Applications of Nanoflowers in Biomedicine

Masoud Negahdary¹,² and Hossein Heli²,*

¹Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; ²Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract: Background: Nanotechnology has opened new windows for biomedical researches and treatment of diseases. Nanostructures with flower-like shapes (nanoflowers) which have exclusive morphology and properties have been interesting for many researchers.

Methods: In this review, various applications of nanoflowers in biomedical researches and patents from various aspects have been investigated and reviewed.

Results: Nanoflowers attracted serious attentions in whole biomedical fields such as cardiovascular diseases, microbiology, sensors and biosensors, biochemical and cellular studies, cancer therapy, healthcare, etc. The competitive power of nanoflowers against other in use technologies provides successful achievements in the progress of mentioned biomedical studies.

Conclusion: The use of nanoflowers in biomedicine leads to improving accuracy, reducing time to achieve the results, reducing costs, creating optimal treatment conditions as well as avoiding side effects of the treatment of specific diseases, and increasing functional strength.

Keywords: Advanced technology, biological nanomaterials, flower-like shape, nanomedicine, nanostructures, specified morphology.

1. INTRODUCTION

Today, using novel technologies in biomedical sciences is an important requirement to create optimal systems, improve efficiency, and reduce the costs [1-3]. Nanotechnology is a new science that aids in achieving many optimized systems [4-6]. One of the major goals of nanotechnology is creating structures of materials in which the arrangement of their molecules is pre-designed [2, 5]. Always, researches and studies on materials are followed in order to find products with more efficient properties [7, 8]. In this regard, the process of mechanical alloying that is one of the most advanced methods of producing nanoscaled materials and attracted the attention of a large number of researchers [9]. Mechanochemical synthesis, as one of the methods of making nanomaterials, is one of the powder processing methods that lead to the production of homogeneous nanomaterials from the primary powder mixture [10]. Severe plastic deformation technique is one of producing methods of nanostructured materials that have been widely considered in the last two decades [11]. These processes are classified according to the shape of the products; due to the severe plastic deformation against a metal, the microstructure changes are found and the metal structure will be accessible in nanoscale. The sol-gel process is a wet chemical method for the synthesis of a variety of nanostructures particularly metal oxide nanoparticles [12]. The sol-gel method is inexpensive and because this method is a low temperature technique, proper controlling on the chemical composition of the products is found [13]. Coating is one of the most important parts of surface engineering, which today is used to make nanostructures with the advancement of thin layer coatings [14]. Different methods are used to create thin layers. One of these methods is the physical vapor deposition layer [15], which is done in a vacuum condition. The chemical synthesis of nanoparticles is encompassing deposition methods of a solution containing pre-materials. This production method occurs on the basis of sediment reactions [16], thermal evaporation [17], etc. Other methods such as lithography [18], polymerization [19], etc. are also used in the production of nanomaterials. Special properties of nanostructured materials can change the predicted routes in biomedical mechanisms [20]. Up to now, nanostructured materials of different shapes have been synthesized, e.g. nanoparticle [21-23], nanoshell [24], nanoflake [25], core@shell [26, 27], nanotube [28], nanosheet [29, 30], nanofibril [31], nanorod [32], nanoshale [33], dot [34], nanoparticles [35] etc. “Nanoflowers” is a term used recently by researchers for a particular group of nanostructures that are similar to flowers in microscopic view [36, 37]. So far, applications of nanoflowers in numerous biomedical studies have been reported. These nanostructures are able to react at cellular level and within the cells, and diagnosis and treat-
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ment of diseases are possible in a perfect manner in a wide range of human and animal bodies [38-40]. In addition, applications of nanoflowers in treatment of heart diseases and cancers, and targeted drug delivery have been reported [41]. In this review, various applications of nanoflowers in biomedical studies have been summarized.

2. NANOFLOWERS FOR THE TREATMENT OF CARDIOVASCULAR DISEASES

Cardiovascular diseases are considered as the main leading cause of death in both men and women in the world [42], with a 80% death rate in low- and middle-income countries. Zinc oxide (ZnO) nanoflowers were synthesized as a therapeutic agent for cardiovascular diseases [43]. Angiogenesis is defined as the formation of new blood vessels from the former vessels [44]. This process plays an important role in various physiological processes such as embryonic development, growth, wound healing, reproduction and so on [45, 46]. Due to the proangiogenic properties of ZnO nanoflowers, they can be used for angiogenesis in ischemic patients and this aim will promote cardiac treatment with tremendous impact. The results showed that the plausible approach for angiogenesis by ZnO nanoflowers was the existence of hydrogen peroxide (H2O2) as a redox signaling molecule that had a contributory role for angiogenesis process [43]. Targeted angiogenesis is very important and considerable issue for ischemic treatment or any other angiogenesis process [47, 48]; targeted angiogenesis by nanoparticles through enhanced permeability and retention proposed by Kim et al. [49] and Patra et al. [43] has also confirmed this mechanism.

Barui et al. used ZnO nanoflowers to fabricate new blood vessels [50]. ZnO nanoflowers are prepared through zinc (II) nitrate and ammonia by a domestic microwave (MW) oven. The results confirmed that the nanoflowers can activate proangiogenic activity in various in vitro and in vivo experiments. Angiogenesis and auxiliary roles (for endothelial cell (EA.hy926 cells) movement in wound healing assays) found for ZnO nanoflowers were explained by the formation of reactive oxygen species (ROS) mechanism.

3. APPLICATION OF NANOFLOWERS IN MICROBIOLOGY

Along with the rapid development of human life, control of the harmful effects of microorganisms is inevitable. Since the launch of antibiotics and applying them in treatment of diseases, bacteria have always tried to be resistant against antibiotics [51]. By expanding nanotechnology in the past decade, good opportunities have been created for discovery of the antibacterial effects of nanostructures [52]. Antibacterial properties of nanostructures are due to the very small size and also their large surface-to-volume ratio, making it possible to interact directly with biological membranes and destroy microbial membranes [53]. The bactericidal effect of silver nanoflowers with 3, 4, and 5-fold chiral symmetries was investigated against Escherichia coli, Staphylococcus aureus, and Candida albicans [54]. The silver nanoflowers were synthesized by oblique angle deposition method that is a kind of physical vapor deposition [55]. Although antimicrobial properties of silver are not a new subject, in spite of using nanotechnology, silver nanostructures with exclusive properties have been produced [56-59]. The 5-fold symmetry-silver nanoflowers showed the most antibacterial property.

In a research, ZnO nanoflowers were produced by a low temperature hydrothermal method and three morphologies including rod flowers (length of ~870 nm), fusiform flowers (length of ~1.5 μm) and petal flowers (length of ~600 nm) were obtained (Fig. 1) [60].

Fig. (1). SEM (1 and 2), TEM (3), and High-resolution transmission electron microscopy (HRTEM) (4) image of ZnO with rod (A), fusiform (B), and petal (C) built flower morphology [60] (Permission is granted from American Chemical Society).
In a patent, nanoflowers (produced from titanium oxide, silicon oxide, zinc oxide, Al, Ag, Au, Cu, Fe, Co, Ni, Cr, Zn, Sn, Pd and Pt materials) were used as an interaction matrix in Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometric and were analyzed by Liang et al. and this analysis was also applied in microbial cultures [61]. In this patent, the attachment of nanoflowers led to detect analyze ions with MALDI mass spectrometry. The authors of this patent introduced their method for use and detect samples from animals, plants, bacteria, algae, fungi and viruses with ultra-high sensitivity.

Then, the effect of ZnO nanoflowers on the bacteria Escherichia coli and Staphylococcus aureus was investigated. The nanoflowers represented a strong antibacterial capability while the petal ZnO nanoflowers had stronger biocidal effect than the two other types of nanoflowers. In addition, fusiform nanoflowers had a stronger biocidal effect than the rod nanoflowers.

The morphology dependent antibacterial effect of nanostuctures has been explored. The antibacterial properties of the nanoparticles are due to their very small size and the high surface to volume ratio; the provided increased interaction surface area allows nanoparticles to be contacted directly with the microbial membranes and destroy them by releasing ions [62-65]. Platinum (Pt) nanoflowers with five different sizes were synthesized, and the results of antibacterial evaluation toward Pseudomonas aeruginosa showed that the nanoflowers of <3 nm are toxic for the bacterium, while those of >5 nm are compatible [66]. In a research, Titanium Dioxide (TiO2) nanoflower was chemically synthesized through a facile one-pot hydrothermal process. This nanos-structure showed 3.2 eV band gap and used as an element of an antibacterial composite (Ag/TiO2/ZnO); this nanoflower was used for fighting against Escherichia coli [67].

Copper oxide (CuO) nanoflower was used in a study as antifungal agent against Candida albicans. This nanoflower was produced via Cu foil in an alkaline solution and the size of this nanostructure was between 40 - 200 nm [68].

4. NANOFLOWERS IN FABRICATION OF SENSORS AND BIOSENSORS

Nowadays, nanostructures have been employed to fabricate many sensors and biosensors leading to valuable improvements in this area [69]. The purpose of the use of nanoflowers in the structure of biosensors is to enhance the level of immobilization of biomaterials leading to increase in the sensitivity and catalytic effects and feasibility of sensors and biosensors. In a research, Zhang et al. fabricated a DNAzyme sensor and the analyte was Pb2+ [70]. The Pb2+ is a pollution agent and toxicity with it is harmful and its detection is applicable in environmental and medical diagnoses [71].

Gold nanoflowers are mostly applicable in immunological assays (compared to the other nanoflowers) because they have higher optical extinction, better colloidal stability, larger total surface area, ability to improve the antibodies’ immobilization and strength in optical extinction. He et al. developed an immunosensor for the detection ofloxacin and they used gold nanoflower as signal enhancer [72]. In (Fig. 2), the applied elements of designed immunosensor are shown.

In (Fig. 2), gold nanoflower has provided a compatible and increased surface for specified capture of analyte. The obtained surface using the mentioned nanoflower led to estabilish a stable environment for attachment of antibodies; then, more active antibodies could be found more analyte and offer successful and specify detection behavior.

Fig. (2). Schematic elements and platform of designed related immunosensor by He et al. [72] (Permission number: 3905761265735).
Ahmed El-Said et al., tried to detect the effects of chemotherapeutic agents versus cancer cells using gold nanoflowers [73]. They could detect anticancer drugs on HepG2 cancer cells. The nanoflowers caused an enhancement in the sensitivity. Gold nanoflowers were also employed to detect aflatoxin B1 [74]. They were combined with immunochromatographic test strips, and detected aflatoxin B1 in a range of 0.5 to 25 pg mL\(^{-1}\) in rice. In a patent an acetone sensor was offered; in this research, NiO/ZnO heterostructured nanoflowers were used [75]. This patent was applied as one of semiconductor oxide gas sensors. The used nanoflowers were produced through water-bath and dipping methods. The researchers on this patent tried to improve the sensor efficiency via catalytic effects of NiO nanoparticles. In real detections, due to avoidance of the damaging effects of heat, the sensor elements were coated with an Al\(_2\)O\(_3\) ceramic pipe. The used nanoflowers were nontoxic, simple producible and enhanced sensing performance against acetone.

In another patent tungsten oxide nanoflower was prepared and used as hydrogen sensor [76]. In this patent, tungsten oxide nanoflowers were prepared as a result after the growth of tungsten powder on a substrate. These nanoflowers were used in this hydrogen sensor due to increment in the sensitivity of it. The specific increased surface area and stable structure, provides this sensor with high capability, crystallinity, response and electrical stability. In addition, the used nanoflowers in this research were produced from tungsten powder after heating and incubating methods.

In (Table 1), some other nanoflowers employed as a biosensor element were summarized.

### Table 1. Various nanoflowers that were used in biosensors.

<table>
<thead>
<tr>
<th>Row</th>
<th>Nanoflowers Type</th>
<th>Nanoflowers Production Method</th>
<th>Nanoflowers Size</th>
<th>Analyte</th>
<th>Electrochemical Detection Method</th>
<th>Detection Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pt nanoflowers</td>
<td>Electrodeposition</td>
<td>--------</td>
<td>Urea</td>
<td>Cyclic voltammetry</td>
<td>up to 20 mM</td>
<td>[77]</td>
</tr>
<tr>
<td>2</td>
<td>CuS nanoflowers</td>
<td>Chemical</td>
<td>--------</td>
<td>H(_2)O(_2) and Glucose</td>
<td>Cyclic voltammetry</td>
<td>H(_2)O(_2): (1 × 10(^{-7}) - 1 × 10(^{-6}) M, 3.64 × 10(^{-6}) μA M(^{-1}))</td>
<td>[78]</td>
</tr>
<tr>
<td>3</td>
<td>ZnO nanoflowers</td>
<td>Chemical</td>
<td>80 nm</td>
<td>Glucose</td>
<td>Cyclic voltammetry</td>
<td>5.0 × 10(^{-1}) mM to 13.15 mM</td>
<td>[79]</td>
</tr>
<tr>
<td>4</td>
<td>ZnO nanoflowers</td>
<td>Chemical</td>
<td>4 μm</td>
<td>H(_2)O(_2)</td>
<td>Cyclic voltammetry</td>
<td>6.0 to 1130 μM</td>
<td>[80]</td>
</tr>
<tr>
<td>5</td>
<td>ZnO nanoflowers</td>
<td>Electrodeposition</td>
<td>350 nm</td>
<td>H(_2)O(_2)</td>
<td>Cyclic voltammetry</td>
<td>9.9 × 10(^{-7}) to 2.9 × 10(^{-3}) M</td>
<td>[81]</td>
</tr>
<tr>
<td>6</td>
<td>CuO nanoflowers</td>
<td>Chemical</td>
<td>100 -300 nm</td>
<td>Glucose</td>
<td>Linear sweep voltammetry</td>
<td>10 μM to 5 mM</td>
<td>[82]</td>
</tr>
<tr>
<td>7</td>
<td>ZnO nanoflowers</td>
<td>Chemical</td>
<td>--------</td>
<td>H(_2)O(_2)</td>
<td>Cyclic voltammetry</td>
<td>1.5 × 10(^{-4}) to 4.5 × 10(^{-4}) M</td>
<td>[83]</td>
</tr>
<tr>
<td>8</td>
<td>MoS(_2) nanoflowers</td>
<td>Chemical</td>
<td>--------</td>
<td>Eugenol</td>
<td>Cyclic voltammetry</td>
<td>0.1 to 440 μM</td>
<td>[84]</td>
</tr>
<tr>
<td>9</td>
<td>NiO nanoflowers</td>
<td>Chemical</td>
<td>2 -3 μm</td>
<td>l-ascorbic acid</td>
<td>Cyclic voltammetry</td>
<td>0.005 to 3.5 mM</td>
<td>[85]</td>
</tr>
<tr>
<td>10</td>
<td>Graphene nanoflowers</td>
<td>Thermal growth</td>
<td>10 to 70 nm</td>
<td>H(_2)O(_2)</td>
<td>Linear sweep voltammetry</td>
<td>1 mM</td>
<td>[86]</td>
</tr>
<tr>
<td>11</td>
<td>ZnO nanoflowers</td>
<td>Electrodeposition</td>
<td>100 nm</td>
<td>DNA</td>
<td>Cyclic voltammetry</td>
<td>5-240 ng μL(^{-1})</td>
<td>[87]</td>
</tr>
<tr>
<td>12</td>
<td>Gold nanoflowers</td>
<td>Chemical</td>
<td>40-100 nm</td>
<td>H(_2)O(_2) and trichloroacetic acid (TCA)</td>
<td>Cyclic voltammetry</td>
<td>H(_2)O(_2): 1.0 to 60 μM TCA: 0.06 to 28 mM</td>
<td>[88]</td>
</tr>
<tr>
<td>13</td>
<td>CuO nanoflowers</td>
<td>Chemical</td>
<td>140 nm</td>
<td>Glucose</td>
<td>Cyclic voltammetry</td>
<td>0.5 μM to 82 μM</td>
<td>[89]</td>
</tr>
<tr>
<td>14</td>
<td>Nickel hydroxide nanoflowers (f-Ni(OH)(_2))</td>
<td>Chemical crystal growth</td>
<td>--------</td>
<td>H(_2)O(_2)</td>
<td>Cyclic voltammetry</td>
<td>0.1 to 1.1 mM</td>
<td>[90]</td>
</tr>
<tr>
<td>15</td>
<td>Mn(PO(_4))(_3) nanoflowers</td>
<td>Chemical</td>
<td>20 ± 2 μm</td>
<td>Ractopamine</td>
<td>Electrochemical impedance spectroscopy</td>
<td>0.1 to 1000 ng mL(^{-1})</td>
<td>[91]</td>
</tr>
<tr>
<td>16</td>
<td>Co(_3)O(_4) nanoflowers</td>
<td>Chemical growth</td>
<td>200 nm</td>
<td>Glucose</td>
<td>Cyclic voltammetry</td>
<td>--------</td>
<td>[92]</td>
</tr>
<tr>
<td>17</td>
<td>Ag-doped ZnO nanoflowers</td>
<td>Chemical</td>
<td>70 ± 20 nm</td>
<td>Phenylhydrazine</td>
<td>Current-voltage (I-V) technique</td>
<td>10(^{6}) M to 1 M</td>
<td>[93]</td>
</tr>
<tr>
<td>18</td>
<td>Cu(OH)(_2) nanoflowers</td>
<td>Chemical</td>
<td>&gt;100 μm</td>
<td>Glucose</td>
<td>Cyclic voltammetry</td>
<td>0 to 6 mM</td>
<td>[94]</td>
</tr>
<tr>
<td>19</td>
<td>CuO nanoflowers</td>
<td>Chemical</td>
<td>400 nm</td>
<td>Ascorbic acid</td>
<td>Cyclic voltammetry</td>
<td>0 to 40 μM</td>
<td>[95]</td>
</tr>
</tbody>
</table>

(Table 1) Contd....
5. NANOFLOWERS IN BIOCHEMICAL AND CELLULAR STUDIES

The topographic effects of ZnO nanoflowers were investigated on MC3T3-E1 osteoblast growth and osseointegration by Park et al. [97]. The nanoflowers were synthesized by hydrothermal method with a mean diameter of 250-300 nm and the length of 3-4 μm. The growth of osteoblasts was more effective, usable and applicable on ZnO nanoflowers, rather than a ZnO film.

A new sonochemical method was developed for the synthesis of bovine serum albumin (BSA) and laccase-inorganic composite nanoflowers [103]. The enzymatic activity of the nanocomposite was evaluated using epinephrine as a substrate and it obtained an increment of ~150% activity for laccase nanoflowers against free laccase with a prolonged activity. The nanocomposite was applied for colorimetric detection of epinephrine. In (Fig. 3), a schematic presentation for the synthesis procedure of the nanoflowers is shown.

Chemical conditions such as concentration of used reagents, pH of solutions, temperature in production steps, production methods and protocols and apparatus are very effective items in resulted morphology of nanoflowers; based on different aims, nanoflowers may be produced as various morphologies and in different sizes.

Yin et al. prepared a nanoflowers hybrid of calcium phosphate and α-chymotrypsin [104]. The nanoflowers introduced a new immobilized enzyme route and the enzyme activity was evaluated with N-benzoyl-L-tyrosine ethyl ester. The immobilized α-chymotrypsin in the nanoflowers structure had about 266% activity enhancement. Another nanoflowers hybrid of calcium phosphate with albumin and glucose oxidase was synthesized as a biomimetic catalyst [105]. The nanoflowers were followed Fenton’s reaction with a high-temperature resistance and opened a new approach against natural catalytic complicated enzymatic reactions.

Due to the surface-enhanced Raman scattering property of gold nanostructures, they are applicable in live cell imaging [106]. Gold nanoflowers were employed for live cell imaging in human hepatocellular carcinoma cancer cell line [107]. Nhung et al. prepared gold nanoflowers through seed-mediated growth method in a mixture with chitosan [108]. Biocompatibility of chitosan [109] and near-IR absorption of gold caused the nanoflowers to have prospect for immobilization of biomolecules, surface enhanced resonance scattering, and biomedical applications.

<table>
<thead>
<tr>
<th>Row</th>
<th>Nanoflowers Type</th>
<th>Nanoflowers Production Method</th>
<th>Nanoflowers Size</th>
<th>Analyte</th>
<th>Electrochemical Detection Method</th>
<th>Detection Range</th>
<th>References</th>
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<tbody>
<tr>
<td>20</td>
<td>Pt nanoflowers</td>
<td>Electrodeposition</td>
<td>960 nm</td>
<td>Glucose</td>
<td>Cyclic voltammetry</td>
<td>1-16 mM</td>
<td>[96]</td>
</tr>
<tr>
<td>21</td>
<td>ZnO nanoflowers</td>
<td>Liquid phase deposition technique</td>
<td>-------</td>
<td>Methanol</td>
<td>--------</td>
<td>0.5-700 ppm</td>
<td>[97]</td>
</tr>
<tr>
<td>22</td>
<td>Gold nanoflowers</td>
<td>Electrodeposition</td>
<td>50-80 nm</td>
<td>HepG2 cell</td>
<td>Cyclic voltammetry</td>
<td>200 μM</td>
<td>[73]</td>
</tr>
<tr>
<td>23</td>
<td>ZnO nanoflowers</td>
<td>Hydrothermal method</td>
<td>0.5-2.5 μm</td>
<td>Pb²⁺</td>
<td>--------</td>
<td>0.5-20 nM</td>
<td>[70]</td>
</tr>
<tr>
<td>24</td>
<td>Gold nanoflowers</td>
<td>Spherical gold seeds</td>
<td>45±5 nm</td>
<td>Ofloxacin</td>
<td>Cyclic voltammetry</td>
<td>0.26 to 25.6 ng mL⁻¹</td>
<td>[72]</td>
</tr>
<tr>
<td>25</td>
<td>Nickel oxide nanostructure</td>
<td>Chemical polyol method</td>
<td>30 nm</td>
<td>Acetylcholine</td>
<td>Cyclic voltammetry</td>
<td>0.25-5.88 mM</td>
<td>[98]</td>
</tr>
<tr>
<td>26</td>
<td>Cobalt nanoflowers</td>
<td>Chemical</td>
<td>5 μm</td>
<td>Sulfite / Nitrite</td>
<td>Cyclic voltammetry</td>
<td>Sulfite: 1.0-7.83 M M Nitrite: 0.1-2.15 M M</td>
<td>[99]</td>
</tr>
<tr>
<td>27</td>
<td>Gold nanoflowers</td>
<td>Chemical</td>
<td>-------</td>
<td>Brucella genome</td>
<td>Differential pulse voltammetry</td>
<td>1.07 × 10⁻¹⁴ to 1.07 × 10⁻¹ ng μL⁻¹</td>
<td>[100]</td>
</tr>
<tr>
<td>28</td>
<td>Nickel hydroxide nanostructure</td>
<td>Hydrothermal method</td>
<td>45 nm</td>
<td>Montelukast</td>
<td>Cyclic voltammetry</td>
<td>0.1-1.77 mM</td>
<td>[101]</td>
</tr>
<tr>
<td>29</td>
<td>Nickel oxide nanoflowers</td>
<td>Chemical</td>
<td>30 nm</td>
<td>Choline</td>
<td>Cyclic voltammetry</td>
<td>0.25-6.98 mM</td>
<td>[102]</td>
</tr>
</tbody>
</table>

**Fig. (3).** Ultrafast sonochemical protein nanoflowers synthesis [103] (Permission number: 3905840547641).
Hybrid nanoflowers of copper phosphate and different biomolecules were synthesized [110, 111]. When horseradish peroxidase was incorporated in the copper phosphate nanoflowers [110], its enzyme activity was enhanced by 300%. Fig. (4) shows scanning electron microscope (SEM) images of nanoflowers of copper phosphate/horseradish peroxidase.

On the other hand, nanoflowers of copper phosphate hybridized with albumin, laccase, α-lactalbumin, lipase and carbonic anhydrase were prepared and the catalytic efficiencies of the nanoflowers were evaluated [111]. In a patent, Thevasahayam et al. tried to apply a group of nanoflowers containing carbon, metal, metal-oxide and silica nanoflowers as an agent for detecting and/or isolating a biomolecule via a group of nanoflowers that were involved to a photolytic structure through a linker [112]. The advantages of this patent were presented as specific diagnosis of biomolecules and ability for determination of a biomolecule in a composite-biomolecule complex. The ability of this designed biosensor was followed in detecting of peptides and nucleic acids.

6. DNA NANOFLOWERS AND THEIR BIOMEDICAL APPLICATIONS

Deoxyribonucleic acid (DNA) is not only a focus element in advanced molecular biology, but also plays an important role as components or building blocks for the production of new materials and devices at the nanoscale [113]. It is a very important structure for nanotechnology applications [38]. Zhu et al. prepared a special self-assembled type of multifunctional DNA nanoflowers through rolling circle replication [114]. Assembling was done via liquid crystallization and dense packaging of building blocks, and the size of the DNA nanoflowers was adjustable by changing the assembling time. These DNA nanoflowers had a high stability against nuclease digestion and structure denaturation. A mixture of the DNA nanoflowers with sgc8 aptamer (a bioimaging agent) and doxorubicin was applied for in vivo biomedical applications.

Mei et al. prepared self-assembled DNA nanoflowers using DNA and magnesium pyrophosphate, and then applied them for targeted cancer treatment [115]. The performance of these nanoflowers (which were incorporated with various aptamers) was investigated by doxorubicin release.

7. CANCER THERAPY AND NANOFLOWERS

Now, interaction between nanotechnology, cancer biology and medical sciences has created a great revolution in detection, treatment and prevention of cancer [116, 117]; these achievements are beginning to reach the clinical applications. Rutile phase of titanium dioxide nanoflowers was synthesized by a hydrothermal method and then employed for the treatment of HeLa cells [118]. The nanoflowers led to good destruction and removal effects.

Layered molybdenum disulfide nanoflowers were used for MW thermal cancer therapy [119]. Recent researches approved that molybdenum disulfide nanoflowers have biocompatibility, high reactivity, selectivity, and can be used in vivo or in vitro [120, 121]. For further enhancement of biocompatibility and physiological status, molybdenum disulfide nanoflowers were modified with BSA. Albumin binding with molybdenum disulfide nanoflowers was performed through benzene rings and disulfides groups. Hemolysis tests indicated high biocompatibility of the nanoflowers. In addition, to investigate cell metabolic activity and cytotoxicity of the nanoflowers, the viability of human cervical cancer (Hela) cells and human hepatocellular liver carcinoma cell line (HepG2) cells was measured and it confirmed low toxicity of the nanoflowers.

Graphene quantum dots conjugated with layered prototanned titanate nanoflowers were introduced as a nanocarrier for targeted drug delivery by Zheng et al. [122]. One of the important applications of graphene is that it can transport the drugs and genes into target positions and this issue is much efficient in drug delivery for many diseases in which the absorption of the drug in the target organ is difficult.
Graphene has a very large surface area which makes it able to transfer large amounts of drugs. Since cancer cells are more acidic than normal cells, with the design of systems with reduced pH, drug release will be increased; this makes the drug be absorbed into graphene and the drug be separated from graphene in patient’s limb and will be released in the tumor sites and treatment will be limited by normal cells. This matter is particularly very important to overcome versus drug resistance in cancer cells [123]. On the other hand, layered protonated titanate has a two-dimensional structure similar to graphene and are also infinite ultrathin. Layered protonated titanate has high density for negative surface charges and is usable due to their immobilizing biomolecules, adsorbents and photocatalysts properties. The graphene-layered protonated titanate nanoflowers were applied to lysis human epidermal growth factor receptor 2 cells. These nanoflowers also improved treatment with nuclear accumulation and cancer imaging, and increased the cytotoxic effects of doxorubicin.

Gold nanoflowers with a mean diameter of 10 nm were synthesized and their position and intensity of plasmon absorption and emission peaks were adjusted at 605 nm by surface adsorption of thiolated polyethylene glycol [124]. The nanoflowers were then applied to bioimaging and cancer therapy. Gold nanoflowers were also synthesized by seed-mediated growth method and coated by a layer of silica [125]. Glass coating caused a red shift in the absorption from 684 nm to 718 nm, and this led them to be highly efficient against cancer cells by photothermal therapy with a rapid doxorubicin release. The synthesis procedure is presented in Fig. (5).

As shown in Fig. (5), gold nanoflower was used as core that coated by silica shell; this special structure applied for advanced drug loading and releasing cycle. This flower like nanostructure has provided a strong drug carrier situation that can be used as one of new targeted drug delivery techniques.

Three morphologies of ZnO nanostructures of rod flow- ers, fusiform flowers and petal flowers represented cytotoxicity against Hela cells with trivial cytotoxic effect against normal cells [60]. The petal ZnO nanoflowers showed the highest capability for Zn2+ release and stronger cytotoxic effect. In (Table 2), characteristics of nanoflowers employed in cancer treatment are summarized.

Table 2. Used nanoflowers and their properties in cancer treatment.

<table>
<thead>
<tr>
<th>Row</th>
<th>Nanoflowers Type</th>
<th>Nanoflowers Size</th>
<th>Application Type</th>
<th>Target Cancer Type</th>
<th>Method Against Target</th>
<th>Finally Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TiO2 nanoflowers</td>
<td>102 nm</td>
<td>Treatment</td>
<td>HeLa cells</td>
<td>UV light/Photo-catalysis</td>
<td>Lead to HeLa cell treatment.</td>
<td>[118]</td>
</tr>
<tr>
<td>2</td>
<td>Gold nanoflowers</td>
<td>100 nm</td>
<td>Diagnosis/ Treatment</td>
<td>4T1 tumor</td>
<td>Multimodal imaging / photothermal therapy</td>
<td>Imaging and therapeutic functionalities.</td>
<td>[126]</td>
</tr>
<tr>
<td>3</td>
<td>Iron oxide nanoflowers</td>
<td>20 nm</td>
<td>Diagnosis</td>
<td>IgG/metastasized cancer cell in the blood</td>
<td>Fluorescence microscopic / Cyclic Voltammetry</td>
<td>Successfully detected.</td>
<td>[127]</td>
</tr>
<tr>
<td>4</td>
<td>MoS2 nanoflowers</td>
<td>130 nm</td>
<td>Treatment</td>
<td>H22 tumor</td>
<td>Microwave (MW) thermal cancer therapy</td>
<td>Successfully treated.</td>
<td>[119]</td>
</tr>
</tbody>
</table>

(Table 2) Contd....
<table>
<thead>
<tr>
<th>Row</th>
<th>Nanoflowers Type</th>
<th>Nanoflowers Size</th>
<th>Application Type</th>
<th>Target Cancer Type</th>
<th>Method Against Target</th>
<th>Finally Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Bovine serum albumin (BSA) incorporated Ag nanoflowers</td>
<td>500 nm</td>
<td>Diagnosis</td>
<td>DLD-1 human colon cancer cells</td>
<td>EIS/DPV</td>
<td>Successfully detected.</td>
<td>[128]</td>
</tr>
<tr>
<td>6</td>
<td>DNA nanoflowers</td>
<td>250 nm</td>
<td>Diagnosis</td>
<td>CEM, HeLa and Ramos cancer cells</td>
<td>Confocal fluorescence images</td>
<td>Successfully detected.</td>
<td>[129]</td>
</tr>
<tr>
<td>7</td>
<td>Gold nanoflowers</td>
<td>&gt;100 nm</td>
<td>Treatment</td>
<td>HeLa cells</td>
<td>Photothermal</td>
<td>Successfully treated.</td>
<td>[130]</td>
</tr>
<tr>
<td>8</td>
<td>Bismuth sulfur (Bi2S3) nanoflowers</td>
<td>300 nm</td>
<td>Treatment</td>
<td>HeLa cells</td>
<td>Photothermal</td>
<td>Successfully treated.</td>
<td>[131]</td>
</tr>
<tr>
<td>9</td>
<td>Gold nanoflowers</td>
<td>450 nm</td>
<td>Treatment</td>
<td>HepG-2 cells</td>
<td>Photothermal</td>
<td>Successfully treated.</td>
<td>[132]</td>
</tr>
<tr>
<td>10</td>
<td>DNA nanoflowers</td>
<td>&lt;200 nm</td>
<td>Treatment</td>
<td>Leukemia and breast cancer cells</td>
<td>Cell Titer 96 cell proliferation assay (MTS assay)/ Flow cytometry</td>
<td>Useful in targeted drug delivery.</td>
<td>[114]</td>
</tr>
<tr>
<td>11</td>
<td>Quantum dots-conjugated titanate nanoflowers</td>
<td>600 nm</td>
<td>Diagnosis/Treatment</td>
<td>MCF7/HER2 cells</td>
<td>Fluorescence imaging</td>
<td>Imaging and therapeutic functionalities.</td>
<td>[122]</td>
</tr>
<tr>
<td>12</td>
<td>DNA nanoflowers</td>
<td>200 nm</td>
<td>Diagnosis/Treatment</td>
<td>CEM and HeLa cancer cells</td>
<td>UV-visible spectrometer/Leica TCS SP5 confocal microscope</td>
<td>High performance.</td>
<td>[133]</td>
</tr>
<tr>
<td>13</td>
<td>Fe3O4 nanoflowers</td>
<td>42±8 nm, 30±5 nm and 19±3 nm</td>
<td>Diagnosis</td>
<td>NIH/3T3 cells</td>
<td>MR image</td>
<td>Successfully detected.</td>
<td>[133]</td>
</tr>
<tr>
<td>14</td>
<td>Gold nanoflowers</td>
<td>310-820 nm</td>
<td>Diagnosis/Treatment</td>
<td>Human lung A549 cancer cells and mouse melanoma B16BL6 cells</td>
<td>Surface enhanced Raman scattering (SERS)</td>
<td>Imaging and therapeutic functionalities.</td>
<td>[134]</td>
</tr>
<tr>
<td>15</td>
<td>Gold nanoflowers</td>
<td>150-200 nm</td>
<td>Treatment</td>
<td>HepG2 cells</td>
<td>Photothermal</td>
<td>Successfully treated.</td>
<td>[119]</td>
</tr>
</tbody>
</table>

In a patent, Zhu et al., tried to use of gold nanoflower and nanoflower/quantum dot composite as a probe in photothermal therapy and biological targeting against cancer cells [135]. In this patent a probe introduced that was used in photothermal therapy due to killing cancer cells through a direct way. The adopted sources of light and enhancement of fluorescence intensity, provide a stronger photothermal power versus tumors. Gold nanoflower (diameter 45-150 nm) provides highest surface and contacting areas and also showed highest biocompatibility. This specific nano-probe was examined several times for in vivo cell therapy.

8. NANOFLOWERS IN HEALTHCARE

By considering the pollutants from harmful effects and damage caused by the growing population and industrial works that they create in the environment, further research in this field and finding the best and most feasible way to solve this problem is necessary [136, 137]. In a research, nickel nanoflowers were prepared by a solvothermal process and employed to remove the dyes in wastewater treatment [136]. Nickel nanoflowers showed an adsorption capacity of 36.8 mg congo red g⁻¹, and the adsorption process completed within 10 min. BSA-copper phosphate, and glucose oxidase-copper phosphate hybrid nanoflowers could decompose the pollutant organic dye rhodamine B by 97% within 4 h [102].

Pharmaceutical pollutants are one of the acute problems in modern life [138]. Drugs are widely released in the environment via drug producers, expired and added to patients’ necessity drugs and also consumed drugs by humans and animals. Zhou et al. introduced a new route to degrade and remove norfloxacin by ZnO nanoflowers prepared by a sol-gel method [139]. Hydroxyapatite nanoflowers were presented in a patent, which are applicable in clinical dentistry, orthopedics, plastic surgery, etc. these nanoflowers were very environmentally friendly [140]. Three-dimensional structure of these nanoflowers led to exhibit special functions and also these nanoflowers are biocompatible, because found in hard tissues in human body.

Controllable and simple method for the production of these nanoflowers was based on several steps such as: Using and dissolving H2N2O4P and CaH8N2O10 in HNO3 solution due to the products with calcium ion ([C] = 40-84 Mm) and phosphate ion ([C] = 24-50 Mm) individually; then urea aqueous solution was used and the calcium/phosphate ratio reached to 1.67, and pH to 3-6 respectively. In next production steps, hydrothermal reactions occurred (90-180°C) for 10-24 hours.

CONCLUSION

In this study, applications of nanostructures with special morphology (nanoflowers) were investigated in biomedical sciences. The huge progress of nanotechnology is due to special morphologies that produces special properties in nanomaterials. The indication rapid and high reactivity, specific function and low-cost production and using, have led to the extensive application of these nanostructures in various fields of biomedical sciences.
CURRENT & FUTURE DEVELOPMENTS

Nanoflowers have very useful and important applications in various fields in biomedical sciences such as diagnosis and treatment of diseases, production of novel antibacterial agents, high sensitive biosensors and other related areas. Various studies have confirmed that nanoflowers’ properties are dependent on their size and production methods. Future studies should focus on low-cost manufacturing techniques, promote faster and precise control of the production process and mastering production of nanoflowers to find these nanostructures with the desired size and production according to specific necessary needs. Along with numerous and applicable advantages of nanoflowers, the safety compliance for living organisms against nanomaterials should be considered.

LIST OF ABBREVIATIONS

BSA = Bovine Serum Albumin  
CEM = Human Acute Lymphoblastic Leukemia Cells  
CuO = Copper Oxide  
CuS = Copper Monosulfide  
CV = Cyclic Voltammetry  
DNA = Deoxyribonucleic Acid  
DPV = Differential Pulse Voltammetry  
EIS = Electrochemical Impedance Spectroscopy  
H₂O₂ = Hydrogen Peroxide  
Hela = Human Cervical Cancer Cells  
HepG2 = Human Hepatocellular Liver Carcinoma Cell Line  
HRTEM = High-resolution Transmission Electron Microscopy  
LSV = Linear Sweep Voltammetry  
MRI = Magnetic Resonance Imaging  
MW = Microwave  
NiO = Nickel(II) Oxide  
Pt = Platinum  
SEM = Scanning Electron Microscope  
SERS = Surface enhanced Raman scattering  
TCA = Trichloroacetic Acid  
TEM = Transmission Electron Microscopy  
TiO₂ = Titanium Dioxide  
UV = Ultraviolet  
ZnO = Zinc oxide

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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AUTHORS’ CONTRIBUTIONS

The manuscript’s first draft was written by Masoud Negahdary; Hossein Heli defined study subject and supervised this work; Masoud Negahdary enabled all the comments of Hossein Heli to attain final manuscript. Masoud Negahdary and Hossein Heli read and confirmed the final manuscript.

REFERENCES


