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RESEARCH ARTICLE

TITANIUM DIOXIDE NANOPARTICLES AS DELIVERY SYSTEM AGAINST INFECTIONS AND CANCER

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ABSTRACT

Many people suffer and demise from chronic, recurrent, drug-resistant or biofilm-developing infections such as leishmaniasis, colitis and carcinogenesis throughout the globe annually. Despite the progress in medical treatment, drug resistance, adverse side effects, inadequate therapeutic index, poor bioavailability, insolubility and toxicity have hampered the disease curing process. Recently, nanotechnology-based metal nanoparticles have emerged as nanomedicine for the treatment of various diseases. Titanium dioxide nanoparticles (TiO_2 NPs), owing to their unique photocatalytic characteristics, high chemical stability, excellent biocompatibility and low toxicity have attracted interest for disease-treatment. TiO_2 NPs generally acts through metal ions liberation inducing oxidative and non-oxidative damages of the infected cells and micro-organisms. The attachments of pharmaceutics and ligands to TiO_2 NPs may improve their targeted delivery to specific sites for sustained release of active ingredients with insignificant side-toxicity overcoming the biological barriers. The review focuses TiO_2 NPs for the consideration as delivery system in combating diseases.

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INTRODUCTION

Intracellular or extracellular infectious diseases such as leishmaniasis, gastrointestinal bowel syndrome and cancer caused by parasitic / microbial organisms or by the exposure of toxicants associated with biofilm-mediated or usages of medical devices have reflected a world-wide problem for public health resulting millions of demise each year. When parasites / microbes or other toxicants enter the host body, the defense mechanisms such as antioxidant defense system and innate and acquired immune system become activated to protect the body from the development of infections. But when defense mechanisms become failure to prevent infection, micro-organisms are easily transmitted followed by multiplication in the host cells leading to disease-development (National Institutes of Health (US), 2007; Mandal, 2018; Mandal, 2018a; Bellmann *et al*., 2015). A lot of antimicrobial and anticarcinogenic agents have been utilized to eradicate the micro-organisms or diseases. However, changes in the environment, society, technology and evolving microbes are contributing to the emergence of new diseases and development of anti-carcinogenic and anti-microbial resistances (Mandal, 2018; Cohen, 2000). The resistances to drugs may be resolved through the development of new drugs and chemical modifications of the existing drugs.

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The use of new and modified drugs may develop repeatedly multi-drug resistances to microbes or cancerous cells probably due to insufficient therapeutic index associated with biological barriers, toxicity, low bioavailability and insolubility, leading to chronic infection or spread of diseases (Huh and Kwon, 2011; Hajipour *et al*., 2012). In this context, there is a need to design a more effective and long-term delivery approach to solve this ever-growing problem. Nanotechnological applications in medicine have attracted attention in the advancement of disease-cure against a range of diseases (Ferrari, 2005). Nanoparticles composed of metal-oxides may be promising anticarcinogenic and antimicrobial agents to which pathogens or cells cannot develop resistance (Mandal, 2018). Metallic nanoparticles are distinguished by their small sizes (10-100 nm) accounting for their significant interactions with cellular biomolecules and on the cell-surfaces, while the matter may be altered at the levels of particles and atoms. TiO_2NPs having unique physico-chemical properties such as semi-conductivity, photodynamic activity, spherical nanosize, chemical stability, low-toxicity, high surface to volume ratio and easy surface-functionalizations are attracting attention for their suitability in biomedical usages. Owing to their nanosizes and suitable physico-chemical features, they can extravasate through enlarged pores of capillary endothelium into the tumor zones through enhanced permeability and retention (EPR) effect, and can penetrate cell membranes producing reactive oxygen species (ROS), and membrane leakage to kill the diseased cells or micro-organisms by neutralizing their

membrane charges and overcoming the biological barriers such as blood-brain-barrier (BBR), enzymatic degradations, and multi-drug resistances (MDRs). For controlled, targeted components-delivery, TiO_2 NPs may be conjugated with selective active cargos, ligands, nucleic acids, sugars, antibodies, lipids, proteins and enzymes to enhance therapeutic efficacies and delivery capabilities at the pathological sites (Mody *et al*., 2010). The synthesizing conditions and the surface-functionalizations of TiO_2 NPs may influence them to deliver active ingredients to the target sites against biological barriers in a sustained release manner accompanied with pH responsive, hyperthermia, photo ablation -applications to get higher pharmacokinetics and maximum therapeutic efficacies. This review demonstrates TiO_2 NPs as delivery system to translocate potential therapeutics to the target sites effectively for the treatment of various diseases.

Synthesis of titanium dioxide nanoparticles: Generally, TiO_2 NPs are synthesized using co-precipitation method into their three common forms such as 7-10 nm anatase, 15-20 nm rutile and 10-15 nm nanotube having 70-150 nm length. The NPs may be synthesized following wet chemical synthesis. Firstly, deionized water (18.2 M) and 50:50 ethanol (99.8%) -mixture is boiled to reflux, while the boiling solution-pH is adjusted to 3.0 by 1N HCl-addition. Titanium isopro-poxide is added gently to the refluxing mixture to get immediate white precipitates-solution followed by stirring at 85^{o}C for 4 h. Then the solution is cooled to room temperature and cleansed several times with ethanol to do dry for getting partially amorphous anatase while the crystallined rutile nanoparticles are obtained by calcining the anatase at 800^{o}C for 2 h. To prepare nanotubes, 0.5 g anatase is mixed to 20 mL 10M NaOH solution, and the mixture is autoclaved at different temperatures ranging from 120-150^{o}C for 20-24 h. The solution is then cooled to room temperature and cleansed several times to detach residual NaOH. The final wash is performed by 1N HCl for neutralization, and partially amorphous titania nanotubes are then subsequently cleansed several times, filtered and dried at 120^{o}C, while the NPs are dispersed in deionized water, PBS or media by sonication or vortexing for further use.

Surface functionalizations of titanium dioxide nanoparticle: TiO_2 nanocomposite may be prepared by loading drug and coating ligands such as chitosan utilizing simple co-precipitation method. In brief, 1g dried TiO_2 NPs powder is suspended in 10 mL deionizing water and stirred at 450 rpm. After that 0.6 g chitosan is added to this mixture and immediate 0.2 g CTAB surfactant is added and stirred to 850 rpm for 3 h and cleansed 3 times with deionizing water for the removal of untreated chitosan. The suspension is diluted by adjoining 10 mL deionizing water and then sudden 2 mL drug addition (1 mg / mL in ethanol). The solution turns orange red which is stirred for 6 h adjusting pH to 7, and cleansed with deionizing water followed by drying in oven at 90^{o}C.

Characterizations of titanium dioxide nanocomposites: The shape, size and morphology of the TiO_2 NPs may be characterized by utilizing high resolution transmission electron microscopy. Their crystalline structure may be confirmed by X-ray diffraction using X'pert PW1827 diffractometer. The capping formation on the TiO_2 NPs-surface and bonding interactions of drug may be confirmed by IR analysis using Perkin Elmer series type spectrometer.

The hydrodynamic sizes and zeta potentials of NPs-suspension may be determined utilizing electrophoretic light scattering spectrophotometer.

Mechanism of action of titanium dioxide nanoparticles: Generally, metal nanoparticles show their anti-carcinogenic and anti-microbial activities through disruption of cell membrane, generation of ROS, penetration of cell membrane and induction of intracellular damage via interactions with cellular organelles, proteins and DNA (Mandal, 2018a; VanVliet *et al*., 2014). These activities may be organized through binding of TiO_2 NPs to phosphorous / sulfer -containing bio-molecules such as DNA and proteins leading to impairment of enzymes, DNA and cell membranes, while some nanoparticles act with mitochondria to impair electron transport chain pathway accompanied with inhibition of ATP synthesis due to damage of mitochondrial proteins. In addition, NPs can also impair lipophosphoglycan and glycoprotein molecules which are significant for microbial infectivity. Another mechanism is ions-liberation from NPs that interact with cysteine-containing proteins to inhibit protein-functionality.

The electronic structure of TiO_2 NPs belongs to the semiconducting metal oxide family showing photo-catalytic activity through electrons-filled valence band (VB) and electron-free conduction band (CB), while energy difference between the bands called band gap or energy gap (Khan *et al*., 2015). The consequence of semiconductor TiO_2 NPs' light irradiation excitation is that electrons (e^-) transfer from VB to CB, leaving behind positive holes (h^+), while specific electrons-holes pairs ($e^- + h^+$) are created (Grcic *et al*., 2013; Fujishima *et al*., 2000; Mills and leHunte, 1997). This bound exciton state lacks stability exhibiting their redox characteristics. Charge carriers (h^+, e^-) may then migrate to the catalyst-TiO_2 NPs-surface where they may be trapped by adsorbate molecules to initiate ROS-generation, while excited negative electrons (e^-) may reduce molecular oxygen (O_2) to form superoxide radical anion ($^{\cdot}O_2^-$). Productive positive holes (h^+) may oxidize water molecules (H_2O) and hydroxide ions (OH^-) to generate hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) implicated in oxidative stress, while H_2O_2 can pass through cell membrane to create cellular damage (Grcic *et al*., 2013; Fujishima *et al*., 2000; Mills and leHunte, 1997; Linsebigler *et al*., 1995).

Titanium dioxide nanoparticles as delivery system: TiO_2 NPs have been reported as potential antimicrobial agent against biofilm through electromagnetic attraction between the microbes and the NPs leading to cell death (Jesline *et al*., 2015). TiO_2 NPs cause microbial DNA compressions, degenerations and fragmentations reducing the physiological genes-activities, while the affinity and binding between NPs and DNA may be performed by molecular docking indicating TiO_2 NPs-targeted DNA rich in G-C (Iram *et al*., 2015). The antimicrobial activity of TiO_2 NPs may be triggered onto microbial cells by their photo-catalytic action resulting enhanced cell permeability leading to cell death through accelerated intracellular components-photo-oxidation (Desai and Meenal, 2009). Many nanosized (<100 nm) TiO_2 materials have been utilized as delivery carriers for various anti-cancer drugs such as cisplatin, doxorubicin (DOX), valproic acid, temozolomide (TMZ), daunorubicin (DNR) and gambogic acid (GA) through non-covalent or covalent conjugations (Yin *et al*., 2013; Li *et al*., 2009; Lopez *et al*., 2006; Uddin *et al*.,

2011; Xu *et al*., 2015; Zhang *et al*., 2012; Qin *et al*., 2011; Ren *et al*., 2013; Kim *et al*., 2012; Liu *et al*., 2015). As the extracellular microenvironment of tumors is more acidic (pH 5 and 6) than that of normal tissues (pH 7.4), the desirable characteristics of TiO_2 NPs are capable to release drug components more rapidly at acidic tumor-sites showing their therapeutic efficiency in cellular death by the induction of caspase-dependent apoptosis or other signalings (Zhang *et al*., 2012; Qin *et al*., 2011; Chang *et al*., 2017). For active targeting, TiO_2 NPs were conjugated with the specific ligands to get abilities for selective binding to cancerous cells, while TiO_2 NPs conjugated with monoclonal antibodies against Her2 loaded with camptothecin, or hyaluronic acid loaded with cisplatin, showed their selective drugs releasing efficiencies to targeted cancer cells (Yin *et al*., 2013; Kim *et al*., 2012; Liu *et al*., 2015). In order to improve the biocompatibility of the TiO_2 NPs, their surfaces were modified with polyethylene glycol (PEG) and functionalized to folic acid ligand for active targeting to folate receptors in cancerous cells and loaded with drugs such as paclitaxel (PTX), DOX, showed more efficient toxic cancer cells-death through controlled and sustained drugs release with insignificant side effects (Venkatasubbu *et al*., 2013; Du *et al*., 2015). In this context, polyethylenimine (PEI) modified porous TiO_2 NPs are also important as they can liberate encapsulated drug molecules to the target cells utilizing their photo-catalytic activities, while UV-illumination triggers TiO_2 NPs to cut off their coating-PEI molecules by the generation of ROS (Liu *et al*., 2015; Wang *et al*., 2015).

Biodistribution, pharmacokinetics and elimination of titanium dioxide nanoparticles: After the administration of TiO_2 NPs in the body, the systemic circulation may distribute the NPs throughout the tissues and different organs accompanying their interactions with plasma-proteins, blood cells and platelets affecting the patterns of distribution and excretion (Deng *et al*., 2009; Hagens *et al*., 2007). The biodistributions of the NPs to different organs may vary depending on their various sizes, shapes, high surface to volume ratios, charges and surface-coatings along with different routes of administrations. Some investigators treated rats with a single intravenous serum suspended TiO_2 NPs injection (70 / 30 anatase / rutile; 20-30 nm; 5 g / kg BW) for 1, 14, and 28 days (Fabian *et al*. 2008).

The estimated NPs levels were monitored highest in the liver followed by spleen, lungs and kidneys in decreasing manner, while the highest organ-burdens were on day1 post exposure. The NPs were retained in the liver for 28 days experimental time while their slight decrement was observed from day1 to days 14 and 28 in the spleen, and a get back to control levels by day 14 in the kidneys and lungs. In this experiment, TiO_2 NPs were not detected in plasma, blood cells, lymph nodes or brain at 1, 14 and 28 days post-exposure indicating their rapid clearance from the blood into reticulo-endothelial system and few accumulations in the liver and spleen upto 28 days-experimental period. In another study, mice were injected intraperitoneally with different dosages of TiO_2 NPs at 0, 324, 648, 972, 1296, 1944, or 2592 mg / kg body weight for two weeks (Chen *et al*., 2009). At 1, 2, 7, and 14 days post-exposure, NPs (80 nm, 100 nm, anatase) were accumulated highest in spleen followed by decrement in liver, kidneys and lungs, while the highest uptake of NPs was monitored in the spleen during the experimental period and few particles were eliminated from the kidneys through urination. Other investigators observed distribution of TiO_2 NPs (5 nm anatase; 5, 10, 50, 100, and 150 mg / kg body weight) in mice injected intraperitoneally daily for 14 days showing an order of uptakes in liver > kidneys > spleen > lungs > brain > heart (Liu *et al*., 2009). It was observed that TiO_2 NPs were eliminated by kidneys and intestinal hepatobiliary tract through passing of urine and feces. However, some larger NPs were accumulated in some organs especially in liver and sequestered for longer period.

Immune responses: Some investigators have demonstrated that TiO_2 NPs (5 nm; 5,10,50,100 and 150 mg/kg body weight; everyday for 14 days) induce hepatotoxicity through inflammations and liver injury (Ma *et al*., 2009). The real-time quantitative PCR (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) exhibited that NPs altered significantly the mRNA and protein inflammatory mediators including TNF-α, NF-kB, IL-6, IL-1β, IL-4 and IL-10. The chronic intragastric administration of TiO_2 NPs (5-6 nm; 2.5, 5 and 10 mg/kg everyday for 90 days-study) to ICR mice showed spleen injury, while immunoglobulin and lymphocyte subsets such as CD3, CD4, CD8, B cells and natural killer cells were diminished, and the levels of IL-6, IL-2, IL-8, IL-18, IL-4, IL-10, IL-1β, interferon-γ were increased significantly (Sang *et al*., 2012). Furthermore, long-term administration of low dosed TiO_2 NPs to mice, caused spleen injury through the alteration of apoptotic and inflammatory cytokines expressions and decrement of immune capability.

Conclusions and future perspectives: The treatments of infectious diseases caused by bacteria, virus and parasites and cancer are hampered mainly by drug-resistance, drug-toxicity and non-selectivity. TiO_2 NPs show good cellular interactions with biomolecules within the cells and on the cell surfaces and can overcome biological barriers to kill cells by generating ROS which are dependent on some factors such as the concentration, shape, size, surface aspect ratio, photo-catalytic activity and preparatory methods of NPs. ROS damage basically DNA and cell membranes of microorganisms, cancerous and infectious cells by their high oxidative potentials, while plasma membranes damages result in cellular necrosis, nucleus and mitochondrial damages induct cellular apoptotic death and endoplasmic reticulum damages induce cell death by autophagy.

The physico-chemical characteristics of TiO_2 NPs may strongly influence their toxicities and bio-availabilities i.e. immune, cyto and geno -toxicities, and high material stability that may also damage healthy cells. Therefore, TiO_2 NPs are being functionalized on their surfaces with potent antibiotics or other drug-components conjugated with ligands such as monoclonal antibodies, peptides, proteins, sugars, lipids and nucleic acids for the site specific targeting to reduce their toxicities to healthy cells. As TiO_2 NPs are immunogenic by their exposure into the body, the size of the particles should be <5 nm (Mandal, 2018) to be eliminated from the body by renal glomerular bed -filtration and accompanied by proper surface-functionalizations with potent cargos and ligands for active targeting as an ideal approach for nanotechnology based delivery system to diminish maximum side effects. However, there should be thorough systematic investigations regarding their biodistributions, pharmacokinetics, eliminations, toxicities, immune responses and efficacies especially for oral and intravenous administrations in *in vivo* biological systems to use them as proper therapeutic future nanomedicines before going to clinics.

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