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NANOTECHNOLOGY IN THE DIAGNOSIS OF PATHOLOGICAL PROCESSES

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Резюме. Розробка та використання матеріалів нанорозмірного діапазону для вирішення медичних проблем і питань, пов’язаних зі здоров'ям, продовжує викликати все більший інтерес. Дослідження в наномедицині охоплюють безліч сфер, включаючи доставку ліків, розробку вакцин, антибактеріальних засобів, засобів діагностики та візуалізації, пристроїв, що носяться, імплантатів, високопродуктивних скринінгових платформ тощо, з використанням біологічних, небіологічних, біоміметичних або гібридних матеріалів. Багато з цих розробок починають перетворюватися на життєздатні клінічні продукти.

Resume. The design and use of materials in the nanoscale size range for addressing medical and health-related issues continues to receive increasing interest. Research in nanomedicine spans a multitude of areas, including drug delivery, vaccine development, antibacterial, diagnosis and imaging tools, wearable devices, implants, high-throughput screening platforms, etc. using biological, nonbiological, biomimetic, or hybrid materials. Many of these developments are starting to be translated into viable clinical products.

Keywords: nanoparticles, laboratory diagnostics, nanodiagnostics, biomarkers, biofunctional polymers.

Introduction. Nanomedicine differs from other types of medicine in that it involves the development and application of materials and technologies with nanometer length scales to function in all the ways described below [1]. Nanoparticles (NPs) are key components of nanomedicine, and currently, a large variety of nanoparticle types exist. However, no standardized nomenclature exists in the literature; therefore, terms such as engineered nanomaterials, nonbiological complex drugs (NBCDs), nanomedicals/nanomedicines, etc. are used freely. Many nanomaterials can replicate some functions of globular biological macromolecules [2]. Examples are lipid micelles [3], different polymeric nanostructures [4], protein constructs [5], ribonucleic acid (RNA) NPs (RNPs) [6] etc. Many of these nanoscale materials have unique size- and shape-

dependent optical, electronic, and magnetic properties, and these properties are dependent upon methods to synthesize, to purify, and to characterize them [7].

Despite the need to standardize characterization methods, NPs are expected to improve the detection and diagnosis of diseases. First, smart NPs can be designed to provide contrast at the zone of interest and report information about the local environment after administration into the body. This information can aid in imaging the anatomical fine structures of organs and labeling tissues with certain markers and enables local readout of the concentrations of molecules of interest, which helps to analyze diseases directly inside the human body. Second, NPs are key components of many high-throughput diagnostics machines that can analyze extracted samples (such as blood, tissue, etc.) outside of the body for rapidly detecting biological makers and molecular alterations. The ability to analyze multiple biomarkers simultaneously may improve diagnostic precision. Moreover, multifunctional or theranostic NPs that can simultaneously diagnose, treat, and even monitor therapeutic efficacy are being engineered [8].

Aim. Analyze literature data on the specific application of materials or systems with nanoscale properties for in vitro diagnostics.

Methods. A literature search was conducted in Google Scholar, PubMed, Scopus and Web of Science databases using keywords.

Results. Nanoparticles can also be used for detection of molecules, cells, and tissues outside the human body. In this diagnostic application, the function of the NP is to identify unique biological molecules in biological fluids that are associated with the health of the patient. The NPs act as transducers and are coated with ligands to enable the biorecognition of unique biological molecules in the fluid in the in vitro sensing applications. For example, AuNPs have been modified with ligands that specifically bind to a complementary protein. The presence of these proteins induces the cross-linking of the NPs. This controlled agglomeration can be observed colometrically by the change of color of the NP solution. These concepts have been later refined, for example in rapid colorimetric DNA sensing. This AuNP-based diagnostic technology has advanced to testing of patient samples and is now used in the clinic [9]. Nanotechnology presents an opportunity to improve the overall diagnostic process by lowering the limit of detection, thus enabling high throughput and multiplexed detections of biological targets with high sensitivity.

Quantum dots are frequently used as fluorescence labels in proteins or nucleic acid assays. One example in this direction is a QD-based fluorescence polarization assay for screening of antigen surface epitopes [10]. In this example, a method for quickly screening and identifying dominant B cell epitopes was developed using hepatitis B virus (HBV) surface antigen as a target. Eleven amino acid fragments from the HBV surface antigen were synthesized by 9-fluorenylmethoxy carbonyl solid-phase peptide synthesis strategy, and then CdTe QDs were used to label the N-terminals of all peptides. After optimizing the factors for this fluorescence polarization (FP) immunoassay, the antigenicities of synthetic peptides were determined by analyzing the recognition and combination of peptides and standard antibody samples. The results of the FP assays confirmed that 10 of the 11 synthetic peptides had distinct antigenicities. In order

to screen dominant antigenic peptides, the FP assays were carried out to investigate the antibodies against the 10 synthetic peptides of the HBV surface antigen in 159 samples of anti-HBV surface antigen-positive antiserum. The results showed that 3 of the 10 antigenic peptides might be immunodominant, because the antibodies against them existed more widely among the samples and their antibody titers were higher than those of other peptides. Using three dominant antigenic peptides, serum samples were detected for HBV infection by the FP assays. The results showed that the antibody-positive ratio was 51.9%, and the sensitivity and specificity were 84.3% and 98.2%, respectively. This QD-based FP assay is a simple, rapid, and convenient method for determining immunodominant antigenic peptides and has potential in applications such as epitope mapping, vaccine designing, or clinical disease diagnosis.

Quantum dots can also be applied for the detection of dihydronicotinamide adenine dinucleotide (NADH). CdSe/ZnS QDs were functionalized with a monolayer of Nile Blue (NB^+), which quenches the fluorescence via an energy transfer mechanism. Freeman et al. demonstrated that NADH, a cofactor generated via the metabolic Krebs cycle, induced reduction of NB^+ to the colorless NBH reduced state, which resulted in the recovery of QD fluorescence. The metabolism of HeLa cells can be inhibited by anticancer drugs, e.g., taxol, which substantially lowers the intracellular NADH concentration to yield low fluorescence. This latter example highlights the potential use of intracellular QDs biosensors for drug screening applications [11].

Researchers developed bifunctional polymer NPs that acted as optrodes to probe intracellular levels of H_2O_2. They modified PEG hydrogel nanospheres (250-350 nm) with horseradish peroxidase (HRP) and the Amplex Red transducer. In the presence of stress-induced and intracellular formation of H_2O_2, the HRP-catalyzed oxidation of Amplex Red proceeds, yielding the fluorescent resorufin transducer.186 The hydrogel NPs were introduced into macrophages, and these responded to exogenous H_2O_2 or endogenous peroxide stimulated by lipopolysaccharides. Similarly, a nanosensor capable of monitoring intracellular glucose levels. Polyacrylamide NPs were implemented as a carrying matrix for glucose oxidase, GOx, and the Ru(II)-tris-bipyridine, $Ru(bpy)_3^{2+}$, transducer. While the $Ru(bpy)_3^{2+}$ fluorescence is quenched by intracellular O_2 levels, the O_2-driven GOx-catalyzed oxidation of glucose depletes the O_2 levels, resulting in the triggered-on fluorescence of the $Ru(bpy)_3^{2+}$ luminescent probe. As the degree of O_2 depletion by the biocatalytic process is controlled by the concentration of glucose, the resulting fluorescence of the transducer provided a quantitative measure for the concentration of glucose [12]. The use of composite polymer NPs carrying enzymes and optical transducers as bifunctional sensing elements is particularly attractive since the products generated by many enzyme-driven processes may activate optical (fluorescent) transducers. The use of such intracellular nanosensors should be implemented with caution, however, since intracellular environmental conditions, e.g., changes in pH, might alter the enzyme activities, thus perturbing the intracellular nanosensor performances.

One of the emerging techniques related to plasmon resonance that demonstrated high sensitivity and versatility is the chiroplasmonic method developed by Kotov

and Xu [13, 14]. This technique is based on the giant polarization rotation characteristic of nanoscale assemblies highly polarizable metallic nanoparticles. The chiroptical effects in these structures are several orders of magnitude higher than in small organic molecules due to high polarizability of the inorganic nanomaterials and larger dimension [15]. Notably, the physics of chiroplasmonic detection differs from that of red-blue plasmon coupling assays or screening with Raman scattering based (see below). Polarization rotation in NP assemblies is primarily based on interactions of the electromagnetic field with asymmetric nanostructures rather than on the formation of so-called "hot spots" or plasmon coupling. This difference is essential for biomedical diagnostics because it enables detection of long strands of DNA and large proteins. For instance, translation-inspiring sensitivity was obtained for prostate specific antigen using this method [16]. Most recently, this method in combination with UCNPs also enabled dual detection of one of the most promising diagnostic targets micro RNAs (miRNA) at the levels sufficient for its application in cancer diagnostics [17].

Nanoenabled tools can be designed for diagnostics and therapies based on the mechanical properties of cells [18]. At the subcellular and cellular levels, many biophysical properties have recently emerged as indicators of cell physiology and pathology, as complementary or regulatory alternatives for disease development. Cell migration, for example, can be traced by removing the trail of adherent cells left on a substrate coated with AuNPs or with QDs. The morphology of the trails correlates with the metastatic potential of the cells. The force that adherent cells exert on a substrate can also be measured. For example, cell-traction-force microscopy based on the deformation of the polymer substrates on which cells are grown is able to spatiotemporally map cell traction force as precise as a nanonewton [19]. It can also be used to uncover previously hidden details of drug–cell interactions and mechanical contributions during cell migration [20]. For example, previously retarded cell migration upon NP uptake was attributed to cytoskeleton disruption. However, the uptake of the NPs actually transforms cells from the motile phenotype into an adhesive phenotype, as revealed by the increased cell traction force and altered patterns of cell traction force. More importantly, ultrasensitive cell traction force microscopy could be competitive to traditional cell biology methods such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, WB, and flow cytometry, since early changes can be readily observed under cell-traction-force microscopy [21].

Proteins can themselves form the basis of another class of drug carriers. The abundant plasma protein, serum albumin, has been successfully converted into a drug transporter and was launched to the market for delivering the anticancer drug paclitaxel. Serum albumin is a nontoxic, nonantigenic, and biodegradable material. In fact, albumins are excellent charge-reversible materials. Albumins have characteristic isoelectric points, due to the existence of both carboxylic acid and amino groups. When the environmental pH is higher than the pI, albumins have more negative charge on their surface, otherwise albumins are more positively charged. In addition, the pI of albumins can be adjusted by changing the ratio between the carboxylic acid and amino groups through chemical modifications. By tuning charge, albumin NPs have been successfully used to

deliver negatively charged nucleic acids as biomolecules for gene therapy [22]. Recent approaches broadened the applicability of this successful platform and there is further development of novel features that aims to advance intelligent drug delivery. Boronic-acid-rich albumin NPs have been equipped with targeting peptides cRGD on the surface and DOX encapsulated inside the NPs. The boronic acid groups enhance the recognition of NPs to cancer cells, since the boronic acids reversibly interact with overexpressed sialic acid residues. Encouraged by the success of albumin NPs, other endogenous proteins are being investigated such as milk casein, or the iron-transport protein ferritin [23]. Proteins in their denatured form reproducibly enable the attachment of PEG chains as well as drug molecules to improve circulation times and solubility. In this way, polypeptide-PEG copolymers of human serum albumin have been obtained that can form small micelles. These micelles were able to transport and to release multiple copies of the drug DOX in leukemia cells via a two-step release mechanism [24].

Similar to protein carriers, ribonucleic acid (RNA) nanotechnology has emerged as a potential platform for drug delivery applications. This use is largely due to the ability to design structures with high thermodynamic stability, and favorable and distinctive in vivo attributes [25]. RNA can act both as a delivery vehicle as well as an active therapeutic agent. As an example, Qiu et al. combined the bacteriophage phi29 DNA packaging motor pRNA three-way junction with folic acid, a near-infrared (NIR) marker, and BRCAA1 (breast cancer associated antigen 1) siRNA [26]. These RNA NPs comprise functionality for targeting, imaging, delivery, gene silencing, and regression of gastric cancer. In vitro assays revealed that the RNA NPs specifically bind to gastric cancer MGC803 cells. The siRNA incorporated in the RNA NPs thereby significantly silences the BRCAA1 gene. The apoptosis of gastric cancer cells was observed as a result of silencing the antiapoptosis factor BCl-2 and up-regulation of the proapoptosis factor Rb and Bax expression. Animal trials using a gastric tumor-bearing nude mice model confirmed that these RNA NPs could be used to image gastric cancer in vivo, while showing little accumulation in crucial organs and tissues several hours postsystemic injection. The growth of the gastric tumor noticeably decreased during the course of treatment. No damage to important organs by the RNA NPs was detected. All results show that RNA nanotechnology can overcome some conventional cancer therapeutic limitations and open new opportunities for specific delivery of therapeutics such as siRNA, miRNA, and chemotherapeutic drugs to stomach cancer. In recent work, siRNA attached to glucose-modified AuNPs were applied in vitro to a luciferase-CMT/167 adenocarcinoma cancer cell line and in vivo to the lungs of B6 albino mice [27]. The siRNA-bearing NPs induced the expression of pro-apoptotic proteins such as Fas/CD95 and caspases 3 and 9 in CMT/167 adenocarcinoma cells in a dose-dependent manner, independent of the inflammatory response. Moreover, in vivo pulmonary delivered siRNA-bearing NPs were capable of targeting c-Myc gene expression (a crucial regulator of cell proliferation and apoptosis) via in vivo RNA interference (RNAi) in tumor tissue. This led to an ~80% reduction in tumor size without associated inflammation. Overall, RNA NPs can improve therapeutic efficacy while reducing toxicity and side effects and therefore provide an avenue for use in clinical tumor therapy in the near future.

Conclusions. New NPs tuned for nanomedicine applications are emerging, especially in the fields of drug delivery, diagnostics, and cancer therapies. Recent studies have shown that the use of multiple nanomaterials (i.e., NDs and proteins) or a single nanoplatform functionalized with several therapeutic agents can successfully image and treat tumors with improved efficacy. NPs-based drug delivery has been included in the rational, biomimetic, and systematic design of optimal therapeutic combinations. Nanomedicine in cellular, preclinical, and clinical studies has led to many important advances, both fundamental and translational. Many of these advances, however, have been in the field of cancer diagnosis and treatment. This disproportionate focus is expected to be addressed in upcoming years with research focuses expanding to other medical challenges such as antibiotic resistance and artificial organs. Nanomedicine is poised to be of benefit in these areas by virtue of the versatility of the nanomaterial platform design, be it through multimodal therapeutic approaches or through highly specialized multifaceted design for relevant biological applications.

Although nanomedicine has raised exciting expectations for many medical problems, scientific challenges have arisen as well, mainly due to the lack of knowledge about the behavior of nanomaterials inside living organisms. However, due to the basic research focused on these issues, we are now closer to solving them and to reaching "real" medical solutions based on nanomedicine.

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VALIDATION OF PHARMACOPHORE MODELS IN VIRTUAL SCREENING OF BIOLOGICALLY ACTIVE MOLECULES

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Introduction. *In silico* screening of biologically active molecules capable of certain therapeutic effects is performed by analyzing the 3D structure and interaction maps of active molecules (ligands) and biological targets (receptors). Using a pharmacophore model allows for pre-filtering ultra-large chemical libraries and selecting hit molecules that meet the 3D pharmacophore parameters. However, one of the problems of pharmacophore-based virtual screening is the essential level of “false positive” results when identified hit ligands might not be biologically active. It is due to a lack of understanding of the specific ligand-receptor interactions and the quality of a pharmacophore model.

Therefore, to build a relevant pharmacophore model of the dual inhibitor of key coronavirus SARS-CoV-2 proteases (main Mpro and papain-like protease PL^{pro}), we used a combined pharmaco-informatic approach based on the 3D structures of the ligand-receptor complex from the Protein Data Bank, activity databases of available inhibitors, and information on specific interactions SARS-CoV-2 proteases with a substrate in physiological conditions. Finally, we validated the developed approach for M^{pro} and PL^{pro} receptors.

Goal. Our study aimed to construct a relevant pharmacophore model of the dual-acting inhibitors of SARS-CoV-2 M^{pro} and PL^{pro} proteases and validate it by benchmarking its predictive ability.

Materials and Methods. The LigandScout program suite [1] was used for constructing pharmacophores, which automatically generates 3D pharmacophore models taking into account six pharmacophore characteristics: hydrogen bond acceptor (A), hydrogen bond donor (D), aromatic rings (R), hydrophobic groups (H), posi-