. ENP clinical translation promises to be the non-incremental switch needed to unlock NP potential in precision medicine, while opening exciting new perspectives in nanotechnology.

Among ENPs, LPs and EVs are so far the best known. LPs are circulating blood NPs, spanning sizes from few nanometers to hundreds of nanometers, primarily known for their role in

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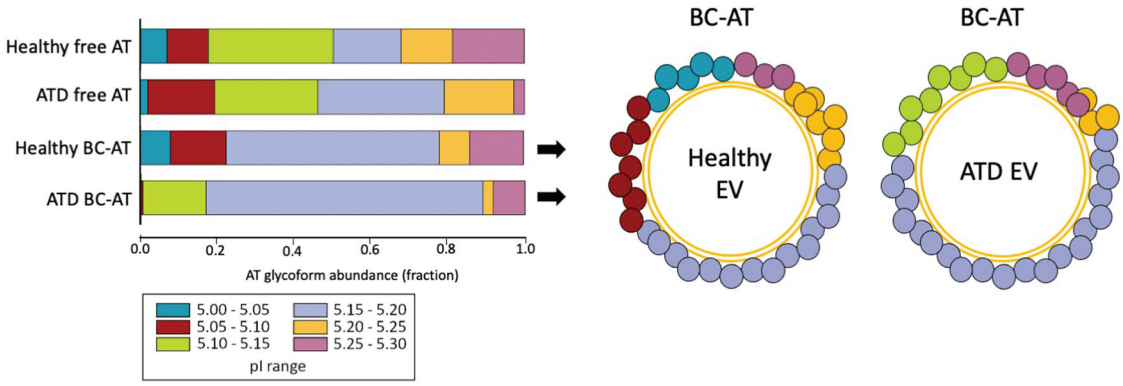

Figure 1. Antithrombin deficiency (ATD), an example of the diagnostic potentiality of the EV-BC. Partition (densitometric profiles, obtained from antithrombin (AT) 2D WB profiles, left, and schematics, right) of the relative amount of AT glycoforms free in plasma and enriched in the EV-BC of healthy subjects and ATD-affected patients (representative of a subject pool). pI range: isoelectric point range. Adapted from Ref. [14].

lipid transport to tissues (chylomicrons, very-low-density LPs, low-density LPs) and for their ability to efflux cholesterol from tissues (high-density LPs, HDL) [9]. They are composed of a hydrophobic core of nonpolar lipids, enveloped in a monolayered amphiphilic membrane made of phospholipids, cholesterol, and proteins. EVs are NPs, with a size range largely overlapping that of LPs, which are released by all cells. They are made by a lipid membrane, which encloses proteins, nucleic acids, and metabolites. EVs are emerging as regulators/mediators of key physiological and pathological intra- and cross-organismal processes, including tissue crosstalk, neurological functions, cancer metastasis, microbiota homeostasis, viral infection, and immunomodulation [10].

The field of ENPs is at the very beginning but rapidly evolving and expanding. The (obvious, with considerable hindsight) understanding that the BC exists and has a role also for ENPs is among the most exciting achievements of the last few years [11].

The BC terminology has not been applied to LPs so far, even if it has been previously shownLPs adsorb on their surface a series of plasma soluble proteins in dynamic exchange with the environment. Indeed, it has been shown that inflammation alters surface protein composition of HDLs and very recently different subspecies of HDLs have been isolated from blood by differential centrifugation, each bearing specific polypeptides adsorbed to their surface [12]. We expect HDL diversity, wider than what described so far, will be discovered upon the application of the advanced molecular and nanoscale characterization methods [8], which are increasingly making their way in the studies of ENPs [9].

The EV-BC is composed of biomolecules (or macromolecular complexes as LDLs) adsorbed onto the external EV surface when nascent EVs are secreted into the extracellular milieu and come in contact with proteins in body fluids [11] or in the extracellular space. Notably, a BC might form also within cells, during the process of EV biogenesis, conferring to each EV-specific properties. EV-BC strongly impacts EV functions, EV–cell interactions, and EV cellular uptake [11,13].

Indeed, removal of the BC from EVs, via washing, has been shown to abolish the abilities of EV in promoting angiogenesis and in modulating T cell activation. Furthermore, it has been demonstrated that the partial depletion of EV-BC impacts the EV immunomodulatory ability [14].

As observed with SNPs, EVs and LPs act as scavengers or concentrators for specific biomolecules [11,15,16], suggesting ENP-BC might be highly considered for biomarker discovery.

For example, the glycoprofiles of proteins adsorbed on HDLs can be modulated by both acute and long-term factors affecting different subjects. Moreover, they can help to differentiate between clinical groups across the different ranges of insulin sensitivity and are expected to be predictive of susceptibility to serious infectious events [16].

In a similar fashion, EV-BC studies are very promising. For example, specific glycoforms of the anticoagulant protein antithrombin (AT) are specifically and differently enriched in the BC of EVs separated from the plasma of healthy subjects and patients affected by qualitative AT deficiency (ATD) (while the profiles of the AT glycoforms in solution of healthy and ATD subjects substantially overlap), evidencing a potential in ATD diagnostic management [15] (Figure 1). Furthermore, EVs, incubated with EV-depleted plasma of patients with rheumatoid arthritis and relative controls, demonstrated the capability to differentiate patients from healthy subjects by enriching for specific plasma proteins [11].

3. What is next?

Several lines of evidence pinpoint the exploitation of ENPs as natural concentrators of biomolecules for early disease identification and stratification.

The major advantage of ENPs with respect to SNPs is that they are biogenic; hence, they do not need to be synthesized, do not need to be incubated with sampled biofluids nor injected *in vivo*. Moreover, being naturally produced, they do not alter/interfere with body physiology. The ENP-BC naturally forms during body circulation and adapts to physiological or pathological changes in the environment. Hence, ENP-BC gives better chances to track *in vivo* dynamic alterations of a biofluid composition, without the risk of interfering/altering it, of triggering toxic effects or immunogenic responses, and overcoming the problem of early clearance.

Another important advantage of ENPs is their intrinsic, large heterogeneity, in terms of biogenesis and source,

which translates into a variety of physicochemical properties as size, surface charge, and surface molecular composition. This entails extraordinary potential to enrich a much higher number of different biomolecules with respect to typical batch of monodispersed SNPs of a given composition.

Moreover, in the recent years, many techniques have been developed to separate different ENP subpopulations; hence, it could be possible to stratify, for example, EV subpopulations into a more restricted groups to possibly identify more specific BC biomarkers.

However, major hurdles need to be faced for the exploitation of ENP-BC as multiplexed biomarkers, starting from the fact that the field suffers from poor understanding of ENP physicochemical and biological properties and functions.

Specifically, unsolved problems and unmet standardization still define the separation of BC from ENPs – this is fundamentally ascribable to the fact that the weak interactions that drive BC formation and structure are analogous, in terms of both mechanism and energy, to those that keep assembled the ENPs, making extremely challenging to precisely 'peel' the ENPs of their BC.

Consequently, this limitation might inevitably affect the identification of useful biomarkers on BC, given the difficulty in predicting which proteins/biomolecules are acquired from the nanoparticle in circulation.

Despite these present hindrances, we believe the game is worth the candle. Awareness is fast increasing on this topic, and together with the application of bioinformatics and molecular modeling studies, knowledge on ENP-BC will rapidly increase [17].

In conclusion, ENP biomolecular corona could surely open new avenues for the diagnostic field. Additional investigation, mostly in terms of tailored analytical methodology and technology, will be essential for diagnostic translation.

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Declaration of interest

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