The interaction in terms of affinity, association and dissociation constant, and the stability of protein adsorbed were analyzed by using a plenty of techniques including isothermal titration calorimetry (ITC), circular dichroism, surface plasmon resonance, quartz crystal microbalance frequently combined together. Gel filtration has been proposed not only to separate the protein-NP complexes in incubation media as previously reported, but also to isolate proteins from the NP surfaces as well as to provide useful information on kinetic exchange rates for adsorbed proteins [58].

Besides proof of the PC-NPs complex presence, defining the PC represents a major topic: several researchers reported the use of electrophoresis as the preferred technology, while protein *quantity determination* was proposed to be characterized by mass spectrometry, in which protein samples were digested into small peptides and simply injected into the analysis instrument [73].

In summary, as evident, the identification and characterization of the PC cannot be obtained by a single analytical protocol, but different and complementary technologies are generally combined. In order to reach the highest level of quality and standardization in PC identification and characterization, the key point relies on the choice of the techniques in function of both the type of nanocarriers and on the analytical parameter to investigate. Using multiple characterization techniques is therefore crucial to analyze different aspects of the PC (i.e., presence of complex, composition of PC, reliability in *in vivo* conditions, etc.) and to get a better understanding of this biological entity. Remarkably, some techniques allow to detect the protein corona *in situ* (ITC), while other procedures require the detachment of bound proteins from the nanocarriers before measurements, thus still representing a controversial issue on PC analysis, since the adoption of purification methods may change equilibrium properties of the PC.

CONCLUSION

In recent years, the advance of nanomedicine as applied science in disease treatments highlighted a deeper need in understanding the interactions between nanocarriers and the biological environment aiming to improve their effectiveness and safety profiles. In this context, the study of the PC connected to any kind of NP drug carrier and its impact on both biodistribution and interaction with the target site is of extreme importance. Relevant information of the PC composition could also be exploited in sample screening at early research stages. This interest generated a wide number of attempted experiments, but at a deeper analysis of the results, even if remarkable, the lack of reproducibility and defined protocols in PC analysis still remain an urgent issue. In particular, as pointed out in this brief review, those data often obtained *in vitro* by simulating biological environments are affected by a high number of variables and seem not to be reliable and predictive for *in vivo* readouts and therefore their translatability. As also pointed out, numerous purification and investigation techniques were applied to evaluate and characterize the PC demonstrating that some technologies could really be useful in analysing some aspects of PC. Thus, in order to concretely exploit PC-NPs complexes data, the scientific path in this field will surely pass through an optimization of the protocols with a rational combination of the different techniques to finalize a systematic and more reproducible PC study approach.

ACKNOWLEDGEMENT

Supported by MAECI grant (PI Tosi, Nanomedicine for BBB-crossing in CNS oncologic pathologies) and Emilia Romagna Region grant (Step-by-step screening: RCUP E56C18002000002).

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