

## Advances in nanomedicine for head and neck cancer

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## 1. ABSTRACT

The quality of life of patients with head and neck squamous cell carcinoma (HNSCC) has been improved because of advances in surgical and radiotherapeutic techniques as well as organ-preservation methods. Despite such progresses, survival rates are dismal because of frequent recurrences, distant metastases and the development of secondary primary tumors. Nanoparticles have distinct characteristics such as a high surface/volume ratio and surface charge and size that can be easily modified. Because of such inherent features, nanoparticles are used in imaging, adjuvant radiotherapy, and drug- or gene-delivery. Thus, nanomedicine holds great promise in the diagnosis and treatment of cancer. In the present review, we summarize recent advances in nanomedicine in the diagnosis and treatment of head and neck cancer. We first review the application of inorganic nanoparticles to photo-thermal and magneto-thermal radiotherapy. We also discuss the use of organic nanoparticles in drug- or gene-delivery during chemotherapy. We then review the application of inorganic nanoparticles as radiotherapy enhancers. Finally, we address the factors that influence the biodistribution of nanoparticles *in vivo*.

## 2. INTRODUCTION

Nanomedicine is the application of nanotechnology in the field of medicine. It uses the physical properties (electronic (1), optical (2), magnetic (3), and catalytic (4)) of nanoscale materials (1–200 nm) to improve human health. In certain aspects, nanoscale materials exhibit special physical properties only at the nanoscale. However, as the size of cellular organelles is approximately 100 to 300 nm and intracellular proteins and molecules are approximately 10 to 50 nm, materials with a size of 1–200 nm can interact in a particular manner with these organelles or molecules.

Head and neck cancers including those of the salivary glands, thyroid, mucosal lining of the oral cavity, pharynx, nasopharynx, and larynx account for 2–6% of all malignancies in the United States. In patients with head and neck squamous cell carcinoma (HNSCC), methods for early diagnosis and therapy, which would improve the survival rate, are significantly limited. Currently available imaging modalities include magnetic resonance imaging (MRI), computed tomography (CT), ultrasonography, and positron emission tomography (PET); however, these

methods require the collection of tissue samples via biopsy or needle aspiration for a definitive diagnosis. The need for sensitive and specific non-invasive molecular tests for staging, screening, and intraoperative diagnosis is therefore important. Currently available therapies consist of combination treatments that include surgery, radiation, chemotherapy, and antibody-blocking therapy. However, the high failure rate and potential organ damage associated with advanced tumors are difficult to overcome. The 5-year survival rate of patients with late stage HNSCC is low (24–33%) especially in patients with distant metastasis, underscoring the need for selective and tumor-specific drug-delivery vectors (5).

According to their chemical composition, there are two types of nanoparticles: organic and inorganic particles. Their application in cancer treatment and diagnosis is focused on three major fields: photo-thermal and magneto-thermal probes, drug- and gene-delivery vectors, and radiation enhancers. In the present review, we discuss these three fields in detail and examine the factors that influence the biodistribution of nanoparticles *in vivo*.

### 3. PHOTO-THERMAL AND MAGNETO-THERMAL PROBES

Photo-thermal and magneto-thermal probes are useful for cancer cell ablation (6). The principle of photo-thermal radiation is based on the use of a photosensitizer such as gold. Once the photosensitizer is excited by a specific wavelength band of light, it will release vibrational energy as heat to ablate the cell that has internalized it. The magnetic particle can generate heat by hysteresis loss under an alternating magnetic field (7). Gordon *et al.* proved this concept by using submicron magnetic particles to selectively destroy cancer cells under an external high-frequency or pulsed electromagnetic field with little effect on normal cells (8). Cell ablation is achieved via different mechanisms according to the temperature used: hyperthermia (42–46°C) can induce apoptosis (9), whereas thermal ablation (>46°C) induces necrosis (10). In addition to their use in cell ablation, these probes can be used for imaging. The clinical application of magnetic resonance imaging (MRI) needs a contrast agent to enhance the distinction between normal and abnormal tissues, and several gold-shelled iron oxide nanoparticles have shown favorable transverse relaxivity (11, 12).

Although these probes have shown effectiveness *in vitro*, their *in vivo* application is associated with several issues. The most important bottleneck is related to the improvement of tumor-specific biodistribution. Antibody conjugation is the preferred choice to enhance tumor-specific biodistribution. Recent studies have shown that the epidermal growth factor receptor (EGFR) is an ideal target for tumor detection because of its high expression level in many kinds of tumors. In 2011, Popovtzer *et al.* reported that the intravenous injection of anti-EGFR-conjugated gold nanoparticles (30 nm) improved the detection of human HNSCC implanted in nude mice by CT imaging compared to non-targeted gold nanoparticles (13). Meanwhile, in 2013, Gupta *et al.* used a GE11-peptide

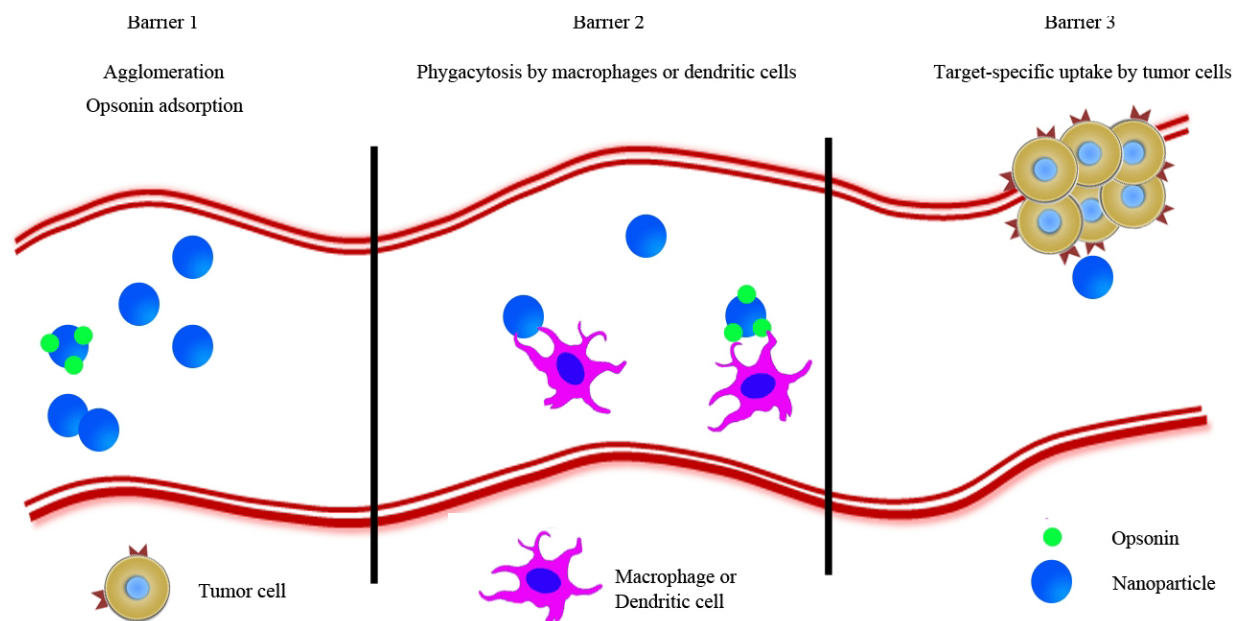
modified, polymeric micelles packaged silicon phthalocyanine-4 (Pc 4) photosensitizer to enhance cell specific ablation of EGFR-overexpressing HNSCCs in a nude mouse tumor xenograft model, and the results showed significantly higher tumor cell ablation efficiency after photodynamic therapy than that achieved with non-targeted nanoparticles (14).

### 4. DRUG- AND GENE-DELIVERY VECTORS

The development of nanovectors for drug and gene delivery is another active research field. Because anti-tumor drugs aimed at killing tumor cells can affect normal cells, the objective of drug delivery is to achieve a high concentration of anti-tumor drug in the tumor region. Although effective drugs have been developed, their specific targeted delivery remains difficult. Nanovectors hold promise as they have enhanced permeability and retention, and are small enough to pass through the blood brain barrier.

Nanovectors for drug delivery consist of inorganic and organic particles. Gold nanoparticles are often used because of their enhanced permeability and retention effects, which improve their accumulation inside tumors. However, the effectiveness of gold nanoparticles is associated with their physical characteristics (size, surface charge) and the administration strategy (injection methods, dosing). For example, in 2012, Tunnell *et al.* examined the relationship of nanoparticle physical properties and dosing strategies with accumulation efficiency. They delivered pegylated gold nanoshells (GNSs) and gold nanorods (GNRs) to tumors using single or multiple dosing strategies, and quantified the gold present in the tumor and liver by neutron activation analysis. Their results suggested that small GNRs accumulate in the tumor at higher levels than large GNSs, and a multiple dosing strategy favors both GNS and GNR accumulation in tumors compared to a single dosing strategy (15). Here, pegylation is important because this type of passive agent can avoid non-specific clearance by the reticuloendothelial system (16). A lipid nanoparticle was recently used as a drug delivery vector to treat colon adenocarcinoma and HNSCC by Peer *et al.* (17, 18). In their studies, they showed that hyaluronan-grafts can enhance the targeting of nanoparticles to CD44-overexpressing tumors.

Gene delivery also provides a strong tool for the treatment of head and neck cancer, especially with the application of recently developed siRNA interference strategies. The gene gun is one of the most frequently used methods for gene delivery. Basically, gold particles coated with a thin layer of DNA or siRNA are used for forced gene delivery into target tissues. Particles containing a DNA vaccine can be delivered into normal tissues to generate immune responses against tumors (19). Genetic materials can also be delivered into tumors. For example, tumor-targeting peptides or transferrin are used to improve the specificity and uptake of cationic liposomes to target HNSCCs (20, 21). A glucosylated polyethylenimine (PEI) nonviral vector has been used to deliver the wide-type P53 gene to HNSCC xenografts to inhibit tumor growth (22).



**Figure 1.** *In vivo* biological barriers of nanoparticles. Nanoparticle delivery by systemic administration should be designed to avoid agglomeration, opsonin adsorption, and opsonin-mediated or non-mediated phagocytosis by macrophages or dendritic cells, and designed to specifically target tumor cells by antibody or peptide conjugation.

## 5. RADIATION ENHANCERS

Gold nanoparticles are ideal as radiation enhancers because of their strong absorption properties. Gold nanoparticles can enhance local radiation doses by more than 200% in tumors (23). Tumor-bearing mice treated with gold nanoparticles have approximately 86% 1-year survival after radiation compared with the non-treated group (24). Titania nanoparticles can also be used to enhance the radiation effect. Moreover, these titania nanoparticles can have an even stronger enhancement effect when doped with rare earth elements such as lanthanides and gadolinium (25).

## 6. THE BIODISTRIBUTION OF NANOPARTICLES

The biodistribution of nanoparticles, which can affect their efficiency and toxicity, is important because of its association with the physico-chemical properties and the administration strategies of the nanoparticles.

For *in vivo* biodistribution, several biological barriers must be overcome before final entry into the target cell (summarized in Figure 1). In addition, several factors affect the biodistribution process. Particle size is an important parameter that determines the circulation and distribution within the organism. Particles larger than 10  $\mu\text{m}$  accumulate passively in the lung after IV injection because venous blood is directed to the lung from the right ventricle of the heart (26). Phagocytosis is another important factor that limits prolonged circulation of particles larger than 0.5  $\mu\text{m}$  (27). By contrast, particles smaller than 5 nm are cleared by the urinary system (28). Nanoparticles with sizes ranging from 150 to 300 nm are

mainly found in the liver and spleen, whereas smaller counterparts extravasate into the bone marrow (29). In addition to the size effect, the shape of nanoparticles also affects *in vivo* biodistribution. A recent study showed that rods are less preferentially taken up by macrophages than their spherical counterparts, which reduces their accumulation in macrophage-rich organs such as the liver and spleen. Meanwhile, gold nanorods showed higher accumulation in tumor tissues than spherical gold nanoparticles (30). The third important factor for biodistribution is the surface charge of nanoparticles. Protein coating immediately upon IV injection and clearance by macrophages occurs at higher rates in positively charged nanoparticles than in negatively charged particles of the same size and shape (31). The mostly widely used strategy to avoid this adsorption is to mask the nanoparticle surface with polymer polyethylene glycol (PEG). This hydrophilic, biocompatible, and nontoxic polymer can minimize interactions of macromolecules such as cytokines and nanoparticles with phagocytic cells of the immune system (32). However, when pegylated nanoparticles are repeatedly used by IV injection, anti-PEG antibodies can significantly diminish the protective effect of PEG (33). The administration method is another factor that can influence the biodistribution of nanoparticles. In the *in vivo* experimental model of HNSCC-bearing nude mice, two administration strategies can be used: IV injection or intra-tumor injection. Xie *et al.* reported that intratumoral administration is a better choice than systemic administration because of its higher intratumoral retention effect and low concentration in other healthy tissues (34). Another report showed that approximately 50% of nanoparticles administered systemically accumulate in organs of the reticuloendothelial system (RES) 10 min after injection, which supports that for superficial tumors such as

head and neck cancer, intra-tumor administration is a better choice than systemic administration (35).

### 7. SUMMARY AND PROSPECTS

After several decades of intensive research effort, nanotechnology is currently an active field of interdisciplinary research with a wide application in medicine. Inorganic nanoparticles are often used for imaging and for photo-thermal, magneto-thermal, and radio-enhancement therapy, whereas organic nanoparticles are commonly used for drug- or gene-delivery.

However, certain issues remain to be solved. 1) The interaction between nanoparticles and complex biological systems is still poorly understood. In vitro experiments have improved our understanding of the uptake and cellular pharmacokinetics of nanoparticles. For example, nanoparticles with a diameter of 50 nm are more efficiently internalized by cells than smaller (15–30 nm) or larger (70–240 nm) particles based on in vitro experiments (36, 37). However, when complex biological media are involved (as in systemic administration), the plasma protein adsorption effect (38), opsonization effect (39), and phagocytosis clearance effect (40) have to be considered during the design of the nanoparticles. Therefore, in vitro experiments and ex vivo models should be combined for the preliminary assessment of these effects on nanoparticles (41). 2) Nanosafety is an important issue that needs to be addressed. Although the nanoparticles used are often biocompatible and have very low acute toxicity, some materials may have long-term toxicity, in particular for organs such as the liver. For example, nanoparticles taken up as agglomerates tend to be less easily degraded by the host and can be detected in macrophages for several months (42). Ye et al. studied the long-term effects of quantum dots containing Cd-Se in rhesus monkeys and found that 90 days after injection, more than 90% of the nanoparticles remained in the organs (43). Therefore, long-term in vivo toxicity and degradation and excretion should be carefully analyzed. 3) Certain methodological issues also need to be carefully considered when drawing conclusions and analyzing results because current data in this field are increasingly contradictory. For example, dynamic light scattering (DLS) is suitable only for the assessment of nanoparticle size in simple media; therefore, for accurate size measurement in complex biological media, the fluorescence single particle-tracking (fSPT) method should be used (44). Analysis of the uptake route of nanoparticle requires the use of pharmacological inhibitors. However, some of these pharmacological inhibitors are not specific and may affect alternative internalization routes and the actin cytoskeleton. Therefore, specific inhibitors should be used and their effects need to be considered when drawing conclusions (45).

In conclusion, nanoparticle research should focus on the identification of a nanoparticle with improved tumor-specific targeting ability and decreased non-specific retention in normal organs.

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**Abbreviation:** HNSCC, head and neck squamous cell carcinoma; MRI, magnetic resonance imaging; CT, computed tomography; PET: positron emission tomography; EGFR, epidermal growth factor receptor; GNSs, gold nanoshells; GNRs, gold nanorods; PEI, polyethylenimine; fSPT, fluorescence single particle-tracking; DLS, dynamic light scattering

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