Multimodal nanovectors with a theranostic potential for cancer treatment

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Abstract. Targeted theranosis (diagnosis combined to therapy) strategies are intended to improve cancer treatment efficacy while decreasing side effects. Nanovectors of hybrid (organic/inorganic) structure allow to combine multiple physical and biological functions and thus are being developed as promising contrast agents for multimodal diagnosis and drug nanocarriers for therapy of cancers. We present here three types of theranostic nanovectors our group develops, all based on combination of magnetic and optical properties with biological targeting and anticancer activity. Development of these nanovectors is being made in the frame of a multidisciplinary research, including biotechnology, chemical synthesis, pharmaceutical technology, physico-chemical and biological analysis in vitro and in vivo. The rational design of our nanovectors has been confirmed by complementary techniques as leading to promising biocompatibility and theranostic potential.

Index terms - Biomedical sensors, Nano medicine

I. INTRODUCTION

Diagnosis of cancers and the image-monitored treatment are the basis of the so called theranostic strategies which are being actively developed. For more efficient and accurate diagnosis, magnetic resonance imaging (MRI), computed tomography (CT), ultrasound (US) and nuclear imaging of positron emission tomography (PET) [1] are being combined in multi-modal approaches [2]. Indeed, spatially resolved 3D detection of tumors with MRI can be advantageously combined with endoscopic detection of superficial tumors by means of optical probes operating in the tissue optical window in Very Near InfraRed (VNIR). Sensitive real-time VNIR detection of small tumors can be achieved using fluorescence emission and/or Raman scattering, in particular if one used surface-enhanced resonance Raman scattering (SERRS) [3]. SERS is as sensitive as fluorescence but provides even higher molecular specificity due to a unique vibrational signature of molecules. Moreover, SERS allows simultaneous (multiplex) detection of several signals within complex biological environments [4].

Hybrid, organic-inorganic nanostructures can be used for multimodal diagnosis since they allow combining multiple nanomaterials (magnetic NPs, plasmonic NPs, drugs, biological targeting ligands) and functionalities [5]. This is the case of nanovectors we developed for bi-modal (MRI-fluorescence) and tri-modal (MRI-SERRS-fluorescence) detection of cancer cells as described below.

The core of our nanovectors is composed of superparamagnetic iron oxide nanoparticles (SPIONs) alone or combined with plasmonic AgNPs. The SPIONs generate a negative contrast on T2*-weighted MRI and are well known to improve the in vivo cancer detection [1]. The plasmonic surface of AgNPs provides enhanced Raman response of adsorbed molecules. For optical detection, we labelled the nanovectors with VNIR fluorophores and/or to Raman reporter molecules attached to the inorganic cores and coated them with biocompatible polymers. The biological ligands like scFv antibody fragments of Herceptin® and folic acid molecules are bound to the external polymeric surface of the nanovectors. These ligands enhance the specificity and the efficiency of the vector delivery to HER-2 overexpressing breast cancers which are the most aggressive breast tumors. Therapeutic agents like chemotherapy drugs and siRNA molecules are also hidden within the polymeric shell of the nanovectors. This structural organization insures the drug/label protection and good biocompatibility of the nanovectors, and corresponds to the rational design strategy.

II. RESULTS

a. Covalently assembled magnetic nanovectors of chemotherapy agent

We generate our covalently assembled nanovectors via silane-mediated binding of native or ligand-modified PEG chains and fluorophores to the SPION surface [6]. For this, one-pot synthesis protocols proposed by our team have the advantage of reducing the number of intermediate steps. To load these nanovectors with anticancer drug doxorubicin, we developed an experimental approach using pre-formed chelate doxorubicin-Fe^{2+} [7]. The interest of this approach consists in the accelerated drug release in acidic environment as it is the case in tumors and in cell lysosomes. Such nanovectors of doxorubicin have been shown to be stealthy [8] and to possess a significant MRI-

fluorescent response, anticancer activity *in vitro* [7] and *in vivo* [9], and reduced hematotoxicity.

From recently, we are working to improve the cancer targeting efficiency of our nanovectors by decorating them with a single-chain variable fragment (scFv, molecular weight ca 27-28 kDa) targeting HER-2. This strategy has several advantages over whole antibodies: (i) the scFv are one-fifth the size of whole IgG antibodies (thus, nanoplatforms functionalised with scFv are much smaller) and they retain full antigen bonding capacity; (ii) unlike whole antibodies, scFv do not contain the Fc constant domain and therefore are not able to trigger potentially harmful immune responses.

b. Electrostatically assembled magnetic nanovectors of siRNA

Short interfering RNAs (siRNA) are a promising tool to treat various human diseases, including cancer. The siRNA act via the RNA interference (RNAi) mechanism. However, the systemic administration of siRNAs does not allow to attain efficiently the site of action. To overcome this difficulty, we develop magnetic siRNA nanovectors (MSNs) based on SPIONs coated with siRNA and chitosan polymer via layer-by layer electrostatic deposition [10]. In addition to protecting siRNAs, chitosan is intended to enhance the efficacy of the MSNs transfection to cancer cells. To improve the formulation and optimize the component quantities, we applied experimental design strategy, respectively a Box-Behnken and a Plackett-Burman design [10]. The method provides surface plots helpful to optimize and predict the component quantities of the MSNs regarding their hydrodynamic diameter (D_H). The results show that the most influent parameter was the order of the components incorporation. The model equations allowed to obtain MSNs with D_H smaller than 100 nm which was desired for their systemic administration.

c. Electrostatically assembled magneto-plasmonic nanoprobes

Controlled electrostatic assembly of SPIONs, AgNPs, nile blue (NB) dye and chitosan (Chi) polymer has been used to obtain stable magneto-plasmonic clusters (AgION-Chi) able to act as MRI-SERRS-active nanoprobes. The interest of the AgION-Chi nanoprobes is in that they are: (i) very rapid and easy to produce without using neither toxic reagents nor conjugation chemistry; (ii) readily biodegradable due to their electrostatic assembly; (iii) colloidally and spectrally stable for nearly one month. The nanoprobes allowed triple fluorescence-SERRS-MRI detection of HeLa cancer cells.

III. DISCUSSION – CONCLUSION

Rationally designed, the nanovectors described here show a good compromise between their physico-chemical characteristics (size ca. 100 nm, nearly neutral surface charge, long-term colloidal stability, sufficient optical and magnetic response) and biological properties (biocompatibility *in vitro* and *in vivo*, ability to recognize cancer cells and to deliver drugs and/or siRNA).
These nanovectors are theranostic and make possible a complementary use of the spatially resolved *in vivo* analysis by MRI with the real time multiplex optical sensing by means of VNIR probing. This is expected to provide more efficient and flexible solution for cancer detection, localization/characterization and therapy.

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