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Nanostructures in Nanomedicine: Critical Issues and Perspectives

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Abstract **-** In the last decades the development of novel smart nanomaterials provide versatile tuneable platforms for the investigation and manipulation of several biological tasks with low invasiveness in tissues and biological systems. As a matter of fact, a large variety of smart integrated nanostructured systems have proven their effectiveness for various types of biomedical applications, including stimuli-responsive organic and metal nanoparticles as well as hybrid (organic/inorganic) nanostructures. These novel nanostructures allow the possibility to include a diagnostic imaging system with the monitoring of the temporal evolution of the response of the disease in patients. The development of integrated medical nano-devices, that includes early diagnostics functions, allow to attain advanced profiling of the health (and disease) of individual patient, thus providing new methods for personalized health monitoring and preventative medicine. However, although the good performance of these novel nano-platform against a large number of specific diseases, a number of inherent drawbacks and critical issues are still present. This circumstance limit their translation in the clinic experience. Much efforts are currently being directed at bridging the gap to put these smart nano-platforms into practice, by a deeper investigation of their safety, therapeutic efficacy, and a detailed understanding of their physico-chemical behaviour.

Keywords**:** Nanomaterials, nanoparticles, targeted delivery, nanomedicine, theranostic approach, personalized medicine

1. Introduction

The introduction of smart nano-platform in biotechnology and nanomedicine has opened unprecedented possibilities of control specific functions at (sub-)cellular level, thus providing functional devices capable of therapeutic, diagnostic, and even theranostic abilities [1-4]. Those nanomedicines technologies make use of (less invasive) nano-devices that can be implanted inside specific part of the body. This approach, finds several applications including cancer therapy, drug delivery, tissue engineering, and even bionics [4, 5]. In particular, smart nanomaterials allow a combination of properties in terms of their architecture, size, shape, and surface functionalities that allow the development of efficient therapeutic applications. Those integrated nanosystems employ nanoparticle-based platform that include: polymers nanocarriers (such as block copolymers [6-9], dendrimers [10-12], hydrogels [13, 14]), lipid nanocarriers [15-19], mesoporous (silica nanoparticles (MSNPs) [20-22] and hybrid (organic/inorganic) nanostructures [22-26]. Moreover, the use of specific (internal and/or external) stimuli allow to manipulate those nanosystems in order to enhance the drug targeting efficacy and reduce unwanted side effects. This favourite the possibility to develop within the same nano-platform a diagnostic imaging system, that is able to follow the temporal evolution and monitoring the disease molecular response for each patient [27-29]. Finally, with the emergence of novel technologies and with the increased knowledge of genomics, new powerful tools are available for the investigation of the molecular profiling and the genetic mapping of a patient. The development of those novel nanomedicine approaches allow to attain a global and early health/disease profiling of individual patients, and provide a set of modern approaches for personalized health monitoring (so called "personalized medicine") and preventative medicine [30]. Despite the development of efficient (therapeutic) drugs delivery systems, has experienced considerable expansion in recent decades, translating the nanotechnology discoveries into the clinical practise results in a huge difficulties and requests deeper investigation and more novel strategies. In Figure 1, we report a schematic representation of various types of advanced nanomedicines. In this article, we describe recent breakthroughs for the design and development of theranostic nano-platforms for therapeutic treatment and their relevance to both basic science and

biomedical and nanomedicine applications. Together with new perspectives, we also highlight the open questions and critical issues that, in our opinion, still limit the clinical applications of those novel nanoplatforms and technologies.

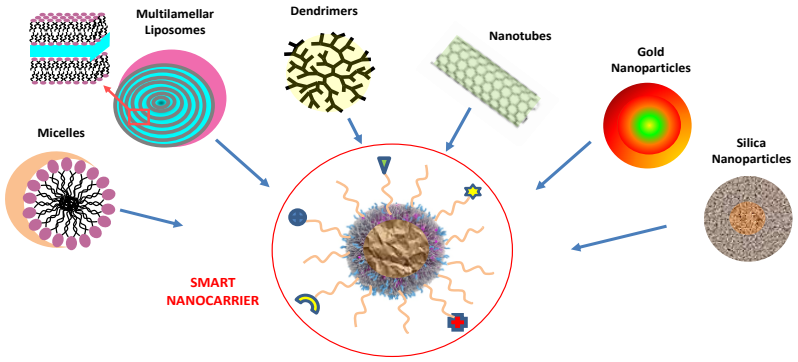

Fig. 1: Schematic representation of some different types of nanomedicines.

2. Passive and active targeting strategies for nanocarriers drug delivery

In drug delivery process the therapeutic compounds are selectively driven to the target sites within the biological system. They should avoid all other (potential) sites of interaction and improve the bio-distribution of therapeutic drug macromolecules to the desired target sites. Traditional drug delivery approaches are often accompanied by (systemic) side effects caused mainly to their nonspecific bio-distribution and uncontrollable drug release characteristics. To overcome these limitations, the use of advanced smart nanocarriers have been developed to achieve the release of drugs at the target sites in a spatial (and eventually temporal) controlled manner. In this respect, those innovative systems provide interesting properties that allow a decrease of the drug concentration, thus reducing the drug toxicities and improving therapeutic efficacy. Two main drug delivery strategies (namely the passive and active targeting) have been developed [27, 31]. The passive targeting (whereby no specific targeting ligands are used) is based on the drug accumulation in the microenvironment areas around the target pathological tissues (and cancer cells), that exhibit a different micro-environment in comparison with the normal cells. In tumour (or inflammatory) tissues the blood vessels have large vascular fenestrations (nanopores with diameters between 50-200 nm) that allow drug-loaded nanocarriers with a smaller size to diffuse outside the blood vessels (extravasation) thus entering the tumor interstitial space and concentrating into the target site [29, 31]. It is worth pointing that vascular permeability depends both on the properties of the specific nanocarrier and the characteristics of the vasculature. Moreover, significant heterogeneity between tumour types (connected with differences in pore dimensions of the vasculature and in vessel structure) may result in heterogeneous extravasation efficiency and delivery and a possible limited impact of drugs nanocarriers.

In the *active targeting* the nanocarriers exploit advanced technologies that allow a “locally activated” drug release actions limited to selective sites (tumors/inflammation tissues) within the body [30-32]. In this case, the control of the active targeting of the diseased tissues is obtained by using specific ligand–receptor mediated interactions, that involve the attachment to the surface of the nanocarriers of high affinity ligands that targets specific receptors [30, 31]. In figure 2, we report a schematic illustration of the intravenous administration of smart nanocarriers, and the possible surface functionalities for a model theranostic nanocrrier system. Despite the relevant number investigations performed in the last years on novel nano-formulations, these nano-structured platforms present lack of toxicity assessment tests, and lack of experience between the pre-clinical and clinical studies, thus resulting in the huge difficulties to obtain regulatory and ethics approval. Moreover, it has been shown that only a very small fraction (<5%) of the total administered nanoparticles formulations are delivered to the target site (tumor accumulation) [32]. Finally, several studies evidenced that active targeting process does not always result in an increased accumulation of the nanoparticles in the tumors region [33, 34], Thus, the simple presence of a of ligand-receptor combination does not ensure a successful active targeting of thee nanocarriers. This indicate that more efficient methods are required in order to improve the response rates to targeted drug delivery therapies.

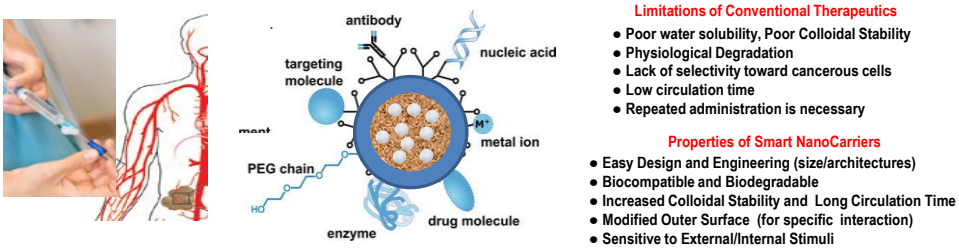

Fig. 2: Scketch of the intravenous administration of smart nanocarriers, and possible surface functionalities of a theranostic nanocrrier

3. Locally activated drug delivery: Stimuli-responsive nanocarriers

Drug-loaded nanocarriers should ensure that the active drugs will not freely extravasate during the regular blood circulation, but they should release the payload only at the specific target sites, where the nanocarriers accumulate by (active/passive) targeting strategy. To fulfil those requirements, various smart nanostructured systems have been developed over the past decades, as stimuli-responsive nano-platforms (figure 3) [35, 36]. Locally activated drug-release processes can occur either by internal-triggered targeting (which is based on the presence of specific enzymes or pH changes at the target sites) or by externally-activated targeting (based on external perturbations, such as light, temperature, magnetic field and ultrasound) [35-37]. More specifically, the endogenous stimuli (such as pH variations, enzyme concentration, hormone level, small bio-molecules, glucose or redox gradient), are related to the pathological characteristics of the specific diseased tissue and/or their microenvironment [37-39]. Owing to these unique characteristics it is possible to exploit the physiology of diseased tissues for the development of stimulus-responsive therapeutic nanoparticles. It is known, in fact, that the tumour region often present a peculiar microenvironment, as it is characterized by unevenness of blood flow, hypoxia and acidic pH. For example, in the presence of ionizable end-groups such as either acidic (e.g. carboxylic and sulfonic acids) or basic (e.g. amines, imidazole and pyridine) moieties that are able of donating (or accepting) H+ ions upon a pH change in the environment, cause an electrostatic charge modification within the system that generates a perturbation (or disruption) of the nanocarrier structure (pH-responsive nanocarrier systems) [35-37]. This process can be exploited for the controlled drug release at the intrinsic low pH (~5.0) level encountered in cancer cells.

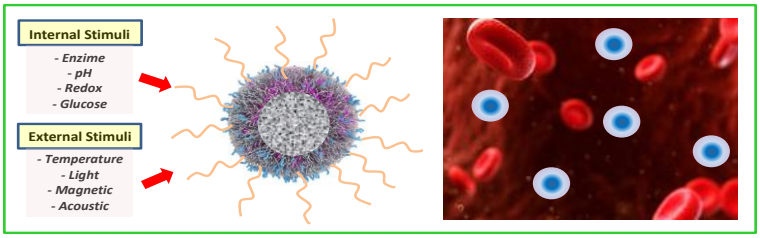

Fig. 3: Schematic illustration of a stimuli-responsive nanocarrier

On the other side, the exogenous stimuli (such as the temperature, magnetic field, ultrasound, light, electric pulse/ high energy radiation), may also be employed to trigger or enhance the drug release at diseased sites or areas [40-43]. For example, in light-responsive nanocarriers the presence of photochromic moieties that undergo photochemical changes (such as photoisomerization, photodimerization or photocleavage) upon light exposure, may induce the structural disruption/disaggregation of the nanocarrier and the release its drug cargo [39, 40]. For the translation of each stimulus from the pre-clinical experimental models to the clinical practice, an intensive activity of optimizations and improvement experiments are necessary [35, 36]. Especially, internal stimuli are indeed very difficult to control because of the peculiar complexity of the biological micro-environment encountered and the large variation from one patient to another. Although the external stimuli (exogenous triggers) responsive systems are much easier to be controlled, they present major problems related to normal tissue damage and tissue-penetration depth. It is worth noticing that exposure to electromagnetic fields may have sensitive influence on cell membrane components [44, 45]. For example, even the exposure to extremely low

electromagnetic fields influences the vibrations of peptide linkages, (thus modifying the secondary structures of α-helix and β-sheet contents) and cause sensitive unfolding process in cell membrane proteins [46].

4. Biomedical application of smart nanocarriers: Critical issues and perspectives

The optimal drug delivery system is able to deliver the active drugs only to the target diseased sites, thus avoiding the healthy tissues. In this respect, novel design concepts and versatile control ability offered by smart nanostructures provide various opportunities in placing any suitable combination of functions into a single scaffold [47, 48]. Those nanostructured system are based on the complex combinations of different (non-covalent) supramolecular interactions, that allow the formation of highly functional materials and devices with remarkable properties [49-55]. More specifically, supramolecular self-assembly between amphiphilic compounds allows the fabrication of a large variety of nanomaterials with emerging complex properties and various architectures [56-59]. These approaches have offered great potential to develop materials with improved therapeutic efficacy including target specificity, controlled drug release, lower therapeutic doses and minimum exposure to normal tissues [60, 61].

However, the dynamic changes at the cellular level and biological events that happen in responses to drug delivery processes are often very difficult to investigate, to describe and to predict. Despite the recent progresses in the study of several diseases and pathologies, the targeted therapy still remains a promise, as in the real clinical experience the effective amount of the drug delivered to tumor targets have been found to be less than 5% at most [32, 33]. Those critical issues are due to complexities of the diffusional barriers in solid tumors, and to the uncertainties that are connected with the enhanced permeability and retention (EPR) effect. Indeed, the tumor-targeting receptors may undergo specific modifications in its surface expression over the time. Moreover, the receptors that are overexpressed in a specific disease or pathology state are often present also (at lower concentrations) in the tissues of healthy cells. Furthermore, the receptor overexpression is often heterogeneous within different cells of a single tumour and also between different patients for the same typology of disease. All those circumstances pose important challenges in the process of detection of those complex phenomena. As a result, the presence of a ligand-receptor combination on nanocarrier systems does not ensure the success of the active targeting process. More predictive investigations and advanced drug delivery methods are then required in order to improve the response rates to targeted therapies [32, 33]. Due to this inherent complexity, the development of advanced targeted drug delivery systems require, then, the investigation of multiple factors such as the dynamic characteristics of tumor (including their spatial and temporal heterogeneity) and the controlled distribution in the blood. The study of model biomembranes and their interaction with nanoparticles has given a strong input to the understanding of the complex processes driven by the interactions that a nanostructured material can develop toward biological systems [62, 63], and highlight the important role of the interaction (electrostatic, hydrogen bond, ion coordination sites, etc.), in the formation of more and more complex morphologies, architectures and dynamic structural transitions [60, 61].

5. Advanced approaches: system biology and personalised medicine

A global profiling of the health (and disease) and an early diagnostics of individual patients can be obtained by using highly sensitive (nano-)analytical techniques for molecular diagnostics in combination with new smart integrated medical nanosystems (such as biosensors). These new approaches for personalized health monitoring and preventative medicine (called "*system biology*"), is based on the collection of many data ("in parallel") using the so called *"-omics"* technologies and prelude to the development of the personalized medicine, which propose an improved approach for the treatment of a wide range of diseases, employing genomics and proteomics technologies [29, 64]. In figure 4 we report a schematic illustration of the principal stages (A-E) of the personalised medicine approach. *Personalized medicine* aims to provide the most appropriate pharmacological treatment and therapy based on the individual profiling for each different patient (*optimized therapy*). This interdisciplinary approach aims to understand the complex interactions and functioning of the individual living systems, by inferring the pathways that regulates the specific biological (physiological or pathological) processes [29, 64].

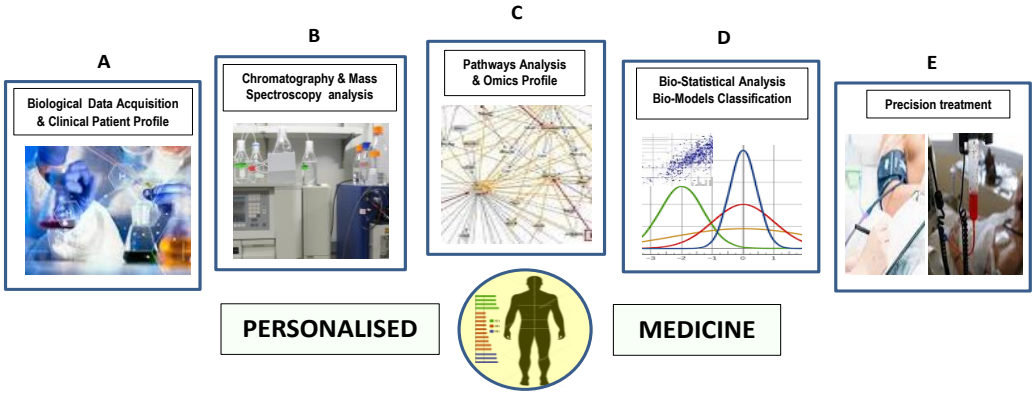

Figure 4. Schematic illustration of the basic stages of the personalised medicine approach.

Although its main activity is focused on the preventative medicine, it will also facilitate earlier disease detection via genomic approach (by using specific disease biomarkers). This program requires a synergistic collaboration between the inter- and cross-disciplinary fields of molecular nano-medicine, biochemistry, bioengineering and biotechnology [29, 64]. However, the complexity of the human genome due to the numerous genes involved (both in disease origins detection and in drug response) is one of the critical issues that impedes the effective, routine clinical application. Moreover, as a large number of genetic variations may exist, their complete identification within the complex genetic map will require a time-consuming and expensive tasks to perform. Although the biocompatibility, toxicological aspects and ethical implications still represent critical issues that are far to be completely resolved, the personalized medicine approach could have an expanding role in the modern approaches and future practice of medicine [29, 64]. With the aim to design the diagnostics and therapeutics that are administered for personalized use, multiple components can be integrated into a single nano-platform. The integration, on the same nanocarrier system, of both nanodiagnostics and drug formulation facilitate greater bioavailability profiles with specific and more effective treatment of diseases at lower doses administered and with a proper pharmacokinetics/pharmacodynamics behaviour, while minimizing the toxicity and the emergence of adverse drug reactions during the clinical practice. The development of a variety of miniature tools and nanostructure platforms is now feasible and cost-effective to produce lab-on-chip approaches, This require multiple steps for the nanocarriers fabrication, such as initial chemical synthesis, the formulation and purification of the final nanostructures. In order to program and achieve these scientific tasks in research and the clinics, large consortiums have to be created, with many institutes working together and in close relationship with regulatory agencies. This will stimulate the translation of the research results to the pharmaceutical industry, thus favouring the commercialization of novel nano-formulations.

6. Conclusion

Nanotechnology enables a large variety of functional nanoparticles that can be synthesized with an high level of control over the size and shape and surface modifications. More specifically, a variety of novel smart nanocarriers have offered great potential to develop nano-platforms with improved therapeutic efficacy including target specificity, controlled drug release, lower therapeutic doses and minimum exposure to normal tissues. Furthermore, the use of stimuli responsive (assembly/disassembly) nanostructured systems in combination with the ligand–receptor recognition processes furnish a large variety of theranostic solutions for the definition of specific bio-medical tasks such as the controlled release of drugs, imaging capabilities and multi-component (multi-functional) therapeutics. The development of integrated medical nano-systems allow to attain an early diagnostics and a global profiling of the health or disease of individual patients, thus providing new approaches for personalized health monitoring and preventative medicine. However, although these nano-platform evidence good performance against a large number of specific diseases, a number of inherent drawbacks and critical issues, limit their translation in the clinic experience. Much efforts are currently being directed at bridging the gap to put these smart nano-platform into practice, by a deeper investigation of their safety, therapeutic efficacy, and a detailed understanding of their physico-chemical behaviour.

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