

*Opšti pregledi/
General reviews*

PHOTOACTIVE NANOPARTICLES FOR
ONCOLOGY

FOTOAKTIVNE NANOČESTICE U
ONKOLOGIJI

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Abstract

In this review, we analyze the basic physical chemistry, as well as biological consequences of visible light absorption of photoactive nanoparticles, with the focus on the subsequent effects that can be exploited for tumor treatment.

*1. DEFINITION AND PROPERTIES OF
PHOTOACTIVE NANOPARTICLES*

Fullerenes, the large carbon cage molecules represent a third carbon allotrope beside graphite and diamond [1]. The most abundant form of fullerenes is buckminsterfullerene (C₆₀) with 60 carbon atoms arranged in a spherical structure (Fig. 1). The shape of the molecule, known as truncated icosahedron, resembles that of a soccer ball, containing 12

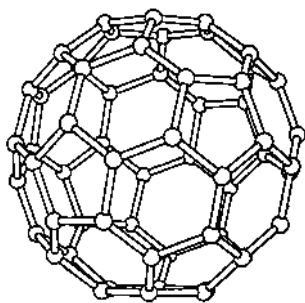


Fig. 1. The structure of C₆₀

pentagons and 20 hexagons, in which every carbon atom forms bond to three other adjacent atoms through sp² hybridization [1,2]. There are two types of bonds in the fullerene: C₅-C₅ single bonds in the pentagons and C₅-C₆ double bonds in the hexagons (Fig. 1). The unique physical and chemical features of C₆₀, the most representative member of the fullerene family, have recently incited a consider-

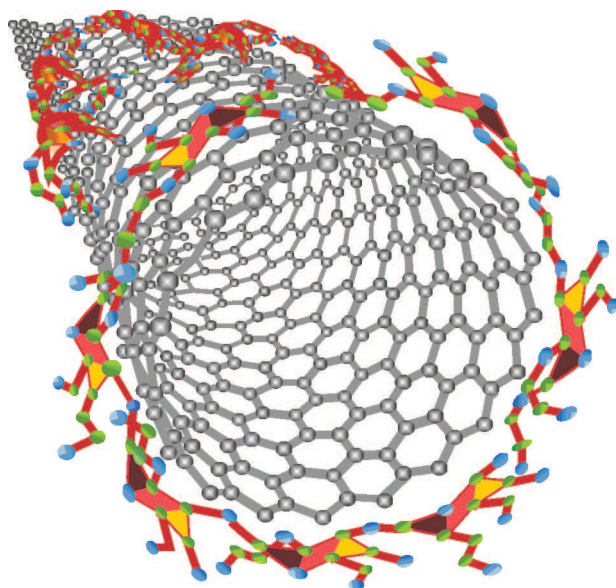
able hope of its possible use in various fields of biomedicine [3]. Many fullerene-based compounds with different biological targets have been synthesized, displaying a range of biological activities potentially useful in anticancer or antimicrobial therapy, cytoprotection, enzyme inhibition, controlled drug delivery and contrast- or radioactivity-based diagnostic imaging (reviewed in Refs. [4,5]).

One of the biologically most relevant features of C₆₀ is the ability to function as a "free radical sponge" and quench various free radicals more efficiently than conventional antioxidants [6], a property that was attributed to a delocalized π double bond system of the fullerene cage. On the other hand, illumination of C₆₀ with visible or UV light fosters its transition to a long-lived triplet excited state and the subsequent energy transfer to molecular oxygen, yielding a highly reactive singlet oxygen (¹O₂) [7,8]. Singlet oxygen and other reactive oxygen species (ROS) react with a wide range of biological targets and are known to be involved in both cellular signaling and cell damage [9]. This dual property of C₆₀ to either quench or generate cell-damaging ROS could be therefore exploited for its development as a cytoprotective or cytotoxic anticancer/antimicrobial agent.

Photodynamic therapy, matured as a feasible medical technology in the 1980s at several institutions throughout the world, is a treatment for cancer involving three key components: a photosensitizer, light, and tissue oxygen. It is an approved treatment for wet macular degeneration, and is also being investigated for treatment of psoriasis.

In the ideal scenario C₆₀ nanoparticle as photosensitizer is applied and after some time it accumulates in tumor

through EPR mechanism. Tumor tissues are known to have leaky vasculature, providing a passive accumulation of nanoparticles; this phenomenon is referred to as enhanced permeability and retention. Upon accumulation of photosezitizer in targeted tumor tissue, it is irradiated with light. This treatment induces coagulative necrosis, a form of cell death that involves protein denaturation and membrane lysis.



● carbon atom from SWCNTs



glucose unit from HEC

Fig 2. The structure of functionalized SWCNT

Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure and one or more graphene walls (single wall and multi wall CNT) [10]. Nanotubes are members of the fullerene structural family, which also includes the spherical buckyballs. The ends of a nanotube might be capped with a hemisphere of the buckyball structure. Their name is derived from their size, since the diameter of a nanotube is on the order of a few nanometers (approximately 1/50,000th of the width of a human hair), while they can be up to several millimeters in length (as of 2008). Nanotubes are categorized as single-walled nanotubes (SWCNTs) and multi-walled nanotubes (MWCNTs).

The nature of the bonding of a nanotube is described by applied quantum chemistry, specifically, orbital hybridization. The chemical bonding of nanotubes is composed entirely of sp^2 bonds, similar to those of graphite. This bonding structure, which is stronger than the sp^3 bonds found in diamonds, provides the molecules with their unique strength. Nanotubes naturally align themselves into "ropes" held together by Van der Waals forces.

CNTs have been proposed and actively explored as multipurpose innovative carriers for drug delivery and diagnostic applications [11]. Their versatile physicochemical features enable the covalent and noncovalent introduction of several pharmaceutically relevant entities and allow for rational design of novel candidate nanoscale constructs for drug development. CNTs can be functionalized with different

functional groups to carry simultaneously several moieties for targeting, imaging, and therapy.

MWCNTs release substantial vibrational energy after exposure to near-infrared radiation (NIR) [12,13]. The release of this energy within a tissue produces localized heating, which can potentially be exploited as a tumor therapy. Furthermore, because biological systems largely lack chromophores that absorb in the NIR region, lesions can be treated without the need for direct access to the tumor site. Although other nanomaterials share some of these properties [14], MWCNTs offer an excellent combination of attributes for the development of a noninvasive photothermal therapy. Thermal ablation is achieved when cells are heated above a temperature threshold, typically 55 °C [15]. Limitations of this procedure include a single point source of thermal energy that results in uneven tumor heating [16].

MWCNTs can be expected to absorb significantly more NIR radiation compared with materials such as SWCNTs, both because MWCNTs have more available electrons for absorption per particle and because, per weight, MWCNTs contain more metallic tubes than SWCNTs given that two-thirds of SWCNTs are semiconducting [17]. This will reduce the amount of NIR radiation (and consequent potential for damage to dermal layers) needed to treat embedded cancers.

What makes CNTs quite unique is their ability, to passively cross membranes of many different types of cells following a translocation mechanism that has been termed the *nanoneedle mechanism*. In that way, CNTs open innumerable possibilities for future drug discovery based on intracellular targets that have been hard to reach until today [18].

Gold Nanoshells are spherical nanoparticles with dielectric core and gold shell. Their properties can be modified by changing either the constituting materials or core-to-shell ratio [19]. The term nanoshell is used specifically because thickness of the gold shell is in the range 1–20 nm. Nanoshell materials can be synthesized practically using any material, like semiconductors, metals and insulators.

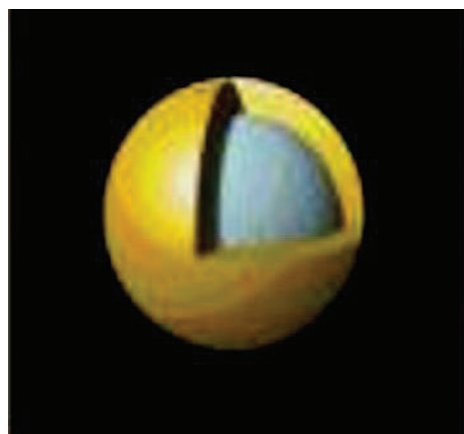


Fig. 3. The structure of gold nanoshell

Usually dielectric materials such as silica and polystyrene are commonly used as core because they are highly stable. They are chemically inert and water-soluble; therefore they can be useful in biological applications. Nanoshell particles can be synthesized in a variety of combinations such as (core-shell) dielectric-metal [20], dielectric-semiconductor [21] and dielectric-dielectric [22].

They are one of the most prominent candidates for development of photothermal cancer therapy and diagnostics in

the past several years [23]. By manipulating nanoparticle shape, researchers can tune the optical resonance of nanoshells to any wavelength of interest. At wavelengths just beyond the visible spectrum in the near-infrared (NIR), blood and tissue are maximally transmissive. When nanoshell resonances are tuned to this region of the spectrum, they become useful contrast agents in the diagnostic imaging of tumors. When illuminated, they can serve as nanoscale heat sources, photothermally inducing cell death and tumor remission. Nanoshell-based photothermal therapy in several animal models of human tumors have produced highly promising results [24].

The tunability of these nanocomplexes in the NIR region (700–900 nm) is highly advantageous since NIR light has been reported to penetrate deeply into soft tissue, nearly 10 cm through breast and 4 cm through brain tissue using microwatt laser sources [25–27]. The therapeutic response of nanoshells results from their ability to absorb NIR light resonant with the nanoshell plasmon energy and convert the light to heat [28]. The heat generated by the nanoshells raises the local temperature of tissues resulting in thermal ablation of cancer cells. Unlike current cancer treatment strategies, such as chemotherapy and radiation therapy, whose toxicity leads to deleterious side effects, these benign, nontoxic nanoshell-based complexes are far less likely to induce side effects in clinical applications.

In imaging applications, nanoshells can be tagged with specific antibodies for diseased tissues or tumors [29]. When these nanoshells are inserted in the body, they get attached to diseased cells and can be imaged. Once the tumor has been located, it is irradiated with resonance wavelength of the nanoshells. This leads to localized heating of the tumor and it is destroyed. The power required for destroying diseased cells is almost half that required to kill healthy cells.

2. ANTICANCER ACTIVITY OF PHOTOACTIVE NANOPARTICLES

The potent ability of fullerenes to photosensitize transition of molecular oxygen to highly reactive ROS makes them promising candidates for the photodynamic killing of cancer cells. The main advantage of this therapeutic approach is selectivity, achieved by tumor-specific activation of photosensitizing agent by highly focused light beam delivered to tumor region at the surface of the body or to internal tumors using optical fibers [30]. There are many studies demonstrating the efficient photodynamic action of various water-soluble C₆₀ derivatives against different types of cultured cancer cell lines (cervical, larynx, lung and colon carcinoma) and malignant tumors in vivo (reviewed in Ref. [31]). A particularly promising approach involves linkage of fullerenes with other photosensitizers, such as porphyrin, exploiting the unique photophysical and redox properties that endow these C₆₀–porphyrin dyads with extremely high capacity for ROS-mediated cytotoxicity even in the relative absence of oxygen due to tumor hypoxia [32]. The observed anticancer activity of fullerene derivatives was apparently dependent on generation of both singlet oxygen and superoxide anion [32,33], and it was inversely correlated with the extent of derivatization of the fullerene cage [33,34]. The latter is consistent with the reduction of the fullerene's ROS-generating capacity that occurs upon increasing the number

of covalently attached functional groups [35,36,37]. Moreover, a closer examination of the structure–activity relationship reveals that C₆₀ derivatives containing more potent ¹O₂-quenching groups (e.g. –OH) display lower photodynamic activity compared to those containing the same number of groups with inferior ¹O₂-quenching ability (e.g. –CH) [33]. This agrees with the assumption that overall ROS production by a C₆₀ derivative is in part determined by the ability of its functional groups to deactivate C₆₀-generated ROS (ref biomaterials). However, some sugar-pendant derivatives displayed different photodynamic efficiencies despite similar production of ¹O₂ [38], while tris-malonic acid C₆₀ was more photocytotoxic than monoadduct in spite of the higher ¹O₂ quantum yield for the latter [35].

These data suggest that, in addition to ¹O₂-producing capacity, other factors, such as degree of cell membrane incorporation and cellular uptake, might profoundly influence the phototoxicity of C₆₀-based agents.

The photodynamic antitumor action of water-soluble C₆₀ derivatives apparently involves induction of the “programmed” cell death (Type I), known as apoptosis [32,33]. This type of cell demise is characterized by activation of the caspase enzyme family and fragmentation of DNA, which occurs without plasma membrane breakdown and is followed by recognition and removal of apoptotic cell by phagocytes in the absence of inflammation [39]. This is consistent with the preferential mitochondrial localization of water-soluble C₆₀ derivatives [40,41], having in mind that ROS induced mitochondrial dysfunction is a key initial step in the “mitochondrial” pathway of apoptosis [39]. Interestingly, C₆₀(OH)_n was able to suppress proliferation and induce apoptosis of tumor cells in the absence of photosensitization and ROS production [42,43,44]. In view of the involvement of redoxsensitive transcription factors, such as NF-κB, in regulation of cell growth and apoptosis [45], these results indicate an interesting possibility that C₆₀ could exert its antiproliferative/pro-apoptotic action not only by producing cell-damaging ROS, but also through antioxidant effects.

Unlike water-soluble C₆₀ derivatives, C₆₀ nanoparticles prepared by addition of conventional surfactants (e.g. SDS, Tween) or polymers (e.g. PEG, PVP) have been only sporadically tested for their photodynamic activity against cancer. This might seem somewhat surprising, as non-derivatized C₆₀ displays higher ¹O₂ quantum yield in comparison with functionalized water-soluble derivatives, so it should be a more efficient photosensitizer. Moreover, the relatively large size of these C₆₀ nanoparticles (up to several hundreds of nm) should presumably provide high intratumor concentration through “enhanced permeability and retention” effect [46], due to abnormally large vascular pores and impaired lymphatic drainage in tumors. Indeed, PEG/C₆₀ conjugate exhibited higher accumulation and more prolonged retention in the tumor tissue than in normal tissues, showing a stronger tumorsuppressive photodynamic effect than conventional photosensitizer Photofrin [47].

Interestingly, the potent ROS-dependent anticancer activity of another nanoparticulate C₆₀ preparation, solvent exchange-prepared THF/C₆₀, was readily initiated at low-level ambient light and could not be further stimulated by

either visible or UV light [48]. The observed effect was oxidative stress-mediated and, in contrast to pro-apoptotic action of water-soluble C₆₀ derivatives, involved “accidental” cell death – necrosis [49]. This type of cell death, unlike apoptosis, is typified by vacuolation of the cytoplasm, breakdown of the plasma membrane and release of cellular contents, resulting in the induction of inflammatory response [36]. The apparent discrepancy regarding the mechanisms of cell death (necrosis vs. apoptosis) could stem from the extremely high ROS production by THF/C₆₀, leading to rapid lipid peroxidation and permeabilization of cell membrane [49], which is consistent with mainly cell membrane vs. mitochondrial accumulation of nanoparticulate vs. water-soluble C₆₀ [50]. However, some amount of THF/C₆₀ probably gained access to cell cytoplasm, as indicated by its ability to influence certain intracellular events involved in necrosis induction, such as activation of mitogen-activated protein kinases and mitochondrial depolarization [51]. In view of the immunostimulatory properties of necrotic cells and resistance of tumor cells to apoptosis, it has been proposed that necrosis might be more efficient than apoptosis in inducing tumor regression [36].

On the other hand, it is more difficult to restrict necrosis to tumors, and THF/C₆₀ was indeed highly toxic to a variety of normal mammalian cells. Nevertheless, it seems conceivable that the large size of THF/C₆₀, which could be easily controlled during preparation, might afford *in vivo* tumor-selectivity through “enhanced permeability and retention” effect. Accordingly, using mouse B16 melanoma model, we have observed that intraperitoneally injected THF/C₆₀ accumulates more in melanoma cells than in normal tissues (Trajkovic et al., unpublished data). In a different approach to selective tumor targeting with C₆₀ nanoparticles, we have demonstrated that noncytotoxic concentrations of THF/C₆₀ and anticancer cytokine tumor necrosis factor (TNF) synergize in inducing oxidative stress and death of TNF-sensitive cancer cells, without harming normal cells [52]. Moreover, it appears that THF/C₆₀, at low doses that do not trigger oxidative stress, might still affect tumor cells by inducing cell cycle arrest and autophagy (programmed cell death Type II) [53], a process of selfcannibalization during which cells digest their own proteins through a lysosomal degradation pathway [36]. While the exact mechanisms underlying these ROS-independent effects are still to be revealed, they are consistent with the ability of C₆₀ nanoparticles to gain access to cell cytoplasm, as indicated by theoretical models and demonstrated in the cellular uptake experiments [54–58]. Importantly, the observed oxidative stress-independent actions of THF/C₆₀ were apparently selective for tumor cells, leaving their nontransformed counterparts mainly unaffected [53].

In order to use CNTs for potential cancer treatment and/or imaging, targeting nanotubes to tumors is highly desirable. Both passive targeting, relying on the enhanced permeability and retention (EPR) effect of cancerous tumors, and active targeting guided by tumor targeting ligands, have been employed for various nanoparticle-based drug delivery systems.

Thus far, there are two published papers reporting *in vivo* tumor targeting by CNTs conjugated with targeting ligands. Dai et al. showed that efficient tumor targeting was

achieved by conjugating a RGD peptide which recognizes integrin $\alpha_v\beta_3$, known to be upregulated on various solid tumor cells and tumor vasculatures – to PEGylated SWNTs [59]. SWNTs with two different PEG coatings conjugated with both RGD peptide and radiolabels were intravenously injected into glioblastoma U87MG tumor-bearing mice, which were monitored by micro-positron emission tomography (micro-PET) over time [60]. RGD-conjugated SWNTs with a long PEG coating (SWNT PEG5400 RGD) exhibited a high tumor uptake of ~13% of injected dose per gram tissue (%ID/g), compared with 4%–5% ID/g obtained with plain SWNTs without RGD (SWNT PEG5400). Interestingly, authors found that efficient tumor targeting could only be realized when SWNTs were coated with long PEG but not with short PEG. The latter had short blood circulation time, and thus lower probability of being trapped in tumors or to bind the tumor receptors. Results suggest that surface functionalization of SWNTs is also important for tumor targeting *in vivo*. Another study carried out by McDevitt et al. [61] showed tumor targeting of CNTs by antibody conjugation.

The first *in vivo* cancer treatment study with CNTs was reported by Zhang et al. using positively charged SWNTs to deliver therapeutic siRNA into cancer cells [62]. However, this was a proof-of-concept study, with SWNT siRNA complexes directly injected into tumors, instead of systemic administration.

Dai et al showed that paclitaxel (PTX), a commonly used chemotherapy drug, may be conjugated to branched PEG functionalized SWNTs via a cleavable ester bond [63]. The SWNTPTX conjugate was tested in a 4T1 murine breast cancer model in mice, exhibiting improved treatment efficacy over the clinical Cremophor-based PTX formulation, Taxol. Pharmacokinetics and biodistribution studies revealed longer blood circulation half-life and higher tumor uptake of SWNT PTX than those of simple PEGylated PTX and Taxol, consistent with the observed efficacies of different PTX formulations. The high passive tumor uptake of SWNT PTX is likely due to the EPR effect. In addition, PTX molecules carried to liver and spleen by SWNTs were rapidly dissociated from nanotubes and excreted, diminishing the RES toxicity of this SWNT-based PTX formulation. Work of Dai and coworkers is the first to show that carbon nanotubes can be used for *in vivo* drug delivery for cancer therapy by systemic administration.

SWNTs have strong optical absorption in the visible and NIR range. Dai et al. and Chakravarty et al. have shown that SWNTs can be utilized as photothermal therapeutic agents to kill cancer cells [64, 65]. NIR laser irradiation was used in both cases to generate heat, causing destruction of cancer cells with specific SWNT internalization.

Beside its potential applications in therapy, the high optical absorption of SWNTs can also be utilized in photoacoustic imaging. Photoacoustic imaging, in which sounds are generated as a result of local heating by the absorption of laser light, has higher spatial resolution than traditional ultrasound, and deeper tissue penetration than fluorescence imaging [66].

In 2003, Lin et al demonstrated selective photothermal therapy using gold nanoparticle immunoconjugates [67]. Lymphocytes incubated with gold nanoparticles conjugated to antibodies and then exposed to short laser pulses (565 nm

wavelength, 20 ns duration) showed cell death with 100 laser pulses at an energy of 0.5 J/cm². The cell death is attributed mainly to the cavitation bubble formation around the nanoparticles. By adjusting the particle number, size, and laser energy, the researchers were able to selectively induce cell death or transiently modify cellular functions without causing cell destruction. In the same year, Zharov et al. [68] studied the threshold and the dynamics of thermal events around the particles incorporated into K562 cancer cells using nanosecond Nd-YAG laser at 532 nm and a photothermal contrast technique. They found that, at an energy level of 2–3 J/cm², only one or three laser pulses are sufficient to damage a cell containing 10–15 particles of 20 nm size, whereas at a lower fluence rate of 0.5 J/cm², at least 50 pulses and approximately 100 particles are required to produce the same harmful effects on the cells. Recently, El-Sayed and coworkers [69,70] demonstrated selective photothermal therapy by using gold nanoparticles with a visible continuous wave laser. In these studies, 40 nm gold nanoparticle were conjugated to anti-EGFR antibodies and then incubated with both human oral cancer cells and nonmalignant skin cells for 30 min. By using dark field light scattering imaging and surface plasmon absorption spectroscopy, it was found that gold nanoparticles were preferentially and specifically bound to the cancer cells, while only a heterogeneous non-specific distribution of the nanoparticles was seen over the healthy cells [71]. The nanoparticle-labeled cells were then exposed to a argon ion laser at 514 nm. It was found that the malignant cells required less than half the laser energy to be killed as compared to the benign cells. No photothermal destruction was observed for any of the cell types without nanoparticle labeling, even at four times the energy required to kill the malignant cells labeled with anti-EGFR/gold nanoparticle conjugates. This selective photodamage of the cancer cells is clearly attributed to the higher gold nanoparticle loading on cancer cells due to the overexpressed EGFR on the cancer cell surface. Higher gold nanoparticle labeling results in a consequently higher optical density. Thus, a lower laser energy is required to raise the temperature above the threshold for destruction, as estimated to be in the range of 70–80°C. This method can be extended to other types of

cancers as well because most types of cancer cells have an overexpression of EGFR receptors. However, the use of visible light absorbing nanospheres is restricted to skin or near-surface type cancers due to the inability of visible light to penetrate through skin and tissue.

Gold nanorods and nanoshells have been demonstrated for selective photothermal therapy using CW NIR lasers mainly by the El-Sayed [72] and Halas groups [73], respectively. By using dark-field light scattering imaging, El-Sayed and coworkers found that gold nanorods conjugated to anti-EGFR antibodies were well organized on the surface of cancer cells with relatively higher binding affinity, while they were randomly distributed nonspecifically on and around the normal cells, similar to the case of the gold nanospheres. A CW Ti:Sapphire laser with a wavelength at 800 nm, was used for the photoirradiation of the cells labeled with the nanorods. It was found that the cancer cells required half the laser energy (10 W/cm²) to be photothermally damaged as compared to the normal cells (20 W/cm²), as attributed to the selective targeting of the overexpressed EGFR on the cancer cell surface by the anti-EGFR conjugated gold nanorods. Later, Takahashi et al [74] in Japan achieved cell death using phosphatidylcholine-passivated gold nanorods and a pulsed Nd-YAG laser at 1,064 nm. Recently, Wei and coworkers at Purdue University [75] demonstrated that gold nanorods conjugated to folate ligands can be used for hyperthermic therapy of KB oral cancer cells with a CW Ti:Sapphire laser. Severe blebbing of cell membranes was observed at laser irradiation with power density as low as 30 J/cm².

3. SUMMARY

Photoactive nanostructures thus show great promise for the selective photodynamic/photothermal treatment for cancer. It is realized that a number of variables need to be further addressed, e.g., stability, biocompatibility, and chemical reactions of nanoparticle bioconjugates in physiological environments, blood retention time, tumor extravasation, the fate of the nanoparticles following therapy, etc.

Apstrakt

U ovom radu su prikazane osnovne fizičko hemijske osobine fotoaktivnih nanočestica. Aktivnosti nanočestica inicirane svetlošću prema ćelijama i tkivima su analizirane sa naglaskom na efekte koji se mogu iskoristiti u terapiji tumora.

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