Applications of Nanomedicine in Oral Cancer

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Abstract

Oral squamous cell carcinoma is the sixth most common cancer for both sexes worldwide. The high mortality rate in cancer such as oral squamous cell carcinoma is commonly attributed to the difficulties in detecting the disease at an early and treatable stage. New methods of nanoengineered materials that are being developed might be effective in detecting the disease at an early treatable stage and treating illnesses and diseases such as cancer. "Nanotechnology" refers to the handling and/or engineering of nano-objects on the scale of molecules. This review deals with some recent developments concerning cancer detection and treatment enabled by nanotechnologies.

Key Words: Nanotechnology, Nanomedicine, Gold Nanoparticles, Quantum Dots, Oral Cancer

Introduction

"Nano" is derived from the Greek word for "dwarf". The term "nanotechnology", coined in 1974, refers to the science of manipulating matter, measured in the billionths of meters or nanometers, roughly the size of two or three atoms [1]. Nanotechnology was envisioned by the physicist and Nobel laureate Richard Feynman in his seminal lecture "There is plenty of room at the bottom" in 1959 [2-4]. After discussing his ideas with a colleague, Feynman offered the first known proposal for a nanomedical procedure to cure heart disease: "A friend of mine [Albert R. Hibbs] suggests an interesting possibility for relatively small machines" [5]. Nanoparticles are being applied in various industries, including medicine, due to their various properties such as increased resistance to wear and the killing of bacteria [6].

Nanomedicine

Convergence of nanotechnology and medicine recently led to an interdisciplinary field, nanomedicine, which brings together engineers, physicists, biologists, chemists, mathematicians, and physicians striving to improve detection, imaging, and drug-delivery devices [7]. Nanomedicine is a subfield of nanotechnology. It is often defined as the repair, construction, and control of human biological systems using devices built upon nanotechnology standards. Basically, nanomedicine is the medical application of nanotechnology [8]. The approaches to nanomedicine range from the medical use of nanomaterials, to nanoelectronic biosensors, and even possible future applications of molecular nanotechnology. The speculative field of molecular nanotechnology believes that cell repair machines could revolutionise medicine [7]. Nanotechnology's health implications can be split into two aspects: (a) the potential for nanotechnological innovations to have medical applications to cure disease and (b) the potential health hazards posed by exposure to nanomaterials [7].

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer for both sexes worldwide. The five-year survival rate of the disease is currently about 50%. At present, a diagnosis of cancer is made on histological evaluation, with possible prior cytological evidence. Currently, clinical examination and histopathological studies are the standard diagnostic method used to ascertain whether biopsied material is a precancerous or cancerous lesion [9,10]. Oral cancer is often diagnosed only after it has advanced to an untreatable stage where the cancer cells have become aggressive and immune to therapeutic drugs [11-14]. Early detection could lead to frequent patient monitoring,

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dietary changes, counselling for cessation of smoking and drinking, preventative drug administration, and lesion removal. Indeed, early diagnosis and treatment of OSCC leads to a mean survival rate of over 80% and a good life quality after treatment [15]. Detecting oral cancer at its earliest is thus vital for improving the survival rate of this disease.

Diagnosis of Oral Cancer

Studies in India have reported that oral visual screening can reduce mortality in high-risk individuals (tobacco and alcohol users). However, visual screening is limited in that it only identifies whether a lesion is present. Visual screening cannot, for example, determine the progression of a stage I or stage II tumour to stages III or IV, or distinguish which leukoplakia or dyplastic lesions will progress to carcinoma [14,16-18].

Recently, an increased amount of effort has been made to develop less invasive early diagnostic modalities for oral cancer, of which the *in vivo* high-resolution imaging of oral epithelial tissues using novel optical systems [19] and the chemical analysis of saliva [20] show great promise as valuable tools.

Advanced optical systems for *in vivo* imaging, such as optical coherence tomography (OCT) and confocal reflectance endomicroscopy, are designed to image cell and stromal morphology for non-invasive clinical diagnosis in real time. However, the contrast between neoplastic and normal tissues is often too low to be of any clinical value [21].

Biomolecular changes can potentially be detected through non-invasive quantitative assessment of the chemical compositions of saliva using techniques such as enzyme-linked immunosorbent assay (ELISA), micro-satellite analysis and high-performance liquid chromatography (HPLC); however, these highly specific techniques in molecular detection are usually labour-intensive procedures and require a lengthy analysis time [20].

Raman spectroscopy is another technique that has long been used to study cancer-related chemical changes in both cancerous tissues as well as biofluids. Saliva contains many macromolecules such as proteins and nucleic acids giving a Raman signature [22]. However, Raman spectra from saliva are inherently weak, making interpretation difficult [23].

Metallic nanoparticles have recently been investigated to overcome the limitations of these imaging and chemical-based diagnostic techniques. Enhancement of the Raman signal using a surface enhanced approach with gold nanoparticles (AuNPs) is being studied. Kah *et al.* (2007) demonstrated the use of gold nanoparticles in surface-enhanced Raman scattering (SERS) to enhance the Raman spectroscopy signal for the analysis of cancer-related chemical changes in saliva. SERS spectra of saliva were obtained and shown to be differentiable between those acquired from normal individuals and those from oral cancer patients, thus showing promise of a simple SERS-based saliva assay for early diagnosis of oral cancer [21]. Use of saliva as a diagnostic fluid would offer a few advantages over previous sera-based counterparts in that saliva is easily accessible, painlessly acquired and presents lower risk of infection compared to serum [21].

Among recent applications of SERS to cancer research, Huang *et al.* (2007) conjugated gold nanorods to anti-epidermal growth factor receptor antibodies to discriminate human oral squamous cancer cells from human nonmalignant epithelial keratinocyte cells [1,24].

Magnetic nanoparticles, quantum-dots (QDs), and AuNPs can be used as alternative contrasting agents [25- 28]. When excited, the surface plasmon resonance (SPR) of AuNPs can scatter and/or absorb light in the visible or the near-infrared (NIR) spectrum. This property is useful for *in vivo* optical imaging techniques such as photoacoustic and two-photon luminescence imaging. These two optical diagnostic techniques specifically generate cellular contrast by tuning the SPR of the AuNPs to the NIR spectrum [29,30].

Opto-acoustic tomography is a novel medical imaging method that uses optical illumination and ultrasonic detection to produce images of deep tissues based on their light absorption. The development of a molecular-based contrast agent composed of gold nanoparticles conjugated to a monoclonal antibody that improves opto-acoustic tomography imaging potentiates its use in imaging deep tumours in early stages of cancer or metastatic lesions [31].

Gold nanoparticles also possess other favourable physicochemical properties for use as optical probes for early detection of oral cancer [32]. They can provide an optical contrast to discriminate between cancerous and normal cells. Their conjugation with antibodies or peptides through electrostatic interaction or coordinate bonding to probe for specific cellular biomarkers

(epidermal growth factor receptor [EGFR] is over-expressed in vast majority of epithelial cancer but not in normal cells [33-36]) with high specificity and affinity allows them to map the expression of relevant biomarkers for molecular imaging [31,37, 38]. Such molecular imaging assists clinicians in diagnosis of precancers [21].

In an *in vitro* study, gold nanoprobes that selectively and sensitively target tumour selective antigens were used to induce a distinct contrast in computerised tomography imaging in head and neck cancer. They used gold nanorods (AuNR) and conjugated them with UM-A9 antibodies which home specifically to squamous cell carcinoma of head and neck region for early detection of oral cancer. This showed that the attenuation coefficient for the molecularly targeted cells is over five times higher than for identical but untargeted cancer cells or normal cells [31].

Semiconductor quantum dots are extremely small particles of cadmium selenide (CdSe) or zinc sulphide whose sizes are in the range of 1 to 10 nm [39]. For biomedical applications, QD surfaces are modified further to target specific cells or molecules. QD–peptide conjugates have been used to target tumour vasculatures and homed to tumour vessels [40]. Antibody-conjugated QDs have been used for real-time imaging and tracking of molecules in living cells and have demonstrated high sensitivity and resolution [32]. Most recently, QD probes have been used for *in vivo* tumour targeting in passive and active modes [39]. In passive targeting, QD probes are delivered and aggregated at tumour sites because of the enhanced permeability and retention effects. In the active mode, QD probes were conjugated with a prostate-specific membrane antigen (PSMA) monoclonal antibody to target PSMA preferentially, which is known as an attractive marker for prostate cancer. After *in vivo* imaging, histologic and immunocytochemical examinations confirmed that the QD signals came from an underlying tumour [39,41]. Similarly, QD probes could be conjugated to monoclonal antibodies to oral cancer-specific antigens, such as epidermal growth factor receptor, to detect oral cancer cells specifically [42].

Quantum dots (nanoparticles with quantum confinement properties, such as size-tunable light emission), when used in conjunction with magnetic resonance imaging, can produce exceptional images of tumour sites. These nanoparticles are much brighter than organic dyes and only need one light source for excitation. This means that the use of fluorescent quantum dots could produce a higher contrast image and at a lower cost than today's organic dyes used as contrast media. However, the downside is that quantum dots are usually made of quite toxic elements [8].

Recently, it has been reported that magnetic nanoparticles are effective contrast-enhancement agents for T2-weighted images [38,43]. T2-weighted images are preferred for the diagnosis of cancer. The magnetic nanoparticles are 3- to 10-nm iron oxide particles that are coated with hydrophilic macromolecules, such as dextran and starch. When the superparamagnetic nanoparticles are injected into target tissue, they create a strongly non-uniform magnetic field that induces dephasing of proton magnetic moment. This results in a significant reduction of T2-relaxation time, which results in enhanced contrast in the T2-weighted image [32,42].

For optimal contrast enhancement in magnetic resonance imaging, AuNPs have been used as a delivery vehicle to convey multiple gadolinium diethyltriaminepentaacetic acid (Gd-DTPA) complexes into selective cellular targets. Dithiolated DTPA (DTDTPA) has been used in place of DTPA to chelate to ionic Gd^{III} and permit conjugation onto 2 to 2.5 nm AuNPs surface. The *in vivo* application and cytotoxicity of the Gd-DTDTPA/AuNPs conjugates have not been fully investigated [30].

Sensor test chips containing thousands of nanowires, able to detect proteins and other biomarkers left behind by cancer cells, could enable the detection and diagnosis of cancer in the early stages from a few drops of a patient's blood [8,44].

The lab-on-chip technology holds the promise of replacing the elaborate techniques with miniaturised, integrated, automated, disposable microfluidic cassettes, inexpensive diagnostic devices with pre-loaded, freeze-dried reagents, and used in conjunction with a hand-held instrument, which would promote early screening and diagnostics during dental visits or routine medical examinations [14]. Micro-fluidics are by definition suited for handling living cells (whose typical diameter is a few micrometers) in a three-dimensional, biologically relevant environment [1]. This microfluidic chip accepts saliva samples, can be operated by minimally trained personnel, and can provide a diagnostic answer in an automated and timely fashion. The detection of oral pre-cancer (dysplastic) and cancer cells within the chip will take advantage of mem-

brane-associated cell proteins that are singularly expressed on the cell membranes of dysplastic and cancer cells and of the unique gene transcription profiles of cancer cells. The measured profile is compared with archived gene transcription profiles to determine the cancer type and stage. As such, this system provides a means for automated, rapid detection and molecular analysis of cancers in a miniaturised format suitable for use in the clinic and/or the operating room [14].

A new technology that uses the novel microchip has been developed in a project led by Professor John McDevitt from Rice University, Houston, USA. Swift detection of oral malignant and premalignant lesions has been made possible by the development of this nano-bio-chip, which is able to detect oral cancer via an immediate, non-invasive technique. It uses the latest techniques in microchip design, nanotechnology, microfluids, image analysis, pattern recognition and biotechnology to shrink many of the main functions of a state-of-the-art clinical pathology laboratory onto a nano-bio-chip the size of a credit card [45,46].

The new test involves removing cells from the cheek or tongue with a brush, placing them on a chip, and inserting the chip into the analyser, leading to a result within 15 minutes. Nano-bio-chips are disposable and slotted like a credit card into a battery-powered analyser. A brush-biopsy sample is placed on the card and microfluidic circuits wash cells from the sample into the reaction chamber. The cells pass through mini-fluidic channels about the size of small veins and come in contact with "biomarkers" that react only with specific types of diseased cells. The machine uses two light-emitting diodes (LEDs) to light up various regions of the cells and cell compartments. Healthy and diseased cells can be distinguished from one another by the way they glow in response to the LEDs [45,46].

This technology has been found to be 97% sensitive and 93% specific in detecting malignant or premalignant lesions. Preliminary studies by the researchers using the diagnostic chip have demonstrated comparable success rates to traditional techniques, such as biopsies, highlighting the new technique as a suitable alternative to the commonly used method [45,46]. Further studies need to be conducted on a large scale to ascertain whether this technology can be used for early diagnosis of malignant lesions. Further trials are needed to establish their effectiveness.

Treatment of Oral Cancer

Nanotechnology is probably the only method that can be used for site-specific action without causing side effects by killing the normal cells. Cancer nanotechnology is the latest trend in cancer therapy [47]. It represents a great hope for improving cancer treatments by acting at least at two main levels: conferring new properties to a pharmaceutical agent (increased stability, modified pharmacokinetics, decreased toxicity, and so on) and targeting the agent directly to the tumour [1].

Nanomaterials for brachytherapy, such as BrachySil™ (Sivida, Boston & Perth, Australia), deliver 32P and are currently being tested in a clinical trial. A drug delivery system that can cross the blood–brain barrier is a vision of the future with this technology. Nanovectors for gene therapy to correct disease at molecular level are at the development stage [6,48].

As with many cancers, there is a need to deliver therapeutic agents with greater efficiency to improve the treatment of OSCC and to improve patient outcomes. The development of polymersomes offers a novel way to deliver therapy directly into tumour cells. Use of PMPC-PDPA (poly 2-(methacryloyloxy)ethyl phosphorylcholine) polymersomes may enhance polymersome-mediated antitumor therapy [49].

QD probes can target and accumulate in tumours both by their enhanced permeability and retention (EPR) effect and by recognition of cancer cell surface biomarkers. Chemotherapeutic agents bound to QD probes that will recognise and bind to cancer cells may offer a new strategy for molecular cancer therapy by avoiding systemic toxicity [41,50].

One of the major advances in minimally invasive therapies for cancer is photodynamic therapy (PDT). First discovered in the early 1900s, it is now an approved cancer treatment for various superficial malignancies, including basal cell carcinoma, oral, oesophageal and lung cancers [41]. The application of QDs-PS complexes as therapeutic PDT agents was first reported by Samia *et al.* (2003) [51]. Quantum dots can be used in PDT as photosensitisers (PS), which can mediate targeted cellular destruction. They can bind to antibody present on surface target cell and when stimulated by ultra violet light, will release reactive oxygen species (ROS); this is lethal to target cells [6,41,52].

A less invasive experimental technique that holds great promise for the treatment of cancer and

related disease conditions is photothermal therapy. It combines two key components: (a) a light source, specifically lasers with a spectral range of 650–900 nm for deep-tissue penetration, and (b) optical absorbing AuNPs, which transform the optical irradiation into heat on a picosecond timescale, thereby inducing photothermal ablation. El-Sayed *et al.* (2005)[37] found that anti-EGFR-AuNPs conjugates bound readily in a homogenous manner to both HOC and HSC oral cancer cell lines overexpressing EGFR. The binding of the anti-EGFR–AuNPs conjugates enabled a clear visualisation of these cells under a microscope. Anti-EGFR–AuNPs conjugates designed for HSC and HOC oral cancer diagnostics used the colour-scattering property of the AuNPs. When illuminated with a white light at specific angles, AuNPs, depending on their size and shape, will scatter light of many colours [30,37].

Hirsch *et al.* (2003) reported that gold nanoshells that were labelled with antibodies specific to oncoprotein were injected and bound to the target carcinoma cells [53]. Subsequent NIR illumination resulted in local heating because of strong absorption by the nanoshells and subsequent destruction of the tumour cells [42].

Potential Health Hazards

Nanoparticles pose a separate problem within the area of toxicology, designated as nanotoxicology. Reducing the size of structure to nanolevel results in distinctly different properties. The literature on toxicological risks of the application of nanotechnology in medical applications is scarce. Biodegradable substances are normally decomposed and their waste products excreted by the kidneys and intestines. However, nonbiodegradable nanoparticles have been studied and it seems that they accumulate in certain organs, especially the liver. The potential harm that they may trigger, or at what dosage, has not been clarified and further investigation is needed [32].

Conclusion

The application of nanotechnology to biomedicine, particularly in cancer diagnosis and treatment, promises to have a profound impact on health care. Many of the technologies involving nanoparticles for early detection of cancer and treatment are in preclinical stages. Nanotechnology applications in cancer detection and treatment have the potential to replace highly invasive conventional cancer detection and treatment, which often includes biopsies, irradiation, and painful therapies. The ability to diagnose malignant disease at the earliest opportunity allows treatment options to be planned as early as possible and hence directly affects the morbidity and mortality of head and neck cancer.

Statement of conflict of interest

As far as the author is aware, there is no conflict of interest.

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