REVIEW

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In vitro and *in vivo* applications of 3D dendritic gold nanostructures

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Abstract

Our review focuses on a new class of materials – three-dimensional (3D) dendritic gold nanostructures – and their potential *in vivo* and *in vitro* application. To better understand this class of materials and its properties, an overview on dendrimers and gold nanoparticles is needed. Dendrimers are known for their biomimetic properties and they are often compared with proteins due to their globular shape. Dendritic structures make excellent drug delivery systems and their ability to detect or target specific cells and to release active agents in a controlled manner when using adequate functionalization was already proven. Moreover, dendrimers can envelop other functionalized nanoparticles and create even more efficient targeting and release systems. Gold nanoparticles are already being used in the biomedical field in applications like sensing, photodynamic therapy, therapeutic agent delivery and diagnostics. Their high applicability is due to their optical properties, also called quantum size effect, given by the interaction between light and electrons onto the surface of the gold nanoparticles. There are three main types of gold dendritic structures: gold–dendrimer nanocomposites, dendrimer-entrapped nanoparticles (DENPs) and gold monocrystalline dendritic growths. Gold nanoparticles (AuNPs)–dendrimer structures combine the therapeutic properties of AuNPs with dendrimer's reactivity and biological membrane crossing ability.

Keywords: dendrimers, gold nanoparticles, gold dendritic nanostructures, gold–dendrimer nanocomposites, dendrimer-entrapped nanoparticles, gold monocrystalline dendritic growths.

Introduction

With the development of nanotechnology and nanomaterials, the medical field, especially prevention, diagnosis and treatment have suffered a radical shift for the better. Nanomedicine is a newly coined term that hides multifarious possibilities. The scientific community has been focusing its attention into finding new cures or new prevention methods by using the unique properties nanomaterials have to offer. This review tries to shed light on the current trends in researching gold dendritic structures with possible applications in medicine.

Dendrimers have unique qualities that offer them biomimetic properties. They are often compared with proteins due to their globular shape. However, proteins are more compact than dendrimers and these macromolecules are not as tightly packed as proteins [1]. Dendrimers are ideal for drug delivery systems that are able to detect or target specific cells and release active agents in a controlled manner. Moreover, dendrimers can envelop other functionalized nanoparticles and create more efficient targeting and release systems.

Gold nanoparticles are the most advantageous metallic structures due to their applications, such as catalysis, biology, sensing, photodynamic therapy, therapeutic agent delivery, diagnostics and electronics [2, 3]. This high applicability is due to their optical properties, also called quantum size effect, given by the interaction between light and electrons onto the surface of the gold nanoparticles [3].

When referring to gold dendritic structures, we have three main types of materials: gold–dendrimer nanocomposites, dendrimer entrapped nanoparticles and gold monocrystalline dendritic growths. Gold nanoparticles (AuNPs)–dendrimer structures combine the therapeutic properties of AuNPs with dendrimer's reactivity and biological membrane crossing ability.

In order to understand the potential applications of these complex three-dimensional (3D) structures, it is wise to first analyze the two components separately.

Dendrimers

Dendritic materials are divided into four major subclasses: dendrimers, dendrons, dendrigraft polymers and

random hyperbranched polymers [4]. Dendrimeric architecture breaks down to three main components: central core – multifunctional, molecules can be trapped at this level, branched units and external capping-groups, which are used for functionalization. Also, the repeating monomer units (branching units) determine the generation of the dendrimer.

One of the major issues in using a novel type of nanomaterial in biomedical applications relates to their biocompatible properties, as these materials have to be nontoxic and non-immunogenic. The cytotoxic character of dendrimers is dependant on their chemical structure. The major factor that has a strong influence is the terminal groups; according to *in vitro* testing results, polyethylene glycol (PEG) and anionic dendrimers are more cytotoxic than cationic dendritic complexes. The positive charge has a destabilization effect on cell membrane, may lead to cellular lysis and have unwanted interaction with blood cells [5–8].

Studies performed to investigate cytotoxicity were made. One study using 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine liposome and human nasopharynx carcinoma (KB) and rat embryo fibroblast (Rat2) cells in culture done on cationic polyamidoamine (PAMAM) dendrimers showed that higher toxicity is met for high generations dendrimers. One possible explanation is that these types of dendrimer have higher surface to interact with cells and thus to induce cell death and membrane lysis [4, 9]. Also, significant toxicity was found in another study using human intestinal adenocarcinoma (CACO-2) cells [10, 11]. PAMAM dendrimers with amino terminal groups show less toxicity than flexible linear amino-polymers, as the globular-like structure presents more rigidity and thus it is less likely to adhere to the cell's surface. Similar results were found for amine terminated polypropylenimine (PPI) dendrimers [12]. However, *in vivo* studies performed on mice showed that amino-terminated PAMAM dendrimers do not present cytotoxicity [13, 14]. For polyester dendrimers, the cell growth inhibition was present at 40 mg/mL, but no tendency for cell apoptosis was present. Upon intraperitoneal injection into mice, there were no signs for acute and chronic toxicity [15]. The hemolytic effect for PAMAM and PPI dendrimers was studied and a substantial decrease was observed in the contents of RBCs (red blood cells), Hb (hemoglobin) and MCH (mean corpuscular hemoglobin). A significant increase was present to the quantity of WBCs (white blood cells) and Hct (hematocrit) comparison between cationic and control samples showed a considerable difference. Positive charged dendrimers exhibit impairments in the hematological component [16].

The immunogenicity investigations on mice found no proof of immunogenic effect or very weak immunogenic response for cationic dendrimers. When testing multiple peptide dendrimers on BALB/c mice models with enzyme-linked immunosorbent assay for antibody monitoring, no humoral immune response was detected. This may translate to dendrimers being regarded as "pseudo-native" for the host immune system [14].

Two approaches could be used to reduce the cytoxicity and hemolytic effects of dendrimers. The first one is to use biodegradable molecules embedded in dendrimers. Biological dendrimers can be synthesized by selecting monomers that can be metabolically processed *via* biological pathways, *i.e.*, lactic acid, glycerol, succinic acid, PEG, amino acids (lysine, arginine, etc.) [17–22]. The second approach is terminal group modification with natural or anionic moieties and thus decreasing their charge toward neutral. The advantage of this method to reduce toxicity is that functionalization agents can be chosen having in mind future applications for dendrimers, *e.g.*, improving the drug release control for a dendritic drug delivery system [23–27]. Amidation of terminal groups showed an enhancing effect on biocompatibility. Dendrimer PEGylation was proved to enhance the functional and biocompatible properties of drug delivery systems (DDS). Loading efficiency and the stability of drug entrapment inside dendrimers were enhanced with the use of hydrophilic PEG chains. Also, targeting specific tissue with a PEG-modified dendrimer can be increased due to the increase in blood circulation efficiency [28]. *In vivo* circulating, long-lived star dendrimers that are able to accumulate in the targeted tissue could be synthesized by combining dendrimers on the terminals of three-arm star PEG [29]. PEG chains used in drug carrier design can prevent macrophage and white cell recognition of the system during blood circulating time. A study using normal and tumor-bearing mice on G6-lysine dendrimer and PEGylated derivatives proved that while the unmodified dendrimer showed rapid clearance from blood stream and non-specific accumulation in the liver and kidney, the PEGylated ones were accumulated in the tumor tissue [30].

Drug delivery systems should meet a number of criteria to be deemed as efficient: *e.g.*, easy control over the size and shape of the "cargo" space, biocompatibility, high-drug loading capacity, non-immunogenicity, adequate biological response (cell adhesion, internalization, etc.), modifiable surface, biodegradation/bioelimination, controlled drug release, etc. [1].

Dendrimers are particularly suited to carry hydrophobic drugs and strong hydrophobic interactions are present between these molecules and dendrimers – the tertiary amines of PPI and PAMAM dendrimers have a lone pair that acts as hydrogen acceptor [31]. 10HCPT (10-hydroxycamptothecin) is an anticancer drug that was successfully encapsulated in dendrimers based on glycerol and succinic acid [32, 33]. Silver acetate embedded in PAMAM dendrimers improved the antimicrobial activity as the release rate of silver ions was slowed and the anti-inflammatory properties of silver nanoparticles were improved when they were loaded into dendrimers. Also, wound healing was promoted [34, 35]. One parameter that can be used to control the macromolecular functionality and the release rate for dendrimer complexes is the type of linker used between host-dendrimer and guest-molecules. For example, *cis*-aconityl-linked doxorubicin (DOX)–dendrimer conjugate displayed successful uptake and DOX release from lysosomes in cancer cells [36]. A nanovector for cancer therapy has been synthesized from a G5 acylated PAMAM dendrimer conjugated to glycidol, folic acid as targeting agent, fluorescein isothiocyanate (FITC) as fluorescent moiety and methotrexate as the active agent. Methotrexate is an antimetabolite used in cancer treatment and autoimmune diseases. An ester linker was used which is sensitive to acid pH and is hydrolyzed in lysosomes,

releasing the drug. *In vitro* studies proved that the dendrimer-drug conjugate was highly selective to KB cells and that the efficiency of the treatment has increased in comparison to free methotrexate [37–39].

Dendrimers designed to hold compounds like gadolinium salts can be used in imaging applications [40, 41]. Small molecule formulation have no tissue specificity, are rapidly excreted and a high dose is required, which makes the administration of dendrimer-based contrast agent more efficient.

New vaccination strategies can be developed using dendrimers. There have been reported studies where they were used as nanovectors for antigens or T-cell helper epitopes [42–44]. Amino-terminated PAMAM and PPI dendrimers show promise in nucleic acid compaction and cell transfection. Their polycationic character helps in forming dendriplexes with polyanionic nucleic acids – dendrimer generation, N:P ratio, pH, solvent type are few of the parameters used to control the stability and morphology of these complexes [45–49].

The rise in drug resistance of microorganism has created the need for alternative antimicrobial therapy. Cationic and amphiphilic dendrimer show potential in this field to their cell disrupting properties which may lead to cell lysis, even cell death: *e.g.*, quaternary ammonium dendrimers are very potent biocides, nitric oxide-releasing dendrimers have shown great results against Gram-positive and Gram-negative pathogenic microorganism [50], polylysine dendrimers with sulfonated naphthyl groups show promise in preventing bacterial vaginosis and transmission of sexually-transmitted diseases (STDs) like genital herpes (HSV-2 – *Herpes simplex* virus-2), HIV (human immunodeficiency virus), HPV (human papilloma virus) [13, 51, 52].

Gold nanoparticles

There are different approaches in synthesis of gold nanoparticles in both aqueous and non-polar organic solutions, and are obtained in the range of 1 to 100 nanometers with various shapes [53]. The most common synthetic route to obtain gold nanoparticles implies the reduction of a gold salt in a solution by various reducing agents in the presence of a stabilizer [54, 55]. Also, gold nanospheres can be easily prepared by reduction of auric acid with sodium citrate, and size can be varied by changing the sodium citrate concentration [56]. This form is very stable and has the advantage that the surface of the gold nanoparticle can be functionalized with various ligands through covalent and non-covalent interactions [56].

We will discuss the most recent applications in medical field and *in vivo* interactions of gold nanoparticles, results from their unique optical properties, mainly in cancer diagnostics and therapy [56]. Nanometric gold particles present different properties, as against bulk gold because of quantum size confinement required by nano-size conditions [55]. The central to all biomedical applications is biocompatibility, in this case biocompatibility of colloidal gold, as well as shape, size, surface chemistry and electrical charge [57].

In order to use the gold nanoparticles in biomedical applications, we need to test them on different cell cultures, so that we study the potential harmful behavior. The toxicity was observed on different compositions and sizes of gold nanoparticles, and compared with each other, ranging from negligible to severe [57]. A study from Pan *et al.* gives more detail about the role size plays. A moderate reduction of the size of nanoparticles can have a drastic effect on their biocompatibility. A decrease in size from 1.8 nm to 1.4 nm increases the cytotoxicity with a factor of four to six [58].

Studies performed by Ding *et al.* illustrate the effect of the nanoparticle's zeta potential (*i.e.*, the electric potential at the interface between nanoparticles and liquid) on cytotoxicity. The increase in positive charge was correlated with more cytotoxic nanoparticles [58].

Surface functionalization was also discovered to impact biocompatibility. Trials done by Massich *et al.* found that citrate, which is an agent commonly used to stabilize nanoparticles, had a cytotoxic effect when combined with gold nanoparticles [58]. In two studies, nanoparticles with no surface modification were used. As they were synthesized using PLAL (pulsed laser ablation in liquids) method, these nanoparticles did not require a stabilizing agent. Salmaso *et al.* found no trace of cytotoxicity for concentration of gold up to 0.74 nM, while Taylor *et al.* could only find signs of toxicity for concentrations 10^{-5} higher [59].

Another important aspect is the study of toxicity for gold nanoparticles. This subject has been investigated using zebra fish [58, 59] and chicken embryos [60, 61] in correlation with chemically derived particles as well as murine embryos [62] employing laser-generated nanoparticles. No unfavorable effects were noted, even though the presence of AuNPs inside the embryos was proven [58, 59, 62]. The impact of gold nanoparticles on embryo development is hard to be analyzed, as the currently available data is limited. The variety of compositions tested remains too narrow to provide sufficient data. Moreover, no long-term studies are available to assess the impact on embryo or fetal development. Particle translocation through the placenta is possible; therefore, the scientific community should understand that an ongoing effort is needed to study and anticipate the effects nanoparticles have on these vulnerable organisms.

Therapeutic drugs tend to disperse in the entire body when they are administered orally or intravenously [63]. The purpose of the targeted drug delivery is to deliver the therapeutics to the site of interest [62, 64]. Other goals include enhancing bioavailability and retention, controlled discharge of drugs, lengthening the period of drug circulation in the body, and with minimum side effects [64]. In order to achieve controlled drug delivery while avoiding drug interaction with surrounding normal cells, targeting utilizes molecular and cellular changes within diseased tissues under pathophysiological conditions [65]. Nanoparticle platforms are being used to control the accumulation of drug molecules or therapeutic agents into the targeted area [64]. Nanoparticles with sizes (<~100 nm) and shapes that allow them to pass through capillaries and penetrate cells have been designed and prepared [65]. Upon suitable surface modification, these particles can circulate for long time in the body undetected by the

immune system before reaching the targeted site [66]. The unique optical, magnetic, and electrical properties of nanoparticles allow to track their intracellular transport and localization [65].

The amount of *in vivo* toxicity studies on gold nanoparticles is limited. All studies have been performed using particles that were relatively similar, but the results are different. Studies using mouse models showed that, in general, for low doses smaller than 400 mg/kg there is no appreciable toxicity [66, 67], but for higher concentrations the results start to differ. The experimental setup plays an important role, as the smallest variations in parameters like frequency, route of administration, type of animal or material, etc. will have an effect on the outcome of the study. Regarding the administration route, studies where made to determine how the toxicity change [68]. The highest toxicity is revealed in the case of oral intake or intraperitoneal injection, while the least harm is present in intravenous injection [69]. There were conducted studies on mice in which is observed the influence of gold nanoparticles size and effect after intraperitoneal injection [69]. As a result, the nanoparticles with a diameter of 50–100 nm did no damage to the mouse, the nanoparticles with sizes between 8–37 nm give sever toxicity and shorts the life expectancy to 21 days [68]. This discovery demonstrates the correlation between particle properties, such as size, and the potential of gold nanoparticles to have harmful effects. The influence other characteristics of nanoparticles have (*i.e.*, surface charge) were not so far studied *in vivo*. However, in a study performed by Cho *et al.* on PEG-coated AuNPs was found that a single 850 mg/kg intravenous injection gives induces liver inflammation [68].

Gold nanoparticles seem to be ideal contrast agents for bioimaging as they are stable under observation, bind strongly to the target without affecting the target activity appreciably, and can be distinguished easily from the surroundings [69]. Large nanoparticles will not enter the cells by endocytosis, because a size of ~150 nm represents the upper limit for passage through caveolae [70]. For this reason, nanoparticles with a size between 30 and 150 nm are the most suitable for use in molecular imaging [71]. In this range, the scattered light from gold metallic nanoparticles is so intense that it enables imaging the location of individual particles with conventional optical microscopy like dark field microscopy (DFM) [71, 72]. Other optical imaging techniques, such as photothermal imaging, optical coherence tomography (OCT), photoacoustic imaging, and two-photon photoluminescence (TPPL) microscopy, use gold nanoparticles as contrasting agents as well [73]. Gold nanoparticles have also been used in electron microscopy (immunostaining), magnetic resonance imaging (MRI), and X-ray imaging (X-ray computed tomography – X-ray CT) [74]. Techniques such as immunostaining and single particle tracking are used for visualizing sub-cellular components/structures, whereas X-ray CT and MRI can be employed for imaging whole cells/organs [72].

Gold dendritic nanostructures

When referring to gold dendritic structures, we have three main types of materials: gold–dendrimer nanocomposites, dendrimer entrapped nanoparticles and gold monocrystalline dendritic growths. Currently, there are limited techniques trying to find the adequate synthesis route for fractal architectures at nanoscale while having control on the generation growth, size and stability. One way of accomplishing this is by directing the growth of gold and polymer, *i.e.*, polyaniline on gold seeds simultaneously.

Gold nanoparticles–dendrimer structures combine the therapeutic properties of AuNPs with dendrimer's reactivity and biological membrane crossing ability. One example is the use of PAMAM-coated gold nanoparticles in cancer therapy, especially in cancer photothermal therapy due to plasmon resonance the AuNPs exhibit, which converts light into heat [75].

The only concern is the human and environmental exposure risks; therefore, it is high priority to study the cytotoxic effect of these nanostructures. One study investigated the cytotoxicity of PAMAM-coated gold nanoparticles in four different models. The toxic response was determined using Neuro2A and Vero (mammalian cell lines), *Chlamydomonas reinhardtii* (a type of green algae) and *Vibrio fischeri* (a type of bacteria). For plant and animal cells, there is a different response triggered by the nanoparticles due to the rigidity of the cells, which favors the intake of smaller and more hydrophilic particles [76]. In microalgae, the cytotoxicity of gold-glycodendrimers is highly dependent to binding to the receptors on the cell wall [77]. The results found for bacteria are similar. *Escherichia coli* was found to be more sensitive to nanosized particles than *Synechocystis*. For cyanobacterial cells, exopolymeric molecules were present which trapped the NPs onto cells [78].

There are a few mechanisms that are thought to induce toxicity and the most common for PAMAM dendrimers are: membrane reduction and reactive oxygen species formation [79–81]. The main parameters that influence these effects are the number of repeated branching cycles (generation), terminal groups (number and type) and surface charge (neutral, negative, positive) [82].

For G0–AuNPs (glycodendrimer-coated gold nanoparticles), there was no toxicity observed for mammalian cell lines, but the algae and bacterial lines were sensitive to the presence of the nanoscaled particles. For the *C. reinhardtii* and *V. fischeri*, no significant discrepancies were found for EC10 and EC50 values. The effect induced by G0–AuNPs was fast and only 30 minutes were needed to see signs of toxicity. The mammalian cells have specific properties like higher membrane amphiphilicity and fluidity compared to microbial cells and this might be a good hypothesis for the different results found. Comparing the results for G0–AuNPs with PAMAM dendrimers, for mammalian cells, it was found that coating the dendrimers with gold nanoparticles significantly reduced their toxicity [83].

Gold nanoparticles entrapped in dendrimers were reported to be used in cancer cell targeting and imaging [83–85]. The advantage of using dendrimer–inorganic nanoparticles structures is that both components can be functionalized to undergo specific functions like cancer cell targeting. Dendrimer-particle complexes have a different hydrodinamic behavior than simple dendrimers and this should be taken into consideration in the func-

tionalization process. One study compared the different behavior of these two types of delivery system. The aim of the study was to observe the difference between "soft" particles and "hard" particles in interacting with cancer cells. The group of researchers used G5 PAMAM dendrimers and entrapped gold nanoparticles inside G5 dendrimers (Au-DENPs) and investigated the processes involved in cancer cell targeting and internalization *via* confocal microscopy. Both dendrimers and Au-DENPs were modified with folic acid (FA) and FITC to have similar surface moieties. The model used for experiments was KB (human epithelial carcinoma cell line) chosen for the high number of FA receptors (FARs). The results showed both types of particles had the same kinetics of targeting and internalization. The molecular dynamic models of the two structures showed that entrapped gold nanoparticles slightly increased total surface area and radius of gyration of the particles, but the folic acid's position in relation to the center of geometry shows no significant changes. This study provided precious information on future design of dendrimer–nanoparticle nanodevices for biomedical applications [86].

Gold–PAMAM complexes can be used for imaging for *in vitro* and *in vivo* experiments. For example, a gene transfection process was monitored: clusters containing DNA and dendrimers formed complexes, which underwent endocytocis after binding with cell surface. In a lipid bilayer, gold–dendrimer with positive charge easily penetrates into the polar zone of the structure. Moreover, by using radioactive gold as guest, the delivery system can be injected into the microvasculature of the tumor and accumulate into the nucleus of neoplastic cells [87].

One study has reported biosensor made from a glycodendrimer functionalized with mannose with a cystamine core and a gold nanoparticle's coated surface which allows biorecognition based on SET (surface energy transfer) process for protein–carbohydrate interactions. The SET approach can be used for quantitative detection of the binding constant of lectin Concanavalin A (Con A) labeled with fluorophore. The binding constant is 100 times higher to gold–dendrimer complexes than to glycodendrimer alone. SET sensing technique using glycodendrimer-coated gold nanoparticles could be used for cancer detection and protein microarray assays [88].

Another possible application for gold–dendrimer nanostructure may be in functional cell studies. Recently, engineered gold nanosurfaces on silicon/glass have been used in an effort to understand the adhesion-mediated environmental sensing abilities of the cells. This type of surfaces usually is made from an inert substrate like PEG, which is coated at specific molecular distances with a cell-binding ligand, *i.e.*, RGD peptide [89]. One study revealed useful information on the interactions that take place between primary human cell and these surfaces. They found the interactions were modulated by molecular spacing and SPR (surface plasmon resonance) technique was used to determine the binding behavior of the protein to the macromolecular arrays. Gold nanoparticle arrays were used as a template for nanoscale polymers. The approach used was based on sequential chemical self-assembly to create nano-patterned devices [90].

The need to develop new DNA biosensors with highly selective and sensitive detection is critical for evaluating genetic risk factors through gene sequencing analysis [91, 92]. DNA biosensors are based on three detection strategies: electrical, optical and piezoelectric transduction [93–96]. The electrochemical method to manufacture microchip based DNA bioassays has become the most attractive one due to low cost, ease of miniaturization, portability and simplicity of the technique [97]. Gold nanostructures provide new perspectives in nanoelectronics, biology and material science [2, 98, 99] and they are building blocks in novel designs of chemical and biological sensors [100–103].

Using electrodeposition method, nanoflower gold nanostructured electrods were made, which were later on used for DNA biosensors [104]. Dendritic gold nanostructure (DenG) prepared *via* electrodeposition was further used to detect DNA strands with a 1 fM sensitivity [105]. Another group of researcher made a biosensor able to detect cauliflower-like mosaic virus gene segments using flower-like gold microspheres, which were homogeneously dispersed and had a hierarchical architecture; these were synthesized using three-step electrodeposition [106].

Using one-step electrochemical method, well-defined hierarchically aloe-like gold (HAG) nanostructures were created without the need of template or surfactant. HAG crystals had a dendritic fractal morphology, hydrophilic surface and very large effective area. These structures were used to design an ultrasensitive DNA biosensor with a detection limit of 12 aM, an area of response 50 aM–1 pM, good selectivity, stability and reusability. The 3D dendritic nanostructures of HAG increased the amount of DNA immobilization. The biosensor had a "sandwich"-like structure. The first step of fabrication was the hybridization of rDNA (ribosomal DNA) with partially complementary tDNA (transferred DNA), which were later used to obtain double-strains DNA functionalized gold nanoparticles. After this, HAG electrode with cDNA (complementary DNA) immobilized on the surface was obtained through thiol–gold interactions, and finally the gold nanoparticles with double-stranded DNA were immobilized on the electrode surface. Positive merged beam (MB) ions were used as an indicator for the electrode's sensitivity and played the role of probes, as these types of ions can bind to the phosphate group in the DNA molecules through electrostatic interactions. If tDNA is not used, then the sandwich structure described cannot be obtained and the cDNA confined at the surface is not hybridized, therefore MB molecules are not easily absorbed to the electrode's surface and only a weak electrochemical signal is given [107].

Nanogold-G4 PAMAM dendrimer nanostructure with horseradish peroxidase-labeled interleukin-6 antibody (HRP–anti-IL-6) was used in the design of a stable conductometric immunosensor for a fast detection of IL-6 with high sensitivity.

The use of colloidal gold and dendrimer compound enabled direct electrochemical processes of the entrapped HRP by decreasing the electron transfer impedance. The sensor is based on one-step antibody–antigen immunoreaction between the immobilized HRP-anti-IL-6 and IL-6 in sample solutions. The sensor's detection limit was 10 pg/mL. The stability of the sensor was investigated

and it was proved that it is stable for eight days in phosphate-buffered saline (PBS) solution (pH 7) at 48^0C [108].

Interleukin-6 has a critical role in the inflammatory response and has been correlated with diseases like psoriasis, rheumatoid arthritis, cardiovascular disease, and inflammatory bowel disease [109–111]. The design of biologically relevant IL-6 behavior models may have high clinical significance and allow the functional assessment of biological responses [112, 113]. The so far data that exists relies on responses to previously encountered infections (*Herpes simplex*) and has to undergo further processing to separate the new antigens and take into account the effector arm of the response and the generation of immunological memory [114].

Conductometric sensors for biosensing devices consist of a planar glass support with interdigitated gold electrode pairs on one surface in a planar configuration [115]. The biochemical reactions in a solution will give a change in electrical resistance between two parallel electrodes and this is the principle of detection within such a biosensor [116–119].

The biocatalytic efficiency of the immobilized enzyme is inhibited by the formation of the antibody–antigen enzyme on the surface of the electrode and the conductivity of the electrolyte in the sample solution is changed. The biosensor registers this difference in electrochemical potential and translates this into signal that is used to assess the quantity of the IL-6 in the sample solution [120]. Another approach can be placing the enzyme label with the antibodies on the transducer's surface and a separation-free one-step immunoassay is obtained. The immunosensor registers a change in the signal due to the limited access between electrode–enzyme active site that is a direct consequence of the antigen–antibody reaction. Also, the use of conductometric immunosensors has other advantages like the ease in miniaturization, large-scale production [121] and the multitude of enzyme reactions that change the ionic composition of the solution due to consumption or production of charged species.

The orientation and the structural configuration of the antibodies on the surface of the sensor can be used to control the sensitivity of the immunosensor. By decreasing the steric hindrance, the detection limit of the sensor is decreased [122]. Therefore, the immobilization method used is of high importance, as it has to create highly organized structure with low steric hindrance. One of such methods can be protein-mediated assembly of nanoparticles [123]. Using the unique chemo-physical features of peptides and proteins, highly tunable nanoparticles can be obtained [124]. Also, using specifically tailored proteins as linkers, protein–nanoparticle conjugates can be obtained [119, 124, 125]. Dendrimers as self-assembled monolayers on solid surface have been widely used in biosensing application and protein micropatterning [126–128].

In the immunosensor designed based on G4 PAMAM dendrimer coated with gold nanoparticles, the nanogold was easily absorbed at the surface of the dendrimer due to large number of amine surface groups (64 amine groups) [129]. The supporting electrolyte used was KI and the immunoassay reactions (*1*) and (*2*) are described below:

Immunoreaction

$$\text{HRP–anti-IL-6} + \text{IL-6} \rightarrow \text{HRP–anti-IL-6–IL-6} \qquad (1)$$

Conductometric measurement

$$HRP_{(O)} + H_2O_2 + 2I^- + H_2O \rightarrow HRP_{®} + 2H_2O + I_2 \qquad (2)$$

To optimize the biosensing device, the parameters of critical importance in the sensor's performance – surface character, electronic transfer, pH, ionic strength and buffer capacity – were investigated. It was determined that optimal pH is neutral, which indicates that the immobilization of HRP in the electrode has not altered the microenvironment of the protein. Ionic strength and buffer capacity did not show any significant influence on the sensor's magnitude. This is another indicator that the sensor obtained has high specificity and sensitivity for the iodine generated in the enzyme reactions. The immunosensor's performance was also assessed on five real serum samples from patients. This was compared with the results of ELISA method and there were no significant differences between the two methods used [110].

The specificity of the sensor was investigating using a solution with IL-2 and IL-6. The conductometric currents were not different in comparison with the results obtained for IL-6 alone. The increase in IL-2 concentration did not lead to a significant current change. The reproducibility was evaluated using five replicate biosensors and the results proved the biosensor has a reproducible behavior [110].

This type of antibody assay can be used for diagnosis and development of therapeutic protocols for autoimmune disease. Also, by replacing the specific antibodies immobilized on the surface of the electrode, the biosensor can be used to assess other analytes, which are indicators to other diseases [110].

Conclusions

Gold dendritic nanostructures are novel nanomaterials that show great promise in biomedical applications. The research directed onto finding new synthesis method is at the beginning and the chances for new applications to emerge are great. So far, these materials have been successfully synthesized and used as drug delivery systems for cancer therapy and have great properties that make them suitable for biosensing devices.

Conflict of interests

The authors declare that they have no conflict of interests.

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