Aniruddha Adhikari, Susmita Mondal, Soumendra Darbar, Samir Kumar Pal*

Role of Nanomedicine in Redox Mediated Healing at Molecular Level

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Abstract: Nanomedicine, the offspring born from the marriage of nanotechnology and medicine, has already brought momentous advances in the fight against a plethora of unmet diseases from cardiovascular and neurodegenerative to diabetes and cancer. Here, we review a conceptual framework that will provide a basic understanding about the molecular mechanism of action of a therapeutic nanomaterial inside biological milieu. In this review, we highlight how the catalytic nature of a transition metal oxide nanomaterial influences the cellular redox homeostasis, supports the cellular antioxidant defence system and reactivates the reactive oxygen species (ROS) mediated signalling to perform normal cell functions like cell cycle, differentiation, apoptosis, inflammation, toxicity, and protein interactions. With numerous examples, we describe the redox modulatory nature of d-block metal oxide nanomaterials and their biomimetic nanozyme activities to protect the mitochondria, the cellular redox mediator which prevents an organism from various diseases. This knowledge will be useful to design new nanomaterials capable of intracellular redox modulation, which in turn can be effective therapeutic agents for treatment of various unmet diseases that are beyond the ability of modern synthetic medicine.

Keywords: Nanotherapy; Cellular redox homeostasis; Nanozyme; Reactive oxygen species; Surface functionalization.

Introduction

In this article, we review a conceptual framework that offers an understanding of how a redox active nanomaterial incorporated into cellular system can function as a therapeutic agent through the modulation of intracellular redox homeostasis. The concept is based upon the nature of the electron exchange processes (i.e., redox: both reduction and oxidation) that evolved into the nanomaterial due to quantum confinement and surface modifications with bioactive ligands. The redox activity is essential for synchronized functioning of whole-body metabolism which is a multi-layered system comprising of intracellular, intercellular, and inter-organ electron exchange processes [1]. This review will provide substantial insight into the chemical and biological basis of nanomedicine action within the body, and may explain the importance of nanomaterial induced redox stress in treatment of human diseases. We hope this review will particularly help in bridging the gap between application based knowledge about nanomedicines and limited understanding of their molecular mechanism of action, which is considerably different from the familiar ‘one drug one target’ paradigm of modern synthetic drugs. Although a variety of nanomaterials can be classified as ‘nanomedicine’ according to the definition provided by the European Science Foundation [2, 3], for the sake of simplicity and understanding, we limit contents of this review to only transition metal oxide nanomaterials which have fascinating potential as nanomedicine.

Cellular redox homeostasis: the good and bad of reactive species

Life nearly in all its aspects is intricately linked to the intracellular redox homeostasis and its modulation. Intracellular redox potential is dependent on an intricate balance between the intrinsic and extrinsic reactive oxygen species (ROS) and the range of antioxidant mechanisms such as glutathione, thioredoxin, nicotinamide adenine...
dinucleotide phosphate (NADPH), and intracellular H\(^+\) content (pH). In this regard, one should remember that there is no overall cellular redox state \([4]\). Rather, a given physiological or pathophysiological situation is characterized by hugely different set points of redox couples (e.g., reduced nicotinamide adenine dinucleotide (NADH) system, reduced glutathione system) operating concurrently. Before going into a detailed discussion, it is worth mentioning that deviations from the aforementioned set points of redox couples are utilized for redox signalling which is critically important in cell cycle, differentiation, apoptosis, inflammation, toxicity, and protein interactions. Conversely, dysregulation of cellular redox balance, particularly to prooxidative states (i.e., oxidative stress), is implicated in the initiation and progression of several disease states including cardiovascular disease, neurodegenerative disease, diabetes, and cancer \([5, 6]\).

Free radicals can be produced from both endogenous and exogenous sources. Amongst them, two major endogenous sources are mitochondria and the family of NADPH oxidases (NOXs) (Figure 1) \([7]\). Amongst the eleven sites within mitochondria that produce ROS, the three best characterized sites are complex I, II, and III within the electron transport chain (or mitochondrial respiratory chain) located in the inner mitochondrial membrane. These complexes generate O\(_2^{-}\) by one-electron reduction of molecular O\(_2\), and release it into the mitochondrial matrix where superoxide dismutase-2 (SOD2) rapidly converts the

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**Figure 1: Cellular maintenance of redox homeostasis - the good and bad of ROSs.** In aerobic organisms, many processes produce O\(_2^{-}\) including cytosolic xanthine oxidase (OX), the cytochrome P450-monoxygenases (CYP) in the endoplasmic reticulum (ER), the mitochondrial electron transport chain (ETC), and NADPH oxidase (NOX). NOX is a membrane-bound enzyme complex that can be found in the plasma membrane as well as within intracellular membrane structures or vesicles. O\(_2^{-}\) produced by the plasma membrane–bound NOX (e.g., NOX2) can act both intra- and extracellularly. H\(_2\)O\(_2\) produced by superoxide dismutase (SOD) outside the cell can transverse into the cell interior in part through aquaporin channels to initiate intracellular signalling, whereas O\(_2^{-}\) could influx through the chloride channel-3. The intracellular NOX complexes produce ROS in the lumen of a vesicular compartment, where ROS acts locally or from which it enters the cytosol. H\(_2\)O\(_2\) has been implicated in ROS signalling through oxidative modification of critical redox-sensitive cysteines in signalling proteins. This helps in maintaining cell survival, growth, metabolism and normal cellular processes. Coordination among different intracellular antioxidant enzyme systems (e.g., SOD, CAT, GR, GPX) provides an important mechanism for fine spatial control of ROS homeostasis and signalling. CAT, catalase; GPX, glutathione peroxidase.
radical to $H_2O_2$. Complex III can also release $O_2^•$ into the intermembrane space. $O_2^•$ can traverse through voltage dependent anion channels located at mitochondrial membrane to the cytosol, and is subsequently converted into $H_2O_2$ by SOD1. NOXs are membrane proteins localized to the plasma membrane, endoplasmic reticulum (ER), and mitochondria. NADPH donates an electron to the core of the NOX catalytic subunit to generate $O_2^•$ through the one-electron reduction of $O_2$. SOD1 in the cytosol converts the $O_2^•$ radicals to $H_2O_2$. Recent studies have showed that $H_2O_2$ functions in ROS signalling through oxidative modification of critical redox-sensitive cysteines in signalling proteins. Excess $H_2O_2$ in the presence of bivalent cations (e.g., Fe$^{2+}$, Cu$^{2+}$) can catalytically decompose (through a Fenton like reaction) into OH$•$ radicals, which in turn can cause lipid peroxidation and DNA damage. On the other hand, $O_2^•$, if not controlled properly, can damage the iron-sulfur clusters of proteins, causing dysregulation of downstream functions. Similar to the functions of SOD1 and SOD2, several other spatial and temporal strategies exist to maintain the delicate levels of intracellular ROS. For example, peroxiredoxins and glutathione peroxidases (GPX) present in the cytosol and mitochondria convert $H_2O_2$ to water. Catalase (CAT) performs the same function in peroxisomes. The aforementioned protective pathways that drive ROS-detoxification as well as ROS mediated signalling are outlined in Figure 1.

A coordinated regulation process also exists for other reactive species like reactive nitrogen species (RNS) (e.g., nitric oxide, nitrogen dioxide, peroxynitrite), reactive sulfur species (RSS) (e.g., persulphides, thiosulfate), reactive carbonyl species (RCS) (e.g., α,β-unsaturated carbonyl compounds, dialdehydes), and reactive selenium species (RSeS) (e.g., selenocysteine, selenomethionine).

Oxidative stress, mitochondria, and disease

As discussed in the earlier section, free radical production occurs continuously in all cells as part of normal cellular function. However, uncontrolled formation of free radicals originating from either exogenous or endogenous sources (i.e., oxidative stress) are associated with a number of pathophysiological conditions.

Figure 1 depicts the inevitable role of mitochondria in the whole process of redox regulation within the cell. For years, mitochondria were known to perform many key roles in the cell, most notably oxidative phosphorylation, central carbon metabolism, and the biosynthesis of intermediates for cell growth [4, 8, 9]. However, in the last few decades our knowledge regarding the cellular function of mitochondria has changed dramatically. It is now clear that mitochondria not only function as cellular power houses, but also participate in nearly all aspects of cell function, affecting processes not traditionally linked with the organelle, including cancer, inflammation, metabolic signalling, cell death, transformation, and fate [8, 10, 11]. Hence, mitochondrial dysfunction could be linked to many common disorders, including neurodegeneration, metabolic disorders, and heart failure (Figure 2) [8, 10, 12]. Thus, therapies targeting mitochondria might be useful as targets to treat these secondary mitochondrial diseases. The repeated findings that mitochondria contribute to the pathology of several diseases by elevated ROS production, oxidative damage, carbon stress, disruption to calcium homeostasis, induction of mitochondrial permeability transition pore (MPTP), accumulation of protein aggregates, and elevated inflammation suggest that a similar pattern of mitochondrial damage underlies contrasting pathologies, enabling mitochondria targeted drugs to be applicable to many diseased conditions [8]. However, several obstacles exist in the development of mitochondria targeted therapeutics, including biological barriers and mitochondrial toxicity [13]. Once the drug has reached the target cell and entered the cytoplasm, it has additional barriers including intracellular diffusion/transport to the mitochondria and outer and inner mitochondrial membranes [13]. Functionalized nanosystems, in this regard, could provide unique and more effective tools to design mitochondrial therapeutics that either target or avoid mitochondria. While nanosystems targeting mitochondria can be used to enhance efficacy in treating mitochondrial diseases, those that avoid mitochondria might be useful in reducing mitochondrial toxicity. Whatever may be the route, the nanosystem has to have a mechanism of reducing mitochondrial oxidative stress (antioxidant action), and the molecular mechanism that the nanosystem takes to achieve the goal is the main focus of this review.

Antioxidants in health and diseases

Given the enormous range of reactivity of oxidants with a particular target, and conversely, given the similar diverse range of targets, multiple strategies have been undertaken by living cells and organisms to counteract oxidative challenge [4]. An antioxidant can either be an exogenous or endogenous molecule or nanomaterial that
Figure 2: Mitochondrial damage due to overproduction of ROS and its contribution to various disease families. Mitochondrial damage can cause pathogenesis to almost every organ systems of the body including neuronal, hepatic and cardiovascular systems.
possesses the ability to reduce or inhibit the autoxidation of certain oxidizable molecules [14, 15]. Autoxidation can be initiated in vitro in a variety of ways that yields radicals. These processes include electron transfer mechanisms involving transition metal oxides and hydroperoxides, or haemolytic cleavage of weak bonds induced by light or heat (Figure 3). The way by which a radical is formed dictates the nature of the radicals (X•), which can be hydroxyl (HO•), alkyl (R•), alkoxyl (RO•), or hydroperoxyl (HOO•) radicals. The initiating radicals yield R• radicals by H-atom abstraction from a substrate RH which further react with oxygen in a diffusion controlled rate to form peroxyl radicals (ROO•). The peroxyl radicals (ROO•) on further reaction with the substrate to generate hydroxides and R• radicals, establishing a chain-reaction. The chain reaction proceeds for several cycles before the interaction of two radicals quench each other, terminating the reaction (Figure 3). Antioxidants prevent free radical induced tissue damage (described earlier) by preventing the formation of radicals, scavenging them, or by promoting their decomposition [16]. Thus, depending upon their mechanism of actions, antioxidants are classified into two groups, preventive and chain-breaking.

Preventive antioxidants, a heterogeneous class of compounds (e.g., sunscreens [17], metal chelating agents [18, 19] and hydroperoxide decomposing molecules [20] and enzymes like GPX, superoxide dismutase (SOD) or their mimics [21]) function by reducing the initiation rate [22]. Another class of antioxidants called chain-breaking or radical–trapping antioxidants reduce or inhibit the process of autoxidation of molecules by competing with the generated radicals in the propagation step, i.e. the peroxyl radicals react more rapidly with the oxidizable molecules than with the substrate. This reaction between the antioxidant (AH) and peroxyl radical (ROO•) also involves a transfer of H atom to yield hydroperoxide and the antioxidant radical (A•). This antioxidant radical further reacts with a peroxyl radical (ROO•) generated during the propagation step, thereby forming a non-radical end product [23]. Most of the members of this class of antioxidants get consumed during the occurrence of the reaction, and thus, act in a stoichiometric fashion. Some popular naturally occurring chain-breaking antioxidants include tocopherols (vitamin E), flavonoids, stilbenes (e.g. resveratrol), and ascorbate (vitamin C) [14, 18].

Just like natural or exogenous antioxidants, nanomaterials can have both attributes, preventive and chain-breaking (Figure 4). However, their antioxidant capacity can be inherent or generated from covalent functionalization (detailed in next part of the article). Although, several studies showed that dietary antioxidants can reduce ROS levels to counteract various diseases, clinical trials provided evidence that the dietary antioxidants are mostly ineffective inside the
One of the possible reasons for this failure is the inability of the antioxidant to reach biologically relevant targets at required concentration. To address this issue, nanoantioxidant formulations have been fabricated so that they are not only biocompatible and target specific, but also have prolonged stability when compared to other small molecules. The vast and widening field of nanoscience dealing with delivery and release of antioxidants covalently bound on the surface (nanoencapsulation [32], inclusion in biodegradable [33, 34], or in solid lipid nanoparticles [35], loading in naotubes [36], or mesoporous materials [37]) is beyond the scope of this review, as their antioxidant capacity is directed by the release of small molecule antioxidant in solution, which has previously been elucidated in greater details. This review focuses on the inorganic nanomaterials which are intrinsically free radical scavengers with or without organic ligands covalently bound to surfaces.

**d-block materials as redox modulatory biomimetic nanozyme**

The d-block metal oxide nanomaterials exhibit some common physicochemical features. Almost all d-block metal oxides in nanoform show somewhat antioxidant activity. Unlike the dietary or natural antioxidants, the free radical scavenging activities of the aforementioned metal oxide nanomaterials are repetitive. To be specific, these metal oxide nanomaterials are catalytic in nature, i.e., they function as an enzyme in the redox cycle. The multivalence nature of the metal moieties plays a crucial role in redox modulation by alternating the surface charge depending upon the cellular microenvironment. The redox active metal oxide nanomaterials can accept electron from free radicals in the oxidized form and become reduced. And depending on the cellular microenvironment they can again be oxidized and restart free radical scavenging activity.
The d-block metal oxides starting from TiO$_2$ to ZnO can display ‘enzyme-like’ catalytic activity (Figure 5), which is useful for several biomedical application like biofilm disruption [38], protection against neurodegeneration [39], and tumor prevention [40]. One of the major advantages of these biomimetic nanozymes over the classical antioxidants are that they do not suffer from the typical translational issues like excretion, metabolism, higher degradation, biocompatibility, and long term adverse effects due to accumulation in organs like liver and spleen. Nowadays, nanozymes are being synthesized for targeted stimuli-driven (the activity depends on their microenvironment) delivery to specific tissues or for a specific function.

**TiO$_2$@CeO$_2$ Core–Shell Nanoparticles.** Artiglia L. et al. have described the activity of TiO$_2$@CeO$_2$ as a peroxidase-like enzyme [41]. They demonstrated that the Ce$^{4+} \leftrightarrow$ Ce$^{3+}$ redox switch is at the basis of an all-inorganic catalytic cycle that can mimic the activity of several natural redox enzymes. The efficiency of these artificial enzymes (nanozymes) strongly depends on the Ce$^{4+}$/Ce$^{3+}$ ratio.

**Fe$_3$O$_4$ nanoparticles.** In 2007, Yang, Perrett, et al. found that Fe$_3$O$_4$ nanoparticles could serve as peroxidase mimics to catalyse the oxidation of a series of substrates [42]. In accordance with the steady state kinetic results, the substrate concentration dependent Lineweaver–Burk plots were parallel. These experimental data illustrated that the catalytic mechanism of Fe$_3$O$_4$ nanoparticles might follow a ping-pong reaction mechanism [43]. Fe$_3$O$_4$ could combine with the first substrate H$_2$O$_2$ to form intermediate HO•. The generated HO• would then capture one H$^+$ from the hydrogen donor, e.g., TMB (Figure 5). Subsequently, by combining electron spin resonance (ESR) measurements with a radical inhibition assay, the possible catalytic mechanism of Fe$_3$O$_4$-based nanomaterials as peroxidase mimics was proposed by Tang et al. [44]. In their work, ESR was used to monitor the production of intermediate HO• during the catalytic reaction. It was found that Fe$_3$O$_4$ nanoparticles could also generate intermediate HO•, indicating the similarity to peroxidase [45].

**V$_2$O$_5$ Nanowires.** Mugesh, D’Silva, and et al. demonstrated that V$_2$O$_5$ nanowires possessed remarkable GPX-like antioxidant activity which could catalyse the
decomposition of H$_2$O$_2$ with the assistance of Reduced glutathione (GSH) under physiological conditions (Figure 5) [46]. The investigation of the mechanism indicated that the surface of V$_2$O$_5$ nanowires could be used as templates for GSH to reduce H$_2$O$_2$ (Figure 5). First, GS$^-$ could generate an unstable sulfenate-bound intermediate 2 through the nucleophilic attack of complex 1 on the peroxide bond. This intermediate could then hydrolyze to produce glutathione sulfenic acid 3 and dihydroxo intermediate 4. After that, intermediate 4 would react with H$_2$O$_2$ to regenerate complex 1. This catalytic mechanism was like natural GPx. V$_2$O$_5$ nanowires showed typical Michaelis–Menten behavior toward both H$_2$O$_2$ and GSH, with $K_m$ values of around 0.11 mM for H$_2$O$_2$ and 2.22 mM for GSH, while the $K_m$ values of GPx1 enzyme isoform were 0.025 mM and 10 mM for H$_2$O$_2$ and GSH, respectively. This difference might be due to the different binding affinity of nanozyme/natural enzyme for corresponding substrates. The $V_{max}$ for the reactions were around 0.43 and 0.83 mM min$^{-1}$ H$_2$O$_2$ and GSH, respectively, and the $k_{cat}$ was 0.065 s$^{-1}$ [45].

Cu$_{1.08}$Zn$_{0.11}$O Nanoparticles. It has been well documented by Nagvenkar et al. [47] that the intrinsic peroxidase activity of Cu$_{1.08}$Zn$_{0.11}$O is superior over CuO against typical substrates like TMB, OPD, and ABTS, in presence of H$_2$O$_2$. Fluorescence and ESR techniques were used to demonstrate the mechanism of action of Zn-CuO. This enhanced activity of the nanoparticles over the metal oxides have a significant contribution to glucose detection, thereby lowering the limit of detection (LOD) significantly.

Co$_{0.0}$ nanoparticles. It has been illustrated by Mu et al. [48] that Co$_{0.0}$ nanoparticle can exhibit both a peroxidase-like and catalase-like activity. The capability of the NPs to transfer electrons among the reducing substrates and H$_2$O$_2$, and not from the H0• radical generated, accounts for its peroxidase activity. As peroxidase mimic Co$_{0.0}$ nanoparticles can efficiently be used for calorimetric determination of H$_2$O$_2$. The peroxidase-like activity of Co$_{0.0}$ NPs follow Michaelis-Menten kinetics and are more stable at an increased concentration of H$_2$O$_2$ than that of horseradish peroxidase (HRP). In comparison to HRP, Co$_{0.0}$ nanoparticle, has several advantages, such as ease of preparation, low-cost, and stability. Co$_{0.0}$ nanoparticle has also been successfully used in the colorimetric determination of H$_2$O$_2$ and glucose, which proves not only to be a seminal work as peroxidase mimics, but also has immense contribution in the exploitation of redox active nanomaterials in biotechnology and for clinical diagnosis.

Jia et al. [49] demonstrated in their study that Co$_{0.0}$ nanoparticles acting as peroxidase mimics can be used to catalyse the oxidation of chromophoric substrates by H$_2$O$_2$. The nanoparticles evolved a platform which can act as a biosensor for H$_2$O$_2$ and glucose detection, which, can be utilised for studying the inhibitory effects of natural antioxidants on peroxidase mimics. Three natural occurring antioxidants, gallic acid, tannic acid, and ascorbic acid, were compared for their antioxidant capabilities and found to inhibit peroxidase-like activity in a concentration dependent fashion with distinct modes of inhibition based on their specific interaction. The inhibiting capability of tannic acid was found to be greatest followed by gallic acid and ascorbic acid. This study proves the hypothesis that nano-enzyme mimics can be used to evaluate the antioxidant capabilities and also to screen enzyme inhibitors.

The curious case of citrate functionalized Mn$_3$O$_4$ nanoparticles

In a series of studies, we have shown that surface functionalized Mn$_3$O$_4$ nanoparticles have unprecedented optical, magnetic, catalytic, and biological activities [50-54]. In a new approach to treat neonatal hyperbilirubinemia and associated neuropathy, we designed a mixed-valence transition metal oxide spinel structured nanomaterial, citrate functionalised Mn$_3$O$_4$ nanoparticles (C-Mn$_3$O$_4$), which have unique catalytic activity towards degradation of bilirubin without any photo-activation (Figure 6). In binary spinel Mn$_3$O$_4$ nanoparticles all the tetrahedral A sites hold a divalent cation, Mn$^{2+}$ (3d$^{5}$), whereas all the octahedral B sites are occupied by trivalent cations, Mn$^{3+}$ (3d$^4$). Owing to the orbital degree of freedom, the e$_g$ orbitals of Mn$^{3+}$ (t$_{2g}$e$_g$) is lifted by strong Jahn–Teller distortion that compels the lattice to be tetragonal (c/a=1.16) below 1433K. Such exciting physical properties together with the ligand to metal charge transfer (LMCT) from the surface coordinating ligands to Mn$_3$O$_4$ nanoparticles lead to such exceptional catalytic ability. Our in vitro studies revealed the role of both ROS and surface mediated catalysis in the bilirubin decomposition mechanism. We further described the remarkable efficacy of the NPs as nanomedicine in symptomatic selective reduction of serum bilirubin level (both conjugated and unconjugated) without influencing other serum markers of toxicity in the CCl$_4$ induced Swiss albino mouse model of neonatal jaundice. Single intraperitoneal dose of 0.25 mg kg$^{-1}$ body weight was able to irreversibly decrease the elevated bilirubin concentration to normal level within 6 h (Figure 6). The results of preclinical studies demonstrated...
that the C-Mn$_3$O$_4$ NPs can be first-of-their-kind nanodrugs for efficient treatment of neonatal jaundice and associated neuralgic disorders with adequate biocompatibility.

The twist in tale comes when route of administration changes. Oral treatment of these NPs efficiently reverses hepatic fibrosis in CCl$_4$-induced mice. The C-Mn$_3$O$_4$ nanoparticle treatment decreases expression of fibrotic markers like α-SMA and hepatic-hydroxyproline, as measured through immune-histochemical analysis, along with improvements in other liver function parameters (e.g., AST, ALT, ALP, GGT) (Figure 7). Further results show that the NPs can mimic cellular antioxidant defense enzymes (SOD, catalase), and protects cells from oxidative damages (Figure 7). It was found that the acidic condition of stomach enhances the antioxidant activity of C-Mn$_3$O$_4$ nanomaterials due to the disproportionation of Mn$^{3+}$ charges present in the nanoparticle surface. Further investigations on mitochondrial membrane potential, mitochondrial permeability transition pore, and cytochrome c oxidase activity revealed the nanoparticles’ protective activity on mitochondria, the cellular mediator of oxidative stress (Figure 7). To best of our knowledge, this is the first study to demonstrate that direct oral treatment of an inorganic NPs (i.e., C-Mn$_3$O$_4$ NPs) without any delivery system can efficiently reduce chronic hepatotoxicity and liver fibrosis through its antioxidant activity.

**Translational Aspects**

The problem with the conventional antioxidants that make their clinical transformation mostly a failure is multifaceted, starting from limited bioavailability to nonspecific biodistribution [24, 25]. Similar problems can be evident even in nanozymes. However, the unimaginable manoeuvrability in controlling the properties of synthesized nanomaterials can be useful in overcoming the limitations.
Figure 7: Mitochondrial redox modulation ability of C-Mn₃O₄ NPs and their application in treatment of hepatic fibrosis. Citrate functionalized Mn₃O₄ nanoparticles can protect mitochondria from oxidative damage through maintaining normal mitochondrial membrane potential, MPTP, and decreasing cytochrome c release into the cytosol, in turn blocks apoptosis. On the other hand, a decrease in ROS levels downregulates the process of hepatic stellate cell activation through TGF-β, in turn decreases fibrotic damages as observed by Mason’s trichrome, Sirius red and α-SMA immunostaining.
The intracellular behavior of conventional antioxidants is more like a double-edged sword [55]. The conventional antioxidants are generally less bioavailable due to low absorption in the gut and nonspecific biodistribution throughout the body, which in turn reduces the local concentration. Thus, most of the time the conventional antioxidants fail to neutralize the excess free radicals produced due to pathophysiological condition. On the other hand, if by some means, the local concentration gets increased, the conventional antioxidants scavenge almost all the free radicals, affecting the ROS mediated signalling pathways which are hugely important in cell-cell communications and maintenance of normal cell function (it has to be noted that a threshold level of ROS inside the cell is always required for cellular homeostasis) [56]. In some cases, it has been observed that at high concentrations in physiological milieu, conventional antioxidants behave like prooxidants, which is detrimental and increases the oxidative damages [55]. Another problem that conventional antioxidants suffer from is the lack of reusability. The conventional antioxidants neutralize the ROS (or other free radicals) through reduction paired with self-oxidation which makes the antioxidant molecule non-reactive for further function. These problems could be overcome in nanosystems. By simply attaching a cell specific ligand (receptor of which is overexpressed in particular tissue type) on the surface of the nanomaterial, the system can be targeted to specific tissues [57, 58]. There exist numerous strategies to functionalize nanozymes (with small molecules, polymers, antibodies, nucleic acids) for targeted delivery (e.g., capping the nanomaterials with folate will help in delivering it specifically to cancer cells, as cancer cells express significantly higher number of folate receptors than normal cells) (Figure 8). The specificity of the surface functionalized nanomaterials provides the ability to fine tune local concentrations in the required tissues so that the cellular redox homeostasis is maintained. Other than surface functionalization, some inherent properties (e.g., size, shape, hydrophobicity, rigidity, composition) of the nanomaterial itself may dictate the biodistribution inside the body (Ref. 57 is an excellent review regarding this). The redox activity, i.e., ability of the nanosystem to be oxidized as well as reduced depending upon the cellular microenvironment, helps to maintain a threshold level of free radicals inside the cells, which is the utmost requirement. The redox nature and enzyme like properties also make nanosystems reusable; hence, very minute concentrations of nanozymes can provide large effects in a sustained way. Another advantage of nanozymes over conventional antioxidants is the stimuli responsive initiation of activity, i.e., the functioning of the nanomaterial inside a cell can be controlled by external stimuli (light, ultrasound, magnetic field). This unique property of nanomaterials is heavily utilized in the case of photodynamic therapy (PDT) of cancer, which is a form of treatment involving light and a photosensitized nanomaterial, used in conjunction with molecular oxygen to elicit cell death [59-62]. The principle of PDT can be used in redox modulation as well, which will make the treatment more target specific without affecting other cells.

Another concern which is important to discuss in the context of translational aspects of biomimetic nanozymes is the associated toxicity. To our understanding, the toxicity of most of the conventional drugs originates from nonspecific distribution and over activity due to high local concentration (the basic principle of toxicology says "dose makes the poison"). We discussed a probable approach to avoid the problem of non-specificity in a previous section. Another factor, dose determination, is probably the most critical factor in the proper functioning of nanomedicines. In this regard, careful pharmacokinetic and pharmacodynamic screening will be helpful. Although the orthodox practice to decide dose of a nanomedicine (or any other drug) depends upon the body weight of a subject (in both human and animal experiments), we think this is not the right way. Maybe, a personalized approach will be more efficacious considering the unique physicochemical properties of the nanomaterials. Moreover, there is no specific guidelines for testing the toxicity of the nanomaterials. The community needs a larger debate on the subject of dose determination and toxicity in the nanoregime.

**Conclusion and future perspective**

For the development of potent nanoparticle designs, clinical translation plays a pivotal role. Although, the study of enzyme mimetic nanoparticles in the field of diagnostics/bioassays is essential, the greater translational challenge lays in the usage of these nanoparticle formulations as topical or injectable solutions, or as components of implants. Some of the discussed agents (i.e., citrate functionalized \( \text{Mn}_3\text{O}_4 \) nanoparticles) have already been successfully trialled in preclinical mouse model. However, the other synthesised nanoparticles need to be tested on animal models, owing to their possible complications in regenerating similar outcomes as obtained in vitro. In addition to the production of therapeutic and other medically favourable outcomes,
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Injected nanoparticles must show minimal side effects and have an easy excretory route. The process of excretion can occur either through metabolism [63] or as renal/fecal excretion [64]. For example, iron oxide nanoparticles are thought to be broken down in vivo and their components metabolised [64]. However, the human system lacks processes to degrade heavier metals like cerium and some nanoparticles such as gold, which cannot be degenerated in vivo. These nanoparticles have to be excreted either via the urine (for nanoparticles less than 6 nm) or via the faeces [64]. Furthermore, the interaction of nanoparticles with biological fluids (ranging from saliva in the mouth to blood and interstitial fluids) are important attributes for characterization of important aspects in terms of topical and systemic use [65]. In-depth characterization of the catalytic activity of nanoparticles on their interaction with biologically relevant solutions in laboratory models mimicking the physiological environment can provide the in vivo outcomes. In vitro or in silico models that correlates the physicochemical properties of the nanoparticle (size, surface area, doping type/concentration, and chemistries) may help in predicting the bioactivity of nanomaterials against target cells or tissues. Another important concern is the biodistribution of catalytic nanoparticles along

Figure 8: Designing nanoparticles for intracellular applications. Nanoparticles can be designed with high level of manoeuvrability using various physicochemical properties ranging from composition to shape, size, and hydrophobicity. Surface functionalization with biocompatible ligands not only is important for target specific delivery but also it reduces toxicity. Reprinted (with minor modification) with permission from Ref. [57] Copyright 2010, Royal Society of Chemistry.
with their level of tissue or biofilm penetration depth. Nanoparticles possesses certain limitations in site specific targeting, owing to their complex interactions with biological fluids and cellular components in physiological systems, thereby causing toxic side effects. Nevertheless, studies have helped in significant expansion of clinically validated and safe nanomedicine drugs and biologics. To date U.S. Food and Drug Administration (USFDA) has approved several nano-sized agents which have therapeutic and imaging applications (51 FDA-approved nanomedicines and 77 products in clinical trials) [65]. Although, these comparatively simpler designs have proved to be a path for providing a template for undertaking initial attempts for the synthesis of catalytic nanomaterials, complicated structures with multicentre nanoenzyme complexes are challenging to be translated, owing to their multisubstrate availability, control over the sequential reactions, optimal substrate access, and cost and complexity of synthesis [66]. Further research on the catalytic activity, efficacy, and therapeutic activity will help in accelerating the process of product development and their subsequent performance in clinical trials.

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References

1. Santolini J, Wootton SA, Jackson AA, Feilsich M. The Redox architecture of physiological function. Current Opinion in Physiology 2019;9:34-47.
2. Webster TJ. Nanomedicine: what’s in a definition? Int J Nanomedicine 2006;1(2):115-116.
3. Foundation ES. Nanomedicine – An ESF–European Medical Research Councils (EMRC) Forward Look Report. France ESF: Strasbourg cedex, 2004.
4. Sies H, Berndt C, Jones DP. Oxidative Stress. Annual Review of Biochemistry 2017;86(1):715-748.
5. Sarkar PK, Halder A, Adhikari A, Polley N, Darbar S, Lemmens P, et al. DNA-based fiber optic sensor for direct in-vivo measurement of oxidative stress. Sensors and Actuators B: Chemical 2018;255:2194-2202.
6. Kalyanaraman B, Darley-Usmar V, Davies KJA, Denney PA, Forman HJ, Grisham MB, et al. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radical Biology and Medicine 2012;52(1):1-6.
7. Glasauer A, Chandel NS. ROS. Current Biology 2013;23(3):R100-R102.
8. Murphy MP, Hartley RC. Mitochondria as a therapeutic target for common pathologies. Nature Reviews Drug Discovery 2018;17:865.
9. Picard M, Wallace DC, Burelle Y. The rise of mitochondria in medicine. Mitochondrion 2016;30:105-116.
10. Nunnari J, Suomalainen A. Mitochondria: In Sickness and in Health. Cell 2012;148(6):1145-1159.
11. Suomalainen A, Battersby BJ. Mitochondrial diseases: the contribution of organelle stress responses to pathology. Nature Reviews Molecular Cell Biology 2017, 19:77.
12. Andreux PA, Houtkooper RH, Auwerx J. Pharmacological approaches to restore mitochondrial function. Nature Reviews Drug Discovery 2013;12:465.
13. Durazo SA, Kompella UB. Functionalized nanosystems for targeted mitochondrial delivery. Mitochondrion 2012;12(2):190-201.
14. Valgimigli L, Baschieri A, Amorati R. Antioxidant activity of nanomaterials. Journal of Materials Chemistry B 2018;6(14):2036-2051.
15. Hanthorn JJ, Valgimigli L, Pratt DA. Preparation of Highly Reactive Pyridine- and Pyrimidine-Containing Diarylamine Antioxidants. The Journal of Organic Chemistry 2012;77(16):6908-6916.
16. Young IS, Woodside IV. Antioxidants in health and disease. Journal of Clinical Pathology 2001;54(3):176-186.
17. Polefka TG, Meyer TA, Agin PP, Bianchini RJ. Effects of Solar Radiation on the Skin. Journal of Cosmetic Dermatology 2012;11(2):134-143.
18. Adhikari A, Darbar S, Chatterjee T, Das M, Polley N, Bhattacharyya M, et al. Spectroscopic Studies on Dual Role of Natural Flavonoids in Detoxification of Lead Poisoning: Bench-to-Bedside Preclinical Trial. ACS Omega 2018;3(11):15975-15987.
19. Perron NR, Brumaghim JL. A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding. Cell Biochemistry and Biophysics 2009;53(2):75-100.
20. Lu J, Holmgren A. The thioredoxin antioxidant system. Free Radical Biology and Medicine 2014;66:75-87.
21. Brand MD, Affoutrit C, Esteves TC, Green K, Lambert AJ, Miwa S, et al. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radical Biology and Medicine 2004;37(6):755-767.
22. Amorati R, Valgimigli L. Advantages and limitations of common testing methods for antioxidants. Free Radical Research 2015;49(5):633-649.
23. Ingold KU, Pratt DA. Advances in Radical-Trapping Antioxidant Chemistry in the 21st Century: A Kinetics and Mechanisms Perspective. Chemical Reviews 2014;114(18):9022-9046.
24. Warnholtz A, Münzel T. Why do antioxidants fail to provide clinical benefit? Trials 2000, 1(1):38.
25. Steinhubl SR: Why Have Antioxidants Failed in Clinical Trials? The American Journal of Cardiology 2008;101(10, Supplement):S14-S19.
26. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. The Lancet 2003;361(9374):2017-2023.
27. Goodman M, Bostick RM, Kucuk O, Jones DP. Clinical trials of antioxidants as cancer prevention agents: Past, present, and future. *Free Radical Biology and Medicine* 2011;51(5):1068-1084.

28. Bin Q, Hu X, Cao Y, Gao F. The role of vitamin E (tocopherol) supplementation in the prevention of stroke. *Thromb Haemost* 2011;105(04):579-585.

29. Oholow MJ, Sohre S, Granold M, Schreckenberger M, Hoosmann B. Why have Clinical Trials of Antioxidants to Prevent Neurodegeneration Failed? - A Cellular Investigation of Novel Phenothiazine-Type Antioxidants Reveals Compelling Objectives for Pharmaceutical Neuroprotection. *Pharmaceutical Research* 2017;34(2):378-393.

30. Sesso HD, Christen WG, Bubes V, Smith JP, MacFadyen J, Schwartz M, et al. Multivitamins in the Prevention of Cardiovascular Disease in Men: The Physicians’ Health Study II Randomized Controlled Trial Multivitamins in Prevention of CVD in Men. *JAMA* 2012;308(17):1751-1760.

31. Persson T, Popescu BO, Cedazo-Minguez A. Oxidative Stress in Alzheimer’s Disease: Why Did Antioxidant Therapy Fail? *Oxidative Medicine and Cellular Longevity* 2014;2014:11.

32. Hu B, Ting Y, Yang X, Tang W, Zeng X, Huang Q. Nanochemoprevention by encapsulation of (-)-epigallocatechin-3-gallate with bioactive peptides/chitosan nanoparticles for enhancement of its bioavailability. *Chemical Communications* 2012;48(18):2421-2423.

33. Luo Q, Shen Y, Li P, Wang C, Zhao Z. Synthesis and characterization of crosslinking waterborne fluorinated polyurethane-acrylate with core-shell structure. *Journal of Applied Polymer Science* 2014;131(21).

34. Astete CE, Dolliver D, Whaley M, Khachatryan L, Sabliov B. Why Have Clinical Trials of Antioxidants to Prevent Neurodegeneration Failed? - A Cellular Investigation of Novel Phenothiazine-Type Antioxidants Reveals Compelling Objectives for Pharmaceutical Neuroprotection. *Pharmaceutical Research* 2017;34(2):378-393.

35. Trombino S, Cassano R, Ferrarelli T, Barone E, Picci N, Mancuso C. Antioxidant Poly(lactic-co-glycolic) Acid Nanoparticles Made with α-Tocopherol–Ascorbic Acid Surfactant. *ACS Nano* 2011;5(12):9313-9325.

36. Polley N, Saha S, Adhikari A, Banerjee S, Darbar S, Das S, et al. Unprecedented catalytic activity of Mn3O4 nanoparticles: potential lead of a sustainable therapeutic agent for hyperbilirubinemia. *RSC Advances* 2014;4(10):5075-5079.

37. Polley N, Saha S, Adhikari A, Banerjee S, Darbar S, Das S, et al. Safe and symptomatic medicinal use of surface-functionalized Mn3O4 nanoparticles for hyperbilirubinemia. *Nanomedicine* 2015;10(15):2349-2363.

38. Adhikari A, Polley N, Darbar S, Bagchi D, Pal SK. Citrate functionalized Mn3O4 in nanotherapy of hepatic fibrosis by oral administration. *Future Science OA* 2016;2(4):FSO146.

39. Adhikari A, Polley N, Darbar S, Bagchi D, Pal SK. Therapeutic Potential of Surface Functionalized Mn3O4 Nanoparticles Against Chronic Liver Diseases in Murine Model. *Materials Focus* 2017, 6(3):280-289.

40. Bouayed J, Bohn T. Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. *Oxidative Medicine and Cellular Longevity* 2010;3(4).

41. Kamata H, Hirata H. Redox Regulation of Cellular Signalling. *Cellular Signalling* 1999;11(1):1-14.

42. Chou LYT, Ming K, Chan WCW. Strategies for the intracellular delivery of nanoparticles. *Chemical Society Reviews* 2011;40(1):233-245.
58. Hua S, de Matos MBC, Metselaar JM, Storm G. Current Trends and Challenges in the Clinical Translation of Nanoparticulate Nanomedicines: Pathways for Translational Development and Commercialization. *Frontiers in Pharmacology* 2018;9(790).

59. Nandi R, Mishra S, Maji TK, Manna K, Kar P, Banerjee S, et al. A novel nanohybrid for cancer theranostics: folate sensitized Fe2O3 nanoparticles for colorectal cancer diagnosis and photodynamic therapy. *Journal of Materials Chemistry B* 2017;5(21):3927-3939.

60. Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nature Reviews Cancer* 2003;3(5):380.

61. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, et al. Photodynamic therapy of cancer: an update. *CA: A Cancer Journal for Clinicians* 2011;61(4):250-281.

62. Wang C, Tao H, Cheng L, Liu Z. Near-infrared light induced in vivo photodynamic therapy of cancer based on upconversion nanoparticles. *Biomaterials* 2011;32(26):6145-6154.

63. Corot C, Robert P, Idée J-M, Port M. Recent advances in iron oxide nanocrystal technology for medical imaging. *Advanced Drug Delivery Reviews* 2006;58(14):1471-1504.

64. Naha PC, Lau KC, Hsu JC, Hajfathalian M, Mian S, Chhour P, et al. Gold silver alloy nanoparticles (GSAN): an imaging probe for breast cancer screening with dual-energy mammography or computed tomography. *Nanoscale* 2016;8(28):13740-13754.

65. Caracciolo G, Farokhzad OC, Mahmoudi M. Biological identity of nanoparticles in vivo: clinical implications of the protein corona. *Trends in Biotechnology* 2017;35(3):257-264.

66. Cormode DP, Gao L, Koo H. Emerging Biomedical Applications of Enzyme-Like Catalytic Nanomaterials. *Trends in Biotechnology* 2018;36(1):15-29.