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Biomedical applications of nano Titaina in theranostics and photodynamic therapy

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Titanium (TiO_2) is one of the most abundantly used nanomaterials for human utility either as food additive, sunscreen, photovoltaic, environmental scavenger or biomedical applications. Nano TiO_2 in biomedical applications are well documented in endoprosthetic implants and early theranostics of neoplastic and non-neoplastic maladies as a photodynamic therapeutic agent and vehicle in nano drug delivery system. Herein we focus on the recent advancements and applications of nano TiO_2 in bio-nanotechnology, nanomedicine and photodynamic therapy (PDT).

1. Introduction

The role of nanotechnology (NT) in our life is inevitable. Naturally, the existence of Bio-nanotechnology (BNT) can be traced back to the initiation of first life on earth. Nano sized bio molecules (low density lipoproteins) inter and intra cellular transfer, neural transmission and memory storage in biological system are the prototype examples of BNT [1, 2]. Similarly biomolecules like proteins, DNA and RNA are in nano-scaled and structural analogues to nanomaterials, which brings the concept of integrating the nanomaterials with bio molecules to understand the cellular signal pathways, functional mechanisms and cellular interaction within living cells and organisms [3]. In nature the existence of dust particles, smoke and ink are the examples of non-biological NT [4]. BNT existed from the beginning of life and its potential has been explored with the advancement in the human civilization by realizing BNT great impact on our day to day life. Similarly, the enzymes, antigen, antibodies and ligand receptors are having 2-20 nm size in either direction, is a structural analogue to the nanomaterials, and thus it provides plenty of room for BNT biomedical applications[5, 6].

The distinctive properties of fabricated inorganic-nanomaterial (INM) including high reactivity, increased surface area, lower melting point, ductility and charge conductivity make them suitable candidate for biomedical application and BNT [7, 8]. Since these INM can readily interact with organic biomolecules by exhibiting excellent conductivity and site recognition within cellular gadgetry, they can be reintroduced to the cells and biological system. Mostly the physico-chemical properties including shape [9], size [10] and surface chemistry [11] decides the fate of INM. Surface modification of INM lead to relevant change in the physico-chemical properties that make them suitable for certain disease treatment, e.g., -COOH functionalized MWCNT nanowhiskers (NW) are more hydrophilic and less toxic than pristine or untreated nanoparticles and whiskers[12].

Titanium is the most abundantly available element in nature with most common metallic state of T, $TiCl_4$ and Titanium dioxide (TiO_2); the latter one exists with well-known crystalline shape of Rutile, Brookite, TiO_2-B and most reactive Anatase [13-15]. It has also been proven that mixed polymorph of TiO_2 are more efficient than single crystal, e.g., Anatase 80 % and rutile 20 % are more efficient than Anatase 100 % [13], similarly TiO_2-B1 and Anatase are reported more reactive [16, 17]. These polymorphs additive effects can be attributed to surface interface among various polymorphs, reducing the recombination of photo generated electron hole pairs and thus resulting in more ROS generation [13].

Currently more than thirteen hundred nanomaterials are globally available [18], among them TiO_2 is the second most abundantly consumed material in our daily life [19]. The food grade TiO_2 is named as E171 in European Union.[20]. In US, the daily consumer statistics reveal that every child under age of ten years is exposed to 1-2 $mg/kg_{body\ weight\ (bwt)}$ of TiO_2, whereas the other age groups exposure rate is 0.2-0.7 mg/kg_{bwt} [20]. TiO_2 has been used as white pigment in ancient times [21], and its long course exposure to human and environment guarantee its less toxic nature.

Despite the TiO_2 extensive use in other fields, in biomedical the history of TiO_2 is relatively new. The first nano TiO_2 biomedical application can be traced back to early nineties of 20th century, when Japanese scientist Fujishima and his coworkers first time reported TiO_2 with photodynamic therapeutic properties by killing cancer cells (HeLa) [22]. At the

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same time another study reported successful application of nano TiO_2 in medical implants by coating TiO_2 as redox agent on their surface [23]. Soon after these reports TiO_2 got momentum in biomedical field applications and number of publications and citations exponentially increased by the start of 21st century. In this review we will mainly focus on the most recent advancements of nano TiO_2 and its bio-applications in the biomedical realms.

2. Nanotechnology and bio-application of TiO_2

Nanotechnology (NT) is based on the engineering science at molecular level used in biomedicine for therapeutics and diagnostics that is collectively termed as Theranostics [24]. Nano drug delivery system, bio-imaging, photodynamic, sonodynamic and photothermal therapy are among few vital applications of BNT. European Union defined nanomaterials as "manufactured, incidental or natural material containing particles as an aggregate, agglomerate or an unbound; where fifty percent exhibit 1-100 nm size in any dimension" [14]. Generally the size of NP must be in range of 1-100 nm in either dimension as described by the National Nanotechnology Initiative; however the term Nano describes the size in several hundred nanometers. Their tiny nano size has the advantage to cross easily through various cell and tissue barriers with desired effect. NP lodging in desired site depends upon the unique microenvironment, i.e. temperature, pH, ROS, metabolic rate, osmotic pressure, etc [25]. In some cases NP are conjugated to the antibodies or active ligands like peptides and folic acid that are highly specific to achieve the sufficient accumulation in desired tissue, this approach is known as active mechanism [26], similarly most of the neoplastic tissues bear enhanced vascular permeability and retention (EPR) effect that can be termed as passive mechanism to achieve desired nano drug concentration [27, 28].(Fig 1)

As one of the most abundantly used nanomaterials, Titanium (TiO_2) has been widely utilized as food additive, sunscreen, photovoltaic, environmental scavenger or biomedical treatments. When TiO_2 is irradiated with certain light band (i.e. UV), it will generate electron-hole pairs that reacts with the surrounding oxygen to form various reactive oxygen species (ROS), i.e., OH^- or O_2^- radicals by redox reactions [29]. The waste water purification via TiO_2 by utilizing the natural sun light is the most valuable application in our day to day life [30, 31]. In fact, the photo generated radicals are used as sanitizers for polluted water with various dyes and phenols, or other organic pollutants in environment [29, 32]. It has been reported that due to wide band gap, TiO_2 photo absorption is limited to UV light spectrum, i.e., 4 percent of total sun light, however, metal ions and nonmetal doping can extend TiO_2 photo absorption to visible range [33]. The aforementioned approaches are the key features of nano-TiO_2 for biological application and modern theranostics. Mostly nano TiO_2 applications depends upon the type of polymorph used (e.g., one dimensional or three dimensional particle), surface modification, size of the particle and the subject system, i.e., biological, or photoelectrical system, etc.[34].

2.1 Nano-TiO_2 fabrication

Various techniques has been used so far for the fabrication of nanomaterials including sol gel method, hydrothermal process, chemical vapor deposition and electrospinning [35, 36], the later one is more favorable for fabrication of one dimensional TiO_2 nanomaterials, e.g., nano whiskers, nanofibers, nanobelts, neck-lace like nanofibers and nanowires, etc. [30, 37]. Moreover the electrochemical adonization technique is considered more favorable for nanotubes synthesis, additional to the chemical template synthesis and alkaline hydrothermal treatment [38], and has a great control over the dimensions during synthesis [39, 40]. The structure of TiO_2 nanotubes is not clearly defined, however, it is assumed that it can be the hydrogen titanate where the TiO_2 sheets are separated by the H^+ ions [41].
TiO_2 nanowhiskers are better than particles in photocatalytic activity [42] and charge transportation that make them suitable candidates for solar cells and wind screens [43]. Moreover, nano TiO_2 better charge recombination rate and oxygen affinity make it suitable remedy for environmental recycling and an efficient catalyst [44].

TiO_2 nanowhiskers are also known for synergistic effect attributed to their photocatalytic activity that make TiO_2 excellent phototherapeutic agent. Moreover its large surface area and reactivity provide scaffolds for excellent drug delivery system. Recently, Gracia-valverde et al reported successful coating of carbon on the TiO_2 nanotubes for its better application in biomedical realms [45]. Similarly, the synergistic effect for carrying anticancer drugs like Daunorubicin (DNR) to the cancer cells (i.e., hepatic carcinoma cells SMMC-7721) and PD therapeutic effect when irradiated with UV light, vouch TiO_2 nanowhiskers could play important role in future cancer and other disease theranostics [46]. (Fig 2)

2.2 Nano TiO_2 pharmacodynamics

TiO_2 nanoparticles are commonly used in industry as consumer products and for biomedical applications, due to their strong catalytic activity and smaller size provide large surface area per unit mass provide maximum number of electrons on the surface to enhance their reactivity [14]. The safe dose for TiO_2 nanoparticles reported by previous studies is 5 g/kg $_{bwt}$ in rat models, with no adverse effects and rapid distribution when administered systemically [47, 48]. The TiO_2 nanoparticles can be administered as an oral, aerosol or systemic parenterals (intravenous, intra muscular or sub cutaneous) without any adverse effects with exception of less to none transdermal penetration when applied topically [49]. It is evident that liver, spleen, kidney and lungs are the main organs for TiO_2 nanoparticles deposition, while blood cells, plasma and brain are least affected [50]. Nevertheless, recently it has been reported that 5 nm TiO_2 nanoparticles with intraperitoneal

injections for 14 days and 80 to 155 nm particles trans-nasal location can cross the blood brain barrier [51-54].(Fig 3).

3.Biomedical applications of nano TiO_2

3.1 Photodynamic treatment

Photodynamic therapy (PDT) is a therapeutic technique with three main components, i.e. Photosensitizer (PS), Light source and Oxygen molecule. A nontoxic photosensitizer (i.e., macromolecular compound [55] or nano sized organic [56] and inorganic particle [57]) can be introduced to the living tissues and cells. These PS are excited by the absorption of photons from the visible light source and transfer energy to oxygen molecule to produce singlet oxygen ($^1O_2$) and reactive oxygen species (ROS) [58, 59]. PDT has more advantage over the conventional theranostics of cancer, i.e., higher safety, minimum invasive, cost effective, highly localized with least to none complications [60].

The PDT is most effective in delicate parts, natural cavities such as bladder, pulmonary tissues, head and neck, brain, pancreas, prostate, mammary tissue and intraperitoneal cavity, where invasive surgery is always a great deal of risk[61-63]. PDT is still an ideal candidate and first choice for dermal neoplastic and non-neoplastic maladies. Moreover, the first ever therapeutic effect of PDT considered was for dermal malignancies [64]. PDT is more effective for the treatment of tiny and superficial tumors, e.g., internal body organ linings, above and just below the skin and exposed body cavities (i.e., oral, vagina, rectum, urethra and bladder, etc.). In deep seated tumors its efficacy is low due to excitation light less penetration to generate ample therapeutic quantity of singlet oxygen ($^1O_2$) and other reactive oxygen species (ROS). PDT potential against dermal and oral microbial infections (e.g. Halitosis) has been proven more efficient [65].

The early diagnosis of benign and malignant tumors is still a challenge for modern therapeutics and the only available treatment is invasive surgery, chemotherapy or radiotherapy. Hence, the fluorescent imaging provides most suitable alternative for early diagnosis, and PDT of cancer [66, 67] as well as other infectious diseases [68, 69]. This fluorescent imaging and therapy in a combination can also be termed as Photodynamic therapy (PDT) [70]. At the beginning of 20th century PDT was rediscovered by Oskar Raaband Hermannvon Tappeiner, although in ancient China, Egypt and Subcontinent PDT was well established for dermal infections and natural sanitizer for common households [65, 71]. The role of PDT is not only vital in onco-therapy but also in non-neoplastic cardiovascular[72-74], pulmonary[75, 76], dermal[77, 78], ophthalmic[79], oral[59, 80] and inflammatory diseases along with rheumatoid arthritis autoimmune reaction suppressant[81-83]. Mostly photosensitizers and drug delivery systems are sensitive to UV light in range of less than 400 nm wavelength [84], however, this range has limited penetration (because of short wave length) and can damage the DNA as an adverse effect [85, 86]. In deep tissue penetration the near infrared light (NIR) range (620-850 nm) is more favored because of less harmful and maximum penetration range [87]. The NIR fall in phototherapeutic window that is ranged from 600 to 1000 nm range [88].

The photosensitive nanocomposites like TiO_2 nanoparticles could be administered systemically to the living tissue and upon attaining certain concentration in desired location, the visible light or NIR is passed through subject tissue. These NPs (PS) absorb the light in a form of photon and transfer it to local oxygen containing molecules that release various kinds of ROS and $^1O_2$. When light energy is passed through PS, the ground state oxygen is excited to $^1O_2$ and then triplet oxygen ($^3O_2$) energy state (Fig 4)[86]. The half-life of singlet oxygen with in the living tissue is estimated ~ 3 μ seconds[89-91]. Moreover once the PS is photoactivated it produces ROS for 18 hours, which is sufficient enough to induce apoptosis with in the target cell and adverse effects in surrounding environment[92].

The pristine nano TiO_2 are susceptible to photoactivation at UV range light (400 nm)[93]. Whereas the WHO approved therapeutic window start with relatively higher wave length (more than 600 nm) due to the fact that proteins, haemoglobin and melanin also excited at UV range, which could cause severe adverse effects[94]. To make pristine TiO_2 amenable for PDT desired effects, it should be either coupled with up converting surface modifiers or coated with materials of desired range. Synthetic or naturally occurring agents that upon excitation with certain light produce reactive oxygen species (ROS) are termed as photosensitizers (PS) [95, 96]. PS are the main and most important components of PDT [83, 97]. Porphyrin derivatives are the most common and prototype PS [98, 99] that are composed of macromolecules with excellent potential of singlet oxygen generation and least known toxicity [100, 101]. The *Porfimer Sodium* is the example of approved PS in US by the Food and Drug Administration (FDA) for clinical applications, whereas Zinc (II) phthalocyanine is an example of second generation of clinically approved PS, with a high tumor cells selectivity and higher reactivity to generate $^{\cdot}O_2$[60].

Most of the PSs are hydrophobic in nature that impedes their parental administration. This bottleneck develops the concept for hydrophilic nano PS such as micelles, liposome, low density lipoproteins[102-105] etc., nevertheless, these PS' hypersensitivity reactions, accumulation in the skin and eye are the leading factors for severe phototoxicity [106]. Therefore, to overcome these snags, the concept of nano drug delivery system is urged due to the promising hydrophilic nature and safe delivery to the target tissue. Most of drug delivery systems are nano silica based, organically modified [57] and selected metallic and non-metallic PS e.g. gold[107, 108], silver [109], zinc [110, 111], carbon fibers and complexes [112, 113], platinum [114, 115], titanium [116], iron [117, 118], cerium [119], ruthenium [120] etc., and/or their hybrid clusters [121-124].

Recently NT combined the inorganic materials with organic macro molecules as PS to achieve their synergistic effects in PDT [125, 126]. Since last few decades the potential of nano TiO_2 as inorganic PS for PDT has been extensively explored [127]. The nano TiO_2 used as PD-therapeutic agent has become popular for the various disease therapies including cancer [128, 129], microbial infections [130] and food safety and sanitation [131]. The reason for utility of TiO_2 as PS in PDT is that it becomes super hydrophilic when exposed to UV light and this functionality can be reversed, depending upon the exposed light [132].

Hou et al designed NIR up converting TiO_2 nanoparticles to explore its potential in PDT for Cancer amelioration [133]. They combined $NaYF_4:Yb^{3+}$, Tm^{3+} @ $NaGdF_4:Yb^{3+}$ as up conversion nanoparticles (UNCP) for the photoactivation of TiO_2 nanoparticles (PS) by NIR as UNCP @ TiO_2. When these UNCP @ TiO_2 were introduced into cancer cells in vitro and photoactivated by NIR, the TiO_2 generated ROS to induced apoptosis by decreasing mitochondrial membrane potential that released cytochrome C to activate caspase 3 for apoptosis initiation in cancer cells. Furthermore, in vitro, these UNCP @ TiO_2 also suppressed the tumor growth in murine models. (Fig. 5)

Nano TiO_2 has also been used in combination with other nanomaterials and macro molecules where it exhibited excellent synergistic effect, e.g., Zheng et al used TiO_2-Fe_3O_4 nano composites for the neoplastic disease theranostics, where nano TiO_2 as PS for PDT and Fe_3O_4 as contrast agent for Magnetic resonance Imaging (MRI) [127]. Tokuoka et al used chlorine e6 in combination with TiO_2 nanoparticles for murine thymic lymphoma cancer cell line (EL-4). The nano TiO_2 combined chlorine e6 had significant effect on the cell line after photoactivation in visible range as compared to the separate treatment with TiO_2 and chlorine e6 [134]. *In vitro* these results could be achieved but *in vivo* biomedical applications may not be promising due to weak electrostatic adsorption force of nano TiO_2 that systemically interact with the plasma proteins in circulation. Although, this concept is challenged by our group as recently we successfully delivered tetra sulphonatophenyl porphyrin (TSPP) to the inflamed joints by the TiO_2 nanowhiskers in the photodynamic therapy for rheumatoid arthritis early diagnosis by fluorescent bio-imaging and treatment[135] .

3.2 Sonodynamic therapy with nano TiO_2

Recently the ultrasound activated sensitizers dragged attention and got popularity in remedy of deep seated tumors and other infectious diseases, attributed to deep penetration ability of ultrasound radiations. In 1989, Umemura et al were the first who gave the concept of sonodynamic therapy by ultrasonically activating hematoporphyrin, which was well known photosensitizer back then[136].

Most of the sonosensitizers used are organic macromolecules (porphyrin derivatives, phthalocyanines, 5- aminolevulinic acid etc.) that produce ROS upon ultrasonic activation[137-139]. Harada and his co-workers found that TiO_2 nanoparticles activation by ultrasound radiation could be utilized to treat neoplastic melanoma C32 *in vitro* as well *in vivo* [140]. They activated the nano TiO_2 by 1 MHz, 1.0 W/cm^2 for two minutes and found significant decrease in the volume and growth suppression of neoplastic tissue (Fig 6).

Shen et al combined Fe_3O_4 @ TiO_2 (core @ shell) nanoparticles and chemotherapeutic agent, i.e., doxorubicin (DOX) to evaluate its potential for successful delivery to the neoplastic tissue and termed as chemo-sonodynamic therapy[141]. They found that TiO_2 irradiation with ultrasound generated the ROS and ensured the safe delivery and retention of chemotherapeutic agent post intravenous injections in murine models. Shen et al also used Fe_3O_4 @ TiO_2 nanoparticle for sonodynamic therapy of cancer cells and found more than 50 % reduction in cancer cell viability[142]. The synergistic effect of chemo-sonodynamic therapy of $NaYF_4$-Fe_3O_4 @ TiO_2 nanocomposites with hyaluronic acid coating for cancer was explored by Shen et al [143]. They reported successful DOX loaded nanocomposites uptake by MCF-7 and KB cells *in vitro*. In xenograft models the combined synergistic inhibitory effect of chemo-sonodynamic therapy was 88.36 % as compared to 38.91 and 28.36 percent for separate sonodynamic and chemotherapy, respectively.

Yamaguchi et al used TiO_2-PEG nanoparticles to explore their potential as sonodynamic and photodynamic therapeutic effect on human glioblastoma cells U251. They activated the TiO_2-PEG sonosensitizers at 5.0 mW/cm^2 and evaluated its effect by MTT assay[144]. Their results demonstrate promising role of TiO_2-PEG sonosensitizers by significantly decreasing the viability and membrane integrity in the subject tumor cell line.

Ninomiya et al reported S1/S2 protein mobilized TiO_2 nanoparticles for sonodynamic therapy of HepG2 cancer cells *in vitro*[145] and xenograft tumor murine models *in vivo* [146]. Ultrasonic activated TiO_2 nanoparticles activating at 0.5 MHz and 1 MHz for 60 seconds induced apoptosis and reduced viability by 46 % in the HepG2 cells. Similarly when 0.1 mg TiO_2 nanoparticles were directly injected to *in vivo* tumor tissue, five times in 13 days the tumor was reduced within 28 days.
The $^1O_2$ singlet oxygen generation by TiO_2 nanoparticles is well known after photoactivation, recently, Harada et al also reported $^1O_2$ oxygen generation by the TiO_2 nanoparticles with core-shell type polyallylamine micelles, when these TiO_2 containing miscalls were introduced to HeLa cancer cells they generated $^1O_2$ along with other ROS after sonoactivation [147]. These findings are very important and can revolutionize the sonodynamic therapeutics in terms of bio-imaging and theranostics especially for neoplastic maladies.

3.3 Nano TiO_2 for medical implants

Medical implants have revolutionized the orthopedic surgery, especially the endoprosthetic surgeries. However the autoimmune reactions against the orthopaedic implants and cardiovascular stents hamper their liberal applications. Various metallic combinations has been used so far, but the Ti and its various alloys are reported most promising in reduction of auto immune reactions, especially the nano TiO_2 surface coated implants are pertinent to mention. The thin passivation layer of nano sized TiO_2 minimize the autoimmune reaction between the underlying bone tissue surfaces and implant [148].
TiO_2 nanotube implants are used in prosthetic articular surgery of orthopedic patients with tibia-tarsal joint (knee) or acetabulum in hip joint fractures, however, their success rate is not very appreciated due to degradation of the materials or promoting the generation of chronic inflammation within the implanted tissues [149, 150]. Nevertheless, some studies suggest that nano TiO_2 provide more safe scaffolds for the tissue recognition in the endo-prosthetic surgery [151, 152]. Many studies reported that TiO_2 nano tubes enhance the bone mineralization, osteoblast adhesion in vitro and strong bone adhesion *in vivo* [153-155].

In biomedical, especially in orthopaedic plating [156, 157] and stenting in cardio vascular system (CVS), it was found that when the surface was coated with nano TiO_2 or submicron particles, the macrophages chemotaxis was appreciably reduced and delayed the chances of thrombosis as post-operative complications, one of the leading cause of stenosis and complication in CVS stenting [158-160]. The Ti-6Al-4V (Ti-Alloy) is most common alloy used in medical implants [161], due to excellent corrosion properties by spontaneous formation of 2-3 nm oxide film on the alloy surface [162-164]. Nevertheless, this corrosion property is limited by native oxides that jeopardize the strength of titanium alloy [165]. Benea et al increased the surface thickness of the Ti- Alloy from 23.06 nm to 123.35 nm by using anodic oxidation technique for deposition of TiO_2 nanoparticles, which resulted in safer and better tribo-corrosion than untreated Ti-Alloy [166]. Jin and his coworkers found that TiO_2 nanotubes (< 100 nm diameter) had excellent osteoblast adhesion properties with elongated nuclei and up regulated alkaline phosphatase enzyme, which suggest the excellent biocompatibility and interaction with bone tissue for orthopedic implants [167].

The concept of antibiotic loading on prosthetic implants as prophylactic therapeutic agent is getting popularity in biomedical realms. Recently, Chennell et al used various types of TiO_2 nano fibers, tubes and smooth surfaces for loading and eluting of the Cefuroxime as antibiotic on the orthopedic implants [168]. They found the peak drug release at two minutes time interval from the TiO_2 nanotubes with highest concentration release, when compared to other nano structures. Some studies also revealed successful loading and eluting of the gentamycin, a broad spectrum antibiotic on the surface of TiO_2 prosthetic implants and found its inhibitory effect up to six days on the *Staphylococcus aureus* during *in vitro* culture sensitivity trail[169]. Kumeria et al and Popat et al loaded gentamycin on the TiO_2 nanotubes array electrochemically anodized on the titanium surface to inhibit the *Staphylococcus epidermis* (Gram positive) bacteria and adhesion to osteoblast cells *in vitro* [170, 171]. They found excellent osteoblast adhesion differentiation and proliferation that should be a key feature for any orthopedic prosthetic implant and keep check on the postoperative bacterial infections. TiO_2 prosthetic implants in hip replacement were coated with hydroxyapatite and poly cyclodextrin to load the two different antibiotics, i.e., tobramycin and rifampicin, to control the postoperative *Staphylococcus aureus* and *Enterobacter cloacae* [172]. This dual drug loading synergistic effect on the TiO_2 implants was found much efficient to keep check on the infective post-surgical complications. These findings provide new approaches for prophylaxis of prosthetic surgery post-operative infections as common complication by using TiO_2 as drug delivery vehicle and core prosthetic implant.
Zhao et al used chitosan hydrogel mesoporous silica nanoparticles on the titanium surface as pH and electro responsive to release the Ibuprofen i.e. non-steroidal anti-inflammatory drug (NSAID) [173]. This approach is amenable to suppress the post-operative inflammation. Moreover, the combined antibiotic and NSAID loading on the same TiO_2 prosthetic implants can efficiently decrease the postoperative complication of infection and inflammation due to prosthetic surgery.

3.4 Nano TiO_2 for theranostics

The therapeutic effect of TiO_2 is solely dependent upon the generation of ROS including $HO_2^{\cdot}$, $O_2^{\cdot-}$, $OH^{\cdot}$ and $^1O_2$, irrespective of their shape or crystalline form. However, the various isomorphs can effect extant of the ROS generation due to their reactivity variation, i.e., anatase has been reported more reactive than rutile. The $^1O_2$ successful generation has been reported from nano TiO_2 with size more than 10 nm by NIR phosphorescence, whereas nano TiO_2 with less than 10 nm size were unable to generate $^1O_2$ due to smaller size particles inability to retain the charge separation [174]. Certain short half-life ROS, i.e., $^1O_2$, $OH^{\cdot}$, can interfere with intracellular signal pathways and induce local membrane lipid per oxidation attributed to weaker diffusion, while the relatively long half-life ROS, i.e., H_2O_2, $O_2^{\cdot-}$, $HO_2^{\cdot}$, can strongly diffuse intercellular with potential to induce apoptosis by interfering with neighbouring cells signal pathways [175].

In some studies the potential of nano TiO_2 as drug delivery vehicle has also been explored because it is easy to adsorb the therapeutic agent and presence of ample number of surface electron provide scaffolds for covalent or ionic bonding to ensure successful delivery and release in the target tissue via special microenvironment trigger, i.e., pH, osmotic pressure, blood flow, EPR etc. The surface highly reactive uncoordinated electrons have strong affinity to the $OH^{\cdot}$ group in oxygen rich molecules[176], that can be one of the excellent approaches to target the specific cell receptors in drug delivery system.

Recently, our group used TiO_2 nanowhiskers to mitigate the adverse effects of TSPP on circulatory and excretory system both *in vitro* and *in vivo*[177]. Porphyrin derivative's well-known adverse effects (e.g. neuro toxicity, photo toxicity, accumulation in other than target tissue etc.) have limited its potential use for biomedical applications. However, TiO_2 nanowhiskers porous nature and excellent vehicle properties reintroduced the porphyrin derivatives to biomedical realms, especially for neoplastic maladies theranostics.

Veerachandra tested various commercially available combinations of TiO_2 isomorphs on the *Escherichia Coli* samples for food sanitation and found that *Degusa* (P25) was more efficient than other combinations, moreover, they also observed that the germicidal effect and sanitation properties of P25 were increased with an increase in light intensity [131].

The titanate, another form of TiO_2 micro- and nano-particles has got popularity in recent years. The monosodium titanate (MST) particles are efficient for biomedical applications, especially as suppressor of human squamous cells carcinoma by holding 30 % mitochondrial activity[178], however, non-lethal effects of MST were reported on the oral bacterial infections [179], and least effective against the murine fibroblasts L929 and human monocytes THP1 [180, 181]. However, Wataha et al reported successful delivery of MST metal ions to the monocytes and fibroblast cells and affected the cellular functionality [182]. Originally the MSTs were designed for the decontamination of nuclear waste materials as they are good ion exchangers. However, later on it was found that they can be efficiently bound to the biologically active metals [183]. Recently Drury et al demonstrated that nano MST are more efficient in biological activities than macro MST [34].

Sandoval et al used Eurpoium-III (Eu^{3+}) doped TiO_2 nanoshells coated with polyethyleneimine for in vitro cancer cells (HeLa) photo diagnosis[184]. This system could provide scaffolds for cancer theranostics, if anticancer agent is coupled with the TiO_2 nanoshells.

Nano TiO_2 as vehicle in drug delivery system is more sensitive to pH change. Zhang et al reported that fabricated TiO_2 nanocomposites were pH sensitive that selectively delivered DNR to the tumor cells. It is well documented that due to higher metabolic rate pH is lower in neoplastic tissue than normal tissues (7.4) [185].

In stem cell biology nano TiO_2 is also getting popularity. Recently Lavenus et al reported successful tailoring of nano TiO_2 on the surface of glass slides with different angles to investigate its effect on the human mesenchymal stem cells. It was observed that subtle deviation in the coating angle of nano TiO_2 influenced the cell behavior that was evaluated by cellular pathways viz differentiation, proliferation, migration and cellular morphology alteration [186]. (Fig 7) Similarly, in neural stem cells the endocytosis and exocytosis of nano TiO_2 irrespective of their shape and size were investigated by wang et al, where they found nano TiO_2 exocytosis within 72 hours. These findings vouch the safety of nano TiO_2 and can be used as land mark for future stem cells investigations[187].

3.5 TiO_2 role in nano drug delivery system

The nano drug delivery system is tailored to be highly specified and targeted to achieve its desired concentration and safe drug release in subject system or tissue. The targeted therapy not only provides scaffolds for safe delivery but also allow combination of various therapies, e.g., combination of photothermal therapeutic agent with chemotherapeutic agent (Doxorubicin, Daunorubicin etc.) or others[99]. Currently gold and silver nanoclusters, MWCNT, platinum, iridium and titanium are in vogue for nano inorganic drug delivery systems (NI-DDS) with multiple therapies. Among them various NI-DDS titanium has been recently reported with promising sonodynamic, photodynamic therapeutic and delivery agent for cancer and other infectious diseases[146]. (Fig 8)

Recently Yin et al conjugated the mesoporous TiO_2 up converting nanoparticles with Hyaluronic acid and Doxorubicin for cancer therapy[188]. These up converting nanoparticles convert the NIR to UV light for the PDT with mesoporous TiO_2 in the tissue and Hyaluronic acid ensure the controlled drug release of chemotherapeutic agent (Doxorubicin) within the neoplastic tissue. Kawashita et al formed TiO_2 microspheres including SiO_2 nanoparticles and loaded with Rhodamine B as model hydrophilic drug and found rapid elution of Rhodamine B for first six hours[189]. They exploited the TiO_2 (Anatase/Rutile polymorphs) potential in drug delivery system, however, for clinical application, the burst release of the loaded drug may be problem that needs to be addressed accordingly [190]. The TiO_2 nanoparticles were also used as photoprotectant in sunscreens and vehicle in drug delivery system to release the terpinen-4-ol during topical applications.

The porous nature of nano TiO_2 has been widely investigated as vehicle for drug delivery system. Recently Wang et al modified porous TiO_2 nanoparticles with polyethylineimine to deliver anticancer drug (paclitaxel) to the neoplastic tissue by additionally conjugating with folic acid for site specify[191]. The TiO_2 was photoactivated by UV light as trigger for drug release by producing super oxides and polyethyleneimine was used to prevent premature drug release until attaining its concentration in desired tumor. The porous TiO_2 nanoparticles not only produced the ROS but also released the chemotherapeutic agent upon photoactivation. This additive property can be efficiently explored for cancer and other maladies amelioration.

Yuan et al developed protocol to ensure safe delivery of photoactivable nanoparticles to the cytoplasm of HeLa cancer cells [192]. They specially designed Fe_3O_4 @ TiO_2 nanoparticles to attach the epidermal growth factor receptor (EGFR) that ensured the safe delivery of nanocomposites to cellular nucleus without interfering and compromising the EGFR and

karyopherin-β interaction, a key protein factor for nucleus translocation. After photoactivation, they found more DNA double strand breakage due to the effect of those photosensitizers which remained in the cytoplasm.

4. Adverse effects of nano TiO_2

Mostly nanomaterials that are known to be inert when injected into the body they perform totally different [193]. Our body exposure to the nanomaterials may be either oral, transdermal, or via inhalation, which exert adverse effects on our various body systems[7] and vital organs including respiratory system [194, 195], gastrointestinal tract [19, 196, 197], reproductive system [7], excretory system (kidney, liver), circulatory and nervous system [198], etc. The length and rigidity of nanomaterials hamper their clearance from pulmonary tissue provoking the release of inflammatory mediators, e.g., multi-walled carbon nano tubes (MWCNT), nano-titania, platiunum etc. [44, 199].

TiO_2 nanomaterials are one of the most commonly studied nanomaterials for pulmonary toxicity. Nano TiO_2 was considered as the safest among all nanomaterials in vogue, until it was reported as potential carcinogen when murine models were exposed *in vivo* for prolonged duration [200] and then it was ranked as 2 B human potential carcinogen [201, 202]. It has been reported that chronic and sub chronic exposure of nano TiO_2 leads to inflammation, epithelial hyperplasia and pulmonary carcinoma in murine models [193]. Moreover, TiO_2 nanoparticles are considered more toxic than nanowhiskers, similarly larger size is reported less toxic than smaller sized TiO_2 [203].

4.1 Sexual health

The widespread use of TiO_2 nanoparticle has led to severe adverse post therapeutic complications in animals in general and advanced primates including humans in particular [204], notably in the reproductive system by decreasing the viability and proliferation of laydig cells [205, 206] and decreased follicle survival rate, less griffin follicles and altered morphology of the follicle and oocyte in murine models [207]. When TiO_2 nanoparticle were injected to the pregnant mice, then their offspring became infertile with abnormal development of seminiferous tubules, decreased steroli cells number and increased spermatozoa primary abnormalities [208, 209].

TiO_2 nanoparticles can cross the blood testis barrier and damage the testicular interstitial tissue or sertoli cells by upregulating the caspase 3 to induce apoptosis and down regulating the anti-oxidative enzymes i.e. Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Catalase (CAT), Glutathione S-Transferase (GST) etc., leading to the increase of Reactive Oxygen Species (ROS) production [7]. Super oxides ($O^{\cdot-}$) are formed when oxygen acquires additional electron, these $O^{\cdot-}$ are scavenged by the SOD, converting them to H_2O_2 and $^{-}O_2$ [210, 211]. The DNA oxidative damage has also been reported by many studies, suggesting that the smaller size of TiO_2 nanoparticles (< 20 nm) can cause the damage to DNA double helix along with micronuclei formation lipid peroxidation of membranes within the cell either in presence or absence of the photoactivation [212, 213].

4.2 Nervous System

In Central Nervous System (CNS) studies it has been reported that intravenous nano TiO_2 induce oxidative stress to brain by increasing the ROS production and decreasing the anti-oxidative enzymes and melatonin level. Moreover, they also activate the caspase 3 that initiates the apoptosis in the brain tissue [214]. The TiO_2 nanoparticles can translocate in (CNS) via olfactory pathways and cause lesions in brain including the neuronal vacuoles and fatty degeneration of hippocampus in murine models *in vivo* [47, 52]. In recent studies Younas et al also reported that intraperitoneal 20 mg/kg $_{bwt}$ TiO_2 nanoparticles at alternate day could alter the neurobehavioral performance, and elevate the serum enzymes (ALT, AST, LDH), indicating the additional hepatic malfunction also in murine models [50].

4.3 Musculoskeletal system

In Musculoskeletal system the role of nano TiO_2 is very vital, because in many orthopedic implants and plates' nano TiO_2 has been used to suppress the auto immune reactions and inflammatory response in the bone tissues. Nevertheless, there are some studies suggesting toxic role of TiO_2 nano particles on the osteoblast cells by generating more ROS, hamper TiO_2 potential use in prosthetic surgeries. Recently it was reported that nano TiO_2 damage the double helix DNA structure of bone marrow cells post oral intake and also promote the proinflammatory genes in the pro-osteoblast cells (MC3T3-E1 cell line) [17, 215]. TiO_2 nanoparticles (15 nm) cause the impairment of SOD1, SOD2 (Mn SOD) and sirtuin 3 protein that is present in mitochondria and responsible for an increased catalytic activity and homeostasis in human osteoblast cells (hFOB 1.19), moreover, the study also revealed ultrastructure alterations in the osteoblast cells [216, 217]. (Fig 9).

The cardiac arrhythmias and compromised myocardial function has been reported after administration of TiO_2 nanoparticles to the rat models[198]. Earlier, it was also reported that TiO_2 nanoparticles less than 100 nm cause endothelial cells leakiness post 60 minutes exposure, whereas the relatively larger size (more than 600 nm) remained inert [218]. This approach can be ideal in the studies where cancer amelioration is targeted by normalizing the tumor vasculature[219]; however, the consequences can be more drastic on the surrounding healthy tissues if TiO_2 accumulate in other than target tissue.

In another study when TiO_2 nanoparticles were used for orthodontic applications, it was found that it accumulated in vacuoles of gingival fibroblast cells without compromising the sensitivity of human oral squamous cellBiomaterialsne (HSC-2)

and cytotoxic effects on the surrounding tissues, however, nano TiO_2 increased the prostaglandin E2 production in synergy to IL-1B as proinflammatory action of TiO_2 nanoparticles [220].

5. Conclusions

In summary, from the aforementioned properties of TiO_2 nanoparticles and nanowhiskers it is evident that TiO_2 can be excellent photosensitizer and oxidizing agent for biomedical applications and as a vital part of nano-biotechnology and photodynamic therapy. The relatively lower toxic effects, existence in various polymorphs, economical, easy availability and excellent biocompatibility to biological systems declare its potential vital role in nanomedicine and biomedical realms. The important role of nano TiO_2 and relevant nano-biotechnology in early theranostics of neoplastic and non-neoplastic maladies and prosthetic surgeries is exponentially increasing every day. Nevertheless, for best treatment efficiency we need to realize the surface functionalization of nano TiO_2 and bring current photoactivation energy transfer band (400 nm) to the therapeutic window that is more than 600 nm excitation wavelength, approved by WHO.

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Figure Captions:

Fig. 1 Schematic presentation of enhanced permeability and retention (EPR) effect in tumor tissue. The nanomolecules can easily cross the blood capillaries endothelial cells whereas micromolecules can only retain in tumor tissue due to larger intercellular space than normal tissue.

Fig. 2 Fluorescence microscopic results showing SMMC-7721 cancer cells treated with DNR (a), DNR-TiO2 nanoparticles (b), DNR-TiO2 Nanowhiskers (c), whereas (d) represent the possible mechanism for cellular uptake of TiO2 nanowhiskers in SMMC-7721 cancer cells. Adapted from ref 46 Copyright © 2009 Elsevier Ltd.

Fig. 3 Brain tissue histopathology (H&E stain) after 60 days TiO2 nanoparticles administration in ICR mice. Here (a) shows normal brain tissue at 100x; (b) demonstrate 5 mg/kgbwt TiO2 nanoparticles administration resulting in higher calcium deposition in brain tissue (400 x); (c) demonstrate 10 mg/kgbwt TiO2 nanoparticles administration result in spongiocyte proliferation indicating pathological lesions (200 x); (d) is 10 mg /kgbwt administered TiO2 nanoparticles indicate proliferating ependyma (100 x); (e) is 50 mg/kgbwt administered TiO2 nanoparticles indicating the calcium deposition (400 x) whereas (f) is 50 mg/kgbwt TiO2 administered group with spongiocyte proliferation. Adapted from ref 52 Copyright 2010 Elsevier Ltd.

Fig. 4 Schematic presentation of ROS and singlet oxygen generation from TiO2 when photoactivated with UV range light (A). Fluorescence generated from nano TiO2 after photoactivation in fibroblast cells, in vitro (B) and murine model rheumatoid arthritis foot in vivo (C).

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Fig. 7 Cell morphology of hMSCs on the different substrates. A. Staining of characteristic cell morphology observed on the surface (Actin in green, nucleus in blue, Scale bar: 25 μm). B. Projected cell area as a function of surface deposition angles. C. Distribution of the cell morphology on the different substrates (Mean ± SEM, *p<0.05/***p<0.001 compared to TCPS). Reprinted after permission of ref 186 Copyright 2015 Elsevier LTD.

Fig. 8 Chemotherapeutic agent intake by the drug resistant leukemia cells (K562) in the presence of nano TiO2. Reprinted after permission of ref 29 Copyright 2006 Elsevier LTD.

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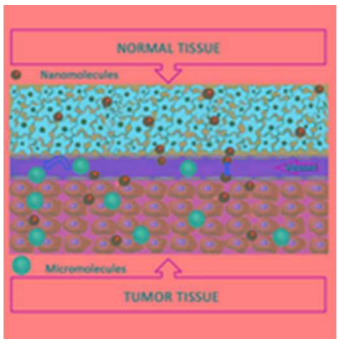

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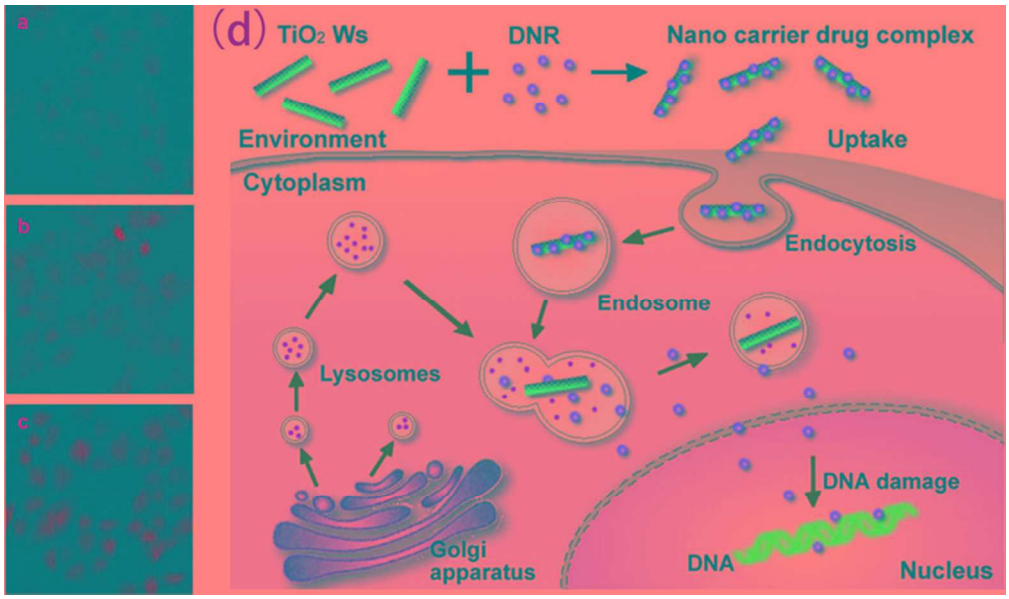

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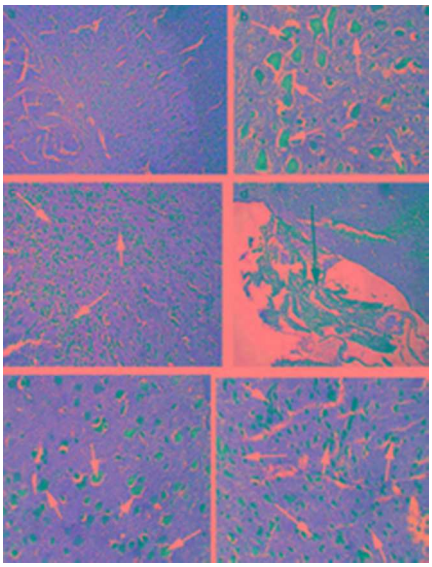

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Fig. 4 Schematic presentation of ROS and singlet oxygen generation from TiO2 when photoactivated with UV range light (A). Fluorescence generated from nano TiO2 after photoactivation in fibroblast cells, in vitro (B) and murine model rheumatoid arthritis foot in vivo (C).
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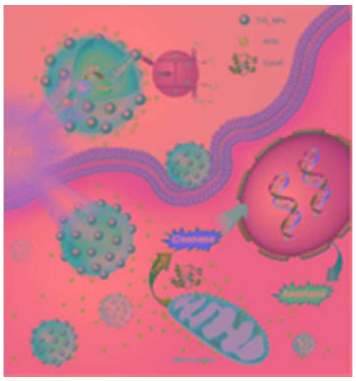

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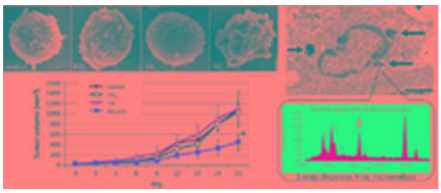

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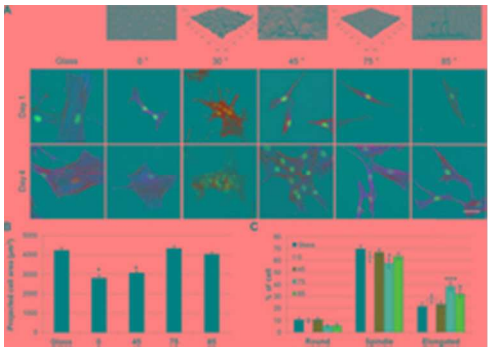

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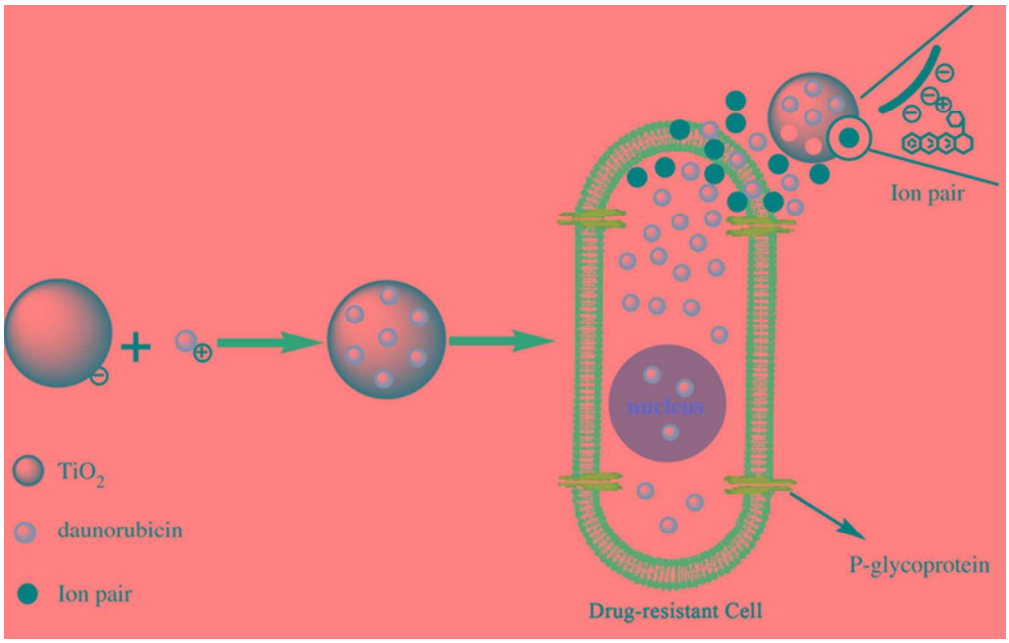

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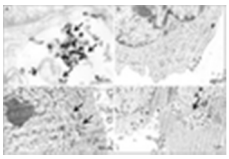

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