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Emerging nanomaterials for targeting peroxisomes

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ABSTRACT

Peroxisomes are single membrane-bound metabolic organelles whose dysfunction can lead to several metabolic disorders. In addition, they have been associated with the pathology of several diseases such as cancer, autoimmune diseases, diabetes, stroke, etc. In the last few decades, research has been focused on detecting peroxisomes in the physiological environment. However, the detection of these peroxisomes was based on fluorescent probes, which had limited success due to their toxicity, photobleaching, poor selectivity, and spontaneous oxidation. Moreover, research has been focused on mimicking the functionality of peroxisomes by fabricating artificial peroxisomes using synthetic materials, which have been limited due to poor stability and biocompatibility. Therefore, a new class of materials, "nanomaterials" has shown promise in overcoming the limitations underlying traditional techniques by providing better optical properties, stability and biocompatibility. Despite the advancement, the field remains in its infancy. Only a handful of studies have reported nanomaterials such as quantum dots, zeolites, liposomes, dendrimers, and nanoparticles to detect and fabricate peroxisomes. This review will provide a general description of peroxisomes and their role in different metabolic activities. The later part will focus on the challenges and progress related to nanomaterials-based peroxisome detection, fabrication, and delivery. This review will also provide insights into the critical research gaps and advances on different strategies utilized to explore peroxisomes, opening new avenues for future research in this field.

1. Introduction

Peroxisomes are single membrane-bound organelles virtually present in all eukaryotic cells except erythrocytes and spermatozoa [1]. They are metabolic organelles known to take part in over 50 enzymatic reactions crucial for cellular functioning [2]. The essential metabolic functions executed by peroxisomes are involved in lipid metabolism, including fatty acid α and β-oxidation [3,4], biosynthesis of C24-bile acids [5], docosahexaenoic acid (DHA) [6,7], ether phospholipids [8] and metabolism of reactive oxygen species (ROS) (especially H_2O_2) [9,10]. This establishes that peroxisomes are critical metabolic organelles whose dysfunction can lead to severe metabolic disorders. Genetic defects in peroxisomal proteins are associated with their functional aberrations, especially those related to metabolism [11].

Knowledge of peroxisome's functionality and its importance in different therapeutic applications has expanded significantly [12]. However, the essential mechanisms underlying these phenomena are still in their initial stages, and there is an urgent need to monitor peroxisome-associated markers [13,14]. Different fluorescent-based probes have been explored to get insight into these mechanisms, which were limited due to their toxicity, photobleaching, poor selectivity, and spontaneous oxidation [15–18]. Improved, although limited understanding of peroxisome associated markers have led to the fabrication of nanomaterials based "Artificial Peroxisomes" (AP), encasing several natural or synthetic enzymes capable of governing specific cellular encapsulated catalytic reactions [19,20]. The potential of these AP's has been showcased in a few applications; however, they are limited due to the partial

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understanding of peroxisome markers and incompetent intracellular delivery methods. Nanomaterials based probes with their tunable and advanced optical properties and biocompatibility offers enormous potential to overcome these limitations, which include:

a) better monitoring of peroxisome associated markers,
b) controlled fabrication of artificial peroxisomes,
c) enhancing the intracellular delivery of artificial peroxisomes or drugs of choice, and
d) understanding the mechanisms underlying these phenomena.

Therefore, in this review, we seek to collectively curate the recent scientific developments in nanomaterial-based detection and fabrication of peroxisomes. We also seek to compile and convey how different nanomaterials-based peroxisomes can pave the path to control metabolic activities, thus regulating various biological processes.

2. Peroxisome biogenesis and its role in metabolism

2.1. Peroxisome biogenesis

Peroxisome biogenesis comprises the acquisition of a lipid membrane, import of matrix proteins, and subsequent organelle expansion. The peroxisome biogenesis factors PEX3 and PEX16 bind with the endoplasmic reticulum membrane, post-exit interacts with PEX19 which is a cycling receptor in the cytosol [21]. It binds with peroxisomal membrane proteins (PMPs), docks with PEX3 to add PMPs to the peroxisomal membrane [22]. Peroxins namely Pex3, Pex16, and Pex19 are essential for peroxisome membrane assembly; and PEX11 isoforms namely Pex11α, Pex11β, and Pex11γ involved in peroxisome division together with dynamin-like protein 1 (D1p), mitochondrial fission factor (Mff) and mitochondrial fission protein 1 (Fis1) [23].

The proliferation of peroxisomes takes place by growth and division, and the division comprises three stages: elongation, constriction, and fission. This is mediated by Pex11β, D1p, Mff, and Fis1 that are localized to peroxisomes. Mff and Fis1 localize at the constrictions of the membranes of elongated peroxisomes, where Mff recruit's dynamin-like protein 1 (D1p). This leads to the formation of a complex consisting of Pex11β, Mff, and DLP1 that promotes Mff-mediated fission during peroxisomal division along with the involvement of Pex11α and Pex11γ and Fis1 that interacts with D1p [23–25]. The daughter peroxisomes become mature and functional by importing matrix and membrane proteins and may re-enter the membrane expansion component of the cycle (Fig. 1).

2.2. Lipid metabolism

Fatty acid oxidation (FAO), critical for balanced production and expenditure of energy, is a tightly regulated process. Peroxisomes are involved in the β-oxidation of very-long-chain fatty acids (VLCFAs) $\geq$ 22 carbons lengths to generate long-chain acyl-CoA fatty acids and acetyl-CoA [26]. Furthermore, peroxisomes are also essential in the oxidation of branched-chain fatty acids such as pristanic acid and di- or tri-hydroxycholestanoic acids (DHCA/THCA) [27–29]. Also, branched-chain fatty acid accumulation in peroxisomal disorder indicated a role of peroxisomes in α-oxidation [30]. In this regard, efficient lipid transport across the peroxisomal membrane remains the most crucial step for any metabolic reaction. Studies have revealed that a particular class of transporters called the peroxisomal ATP-binding cassette (ABC) transporter subfamily D including members ABCD1 (adrenoleukodystrophy protein (ALDP), ABCD2 (adrenoleukodystrophy-related protein (ALDRP), and ABCD3 (70 kDa peroxisomal membrane protein (PMP70)) predominantly mediate the transport of various lipid substrates into peroxisome for shortening by β -oxidation [28]. The defects of ABCD1 and ABCD3 transporters are responsible for two genetic disorders called X-linked adrenoleukodystrophy and congenital bile acid synthesis defect 5, respectively [31,32]. Inactivation of ABCD2 causes cerebellar and sensory ataxia with progressive neuronal degeneration presumably caused by chronic accumulation of VLCFAs and/or other lipid metabolites [33]. ABCD1 and ABCD3 defects occur in patients, whereas inactivation of ABCD2 was only done in mice.

VLCFAs can also serve as ligands for the activation of nuclear peroxisome proliferator-activated receptor (PPAR) signaling, promoting their own β-oxidation [34]. The main enzyme involved in peroxisomal β-oxidation of VLCFAs and dicarboxylic acids (DCAs) is acyl-CoA oxidase 1 (ACOX1) and acyl-CoA oxidase 2 (ACOX2) associated with the oxidation of pristanic acid and DHCA/THCA.

The branched-chain fatty acids have a methyl group at γ-position on the third carbon atom, which prevents their β -oxidation [27]. The terminal carboxyl group is removed as CO_2 by α-oxidation in peroxisomes. β-Oxidation of fatty acids utilize fatty acids only in the (S) isomeric conformation [35]. However, α-oxidation is a non-stereospecific process, accepting both (R) and (S) fatty acid isomers [36]. Thus, during the β-oxidation of (R) form of phytanoyl-CoA, α -oxidation comes into play and converts phytanoyl-CoA (R) form to the (S) form by the action of α-methylacyl-CoA racemase (AMACR) [37]. The process is carried out in three consecutive steps: (a) The enzyme phytanoyl-CoA hydroxylase (PHYH) converts phytanoyl-CoA to 2-hydroxy-phytanoyl-CoA; (b) then, 2-hydroxy-phytanoyl-CoA is cleaved into pristanal and formyl-CoA by 2-hydroxy-phytanoyl-CoA lyase; (c) finally, pristanal is converted into pristanic acid by the action of the enzyme pristanal dehydrogenase. The end product pristanic acid can undergo peroxisomal β-oxidation. Formyl-CoA, produced in the second step, is further broken down into formate and, eventually, CO_2 (Fig. 2).

2.3. Peroxisomal metabolism of ROS/RNS

Peroxisomes, one of the most dynamic and metabolically active organelles and reported to be an essential source of reactive oxygen species (ROS) such as H_2O_2, superoxide ($O\bullet^-$), hydroxyl (•OH), and reactive nitrogen species (RNS), including nitric oxide (NO•) radicals [13]. Research on rat models suggests that peroxisomes consume about 20% oxygen and are sole producers of 35% H_2O_2 [38,39]. These radicals are primarily produced as by-products during the normal catalytic function of the enzymes participating in peroxisomal fatty acid metabolism [40]. H_2O_2 in peroxisomes is produced mainly by the ACOX family of enzymes and other peroxisomal enzymes, such as L-α-hydroxyacid oxidase 1 and 2, and xanthine oxidase (XDH) [41]. The enzymes XDH and inducible form of nitric oxide synthase (NOS2) are the two sources of superoxide (O•−) and nitric oxide (NO•) radicals in peroxisomes. H_2O_2 and O•− are produced as the by-products of the XDH catalytic cycle [42]. The enzyme NOS2 is a homodimer catalyzing the oxidation of L-arginine, creating NO• and citrulline [43]. Moreover, although there is no evidence supporting the existence of enzymes that produce •OH or ONOO− in peroxisomes, however, it may be assumed that the peroxisomal H_2O_2 may be responsible for the production of •OH through the Fenton reaction. In addition, the presence of enzymatic sources of O2•− and NO• generates ONOO−, a kinetically and thermodynamically favored reaction [44].

A high quantity of ROS/RNS developed in the peroxisomes raises additional concern about how these metabolic specialists counterbalance the ROS leakage or unmanageable ROS-mediated

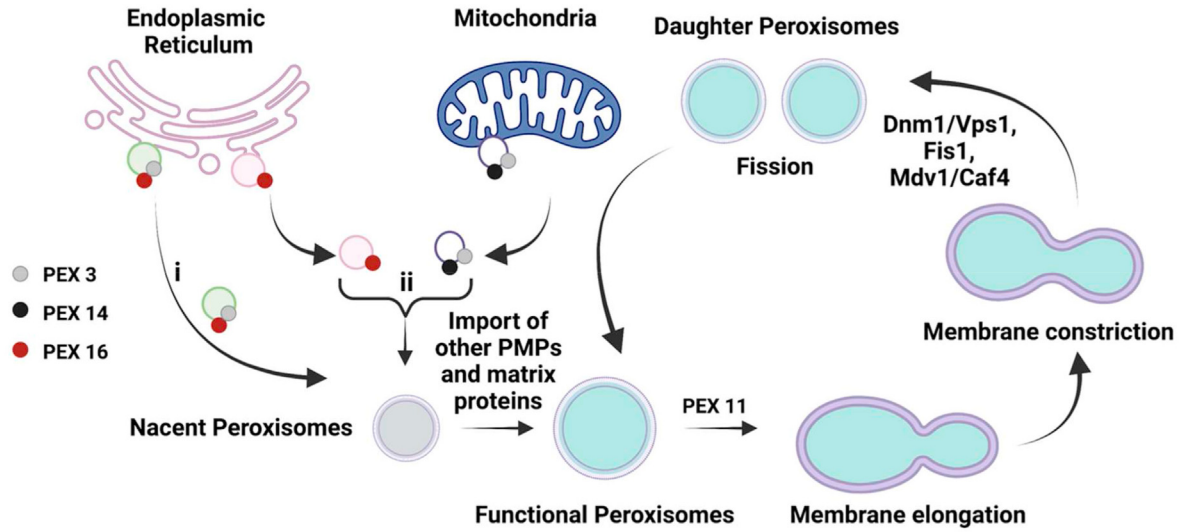

Fig. 1. Schematic of the peroxisomal biogenesis pathway. ER-mediated de novo biogenesis involves the formation of peroxisomes from ER-derived vesicles containing both PEX3 and PEX16. Peroxisome de novo biogenesis involving both ER and mitochondria involves the formation of pre-peroxisomal vesicles, which is a fusion of PEX-bound vesicles originating from both the mitochondria (PEX3 and PEX14-bound) and the ER (PEX16-bound). Both pathways of peroxisome de novo biogenesis are followed by the import of peroxisomal membrane protein (PMPs, e.g., PEX19, PEX5, PEX7, PEX1, PEX6). The import of PMP gives rise to functional peroxisomes. Steps of peroxisome fission-in the elongation/tubulation stage, PEX11 congregates on one end of the peroxisome amongst other PMPs, giving rise to a narrow, tubular structure. During constriction, Fis1 then recruits Dnm1 to its site of action for the final scission of peroxisomes. Mdv1 and Caf4 are two additional adapter proteins required in yeast. These proteins cluster near PEX11 proteins and form a "pinch" on the peroxisome. This leads to fission into two asymmetric daughter peroxisomes. The daughter peroxisomes grow in size by importing matrix and membrane proteins and mature into functional peroxisomes, which may re-enter the membrane expansion component of the cycle.

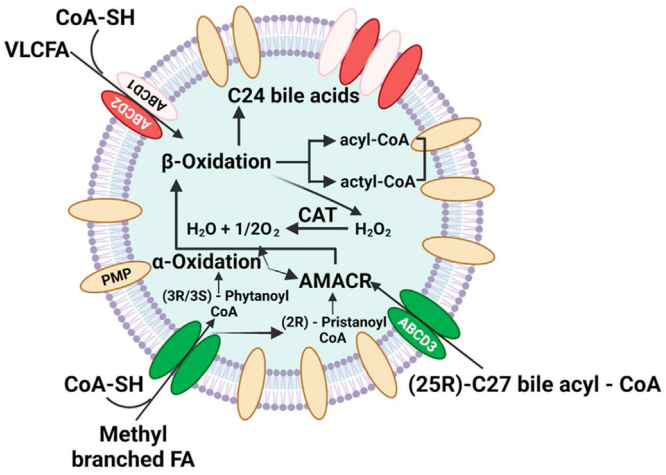

Fig. 2. Peroxisomal metabolic pathways. Very-long chain fatty acids (VLCFAs) with CoA-SH enter the β-oxidation pathway generating H_2O_2 as a by-product which may exit the peroxisomes. The end products of VLCFA β-oxidation, acetyl-CoA, and acetyl-CoA. (25R)- C27 bile acyl-CoA undergoes β-oxidation to form primary C24 bile acid conjugates.

damage to different cellular compartments [45]. The peroxisome is equipped with at least six proteins with antioxidant functions, including superoxide dismutase 1 (SOD1), catalase (CAT), epoxide hydrolase 2 (EPHX2), 'microsomal' glutathione-S-transferase-1 (MGST1), peroxiredoxin-5 (PRDX5), and glutathione-S-transferase kappa 1 (GSTK1) [46]. Catalase, the most widely documented peroxisomal enzyme with antioxidant activity, forms a tetramer, accompanied by four heme groups that enable its enzymatic activity [47]. Its primary function is the quenching of peroxisomal H_2O_2 in a catalatic ($2H_2O_2 \rightarrow 2H_2O + O_2$) or peroxidatic ($H_2O_2 + AH_2 \rightarrow A + 2H_2O$) manner [48]. SOD1, a homodimeric enzyme found in the cytosol, the nucleus, and mitochondria, mediates the conversion of $O_2\bullet-$ to O_2 and H_2O_2 ($2O_2\bullet- + 2H^+ \rightarrow O_2 + H_2O_2$). It has been reported that this enzyme can be imported into peroxisomes by a complex with 'copper chaperone for SOD1' via a piggyback mechanism [49]. Glutathione S-transferases (GST) superfamily of enzymes catalyze xenobiotics' conjugation and potentially damage oxidative metabolites with glutathione [50]. Studies suggest that both GSTK1 and MGST1 might be partially located in peroxisomes, executing xenobiotic detoxification and lipid peroxide products [51,52]. Another enzyme, PRDX5, which resides in peroxisomal matrix, helps in by thiol-dependent reduction of H_2O_2 to H_2O, alkyl hydroperoxides (ROOH) to their respective alcohols (ROH), and $ONOO-$ to nitrite ($ONO-$) [53]. EPHX2, a homodimeric enzyme, binds to epoxides converting them to the corresponding dihydrothiols [54]. These enzymes together are also known for ROS elimination action. Besides these reported enzymes, evidence suggests that peroxisomes also employ low molecular weight, non-enzymatic antioxidant compounds such as glutathione and ascorbic acid to combat ROS stress (Fig. 3).

3. Nanomaterials based detection of peroxisomes associated markers

3.1. Detection of intracellular ROS

ROS plays a pivotal role in regulating a wide range of metabolic processes [12,58]. ROS can be classified into two types: a) weak ROS (hydrogen peroxide (H_2O_2) and superoxidase (O_2^-)) [59], b) high ROS (hydroxyl radical ($\cdot OH$), hypochlorite (OCl^-) and peroxynitrile ($ONOO^-$)) [60,61]. It is well established that a low level of ROS increases cell proliferation and survival through post-transitional modifications; a moderate level of ROS leads to stress tolerance [62]. In contrast, a high level of ROS leads to cell senescence or cell death [63,64]. Recent studies have established the importance of peroxisome-generated redox metabolism in regulating several diseases, including neurodegeneration, diabetes, and cancer, making peroxisomes an ideal platform for intracellular redox signaling

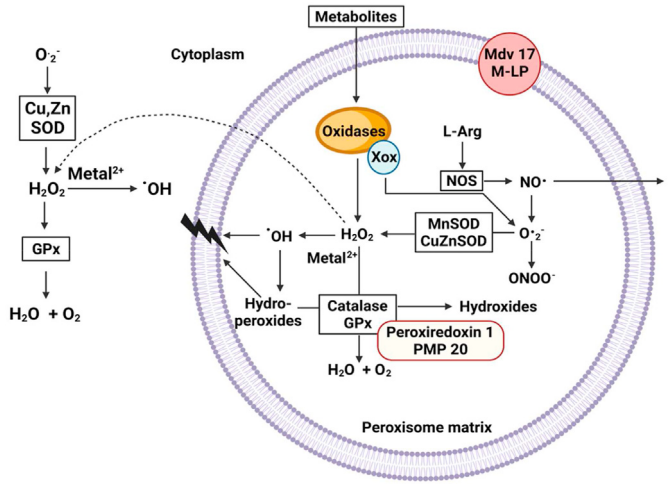

Fig. 3. Schematic overview of peroxisomal enzymes involved in ROS production/degradation. Several peroxisomal oxidases (e. g., acetyl-CoA oxidase) produce H_2O_2, which is either converted to hydroxyl radicals (·OH) or decomposed by catalase and glutathione-peroxidase (GPx). ·OH radicals can damage the peroxisomal membrane by lipid peroxidation of unsaturated fatty acids. Hydroperoxides formed as a result are decomposed by catalase and GPx. Peroxisomal oxidases (e.g., xanthine oxidase (XOx)) generates superoxide anions ($O_2\cdot^-$) which are scavenged by copper-zinc superoxide-dismutase (CuZnSOD). The oxidation of L-arginine (l-Arg) to nitric oxide (NO·) is catalyzed by nitric oxide synthase (NOS). NO· reacts with $O_2\cdot^-$ radicals to form powerful oxidant peroxynitrite ($ONOO^-$). H_2O_2 and NO· can penetrate the peroxisomal membrane and participate in cellular signaling pathways. Peroxiredoxin 1 and peroxisomal membrane protein 20 (PMP20) play a role in the degradation of H_2O_2. PMP20 possess thio-specific antioxidant and peroxidase activity [55]. Intracellular ROS is regulated by Mpv17 [56]. Mpv17 and L-MP are localized in the peroxisomes. Additionally, Mpv17 is also expressed in mitochondria and endosomes [57]. The peroxisomal localization of Mpv17 is still disputed, therefore, further research is required to address the correct localization of Mpv17.

[12]. However, the molecular mechanisms underlying these events are still in their initial stages [13,14]. Unfolding these mechanisms requires answering a few open questions: "how different redox species that are generated in peroxisomes are transported through the peroxisome membrane?"; "what are the potential and specific molecular targets of peroxisome derived H_2O_2?"; and "the mechanism underlying peroxisome derived H_2O_2 in cellular redox signaling?". Unraveling these questions require unique tools and methods that can measure the different level of ROS and redox reaction related to post-transitional modifications. Different fluorescent probes have been extensively explored over the last few decades to detect various types of intracellular ROS [15–18]. These fluorescent probes were limited due to toxicity, photobleaching, poor selectivity, and spontaneous oxidation. Therefore, an urgent need for better tools and methods has focused the current research towards a new class of materials called "nanomaterials". Several nanomaterials-based probes with improved biocompatibility and optical properties have been proposed for detecting intracellular ROS [65,66]. However, none of these probes reported specific detection of peroxisome-generated ROS. This review will only focus on summarizing nanomaterials-based probes that have been used to detect markers associated with peroxisomes.

3.2. Peroxisome proliferation

Peroxisome proliferation is regulated by signaling events occurring in the nucleus where transcription factors can enhance the expression of peroxisomal genes. These signaling mechanisms are triggered by either fatty acids or hypolipidemic drugs like fibrates that leads to an increased number of the peroxisomes [67,68].

Peroxisome proliferator-activated receptor-α (PPAR-α), one of the three isotypes of PPAR family, can significantly increase the peroxisome number and the levels of fatty acid β-oxidation [69–72]. Importantly, peroxisome proliferation has been well-studied in different species responding with variable intensities to peroxisome proliferators, inducing peroxisome proliferation, expression of β-oxidation enzymes, and the formation of liver tumors in rodent species [72].

It is widely accepted that peroxisome proliferation is highly susceptible in mouse and rat. However, humans exhibit little or no increase in peroxisome number or increased peroxisomal enzyme expression. There is conflicting data regarding the incidence of peroxisome proliferation in nonhuman primates. Interestingly, an increase in peroxisomal enzyme activities was reported by Reddy and colleagues in rhesus and cynomolgus monkey studies, while other investigators found little or no increase in peroxisome number or size in the primate, using either hepatocytes *in vitro* or in whole animals [73–81].

The last few decades have marked the advancement of different techniques, including gene expression analysis or enzymatic activity measurement for detection of peroxisome proliferation both *in vitro* and *in vivo*; however, with little success in exact quantification of peroxisomes [73,82–85]. The current gold standard for quantifying peroxisome proliferation involves immuno-labeling of peroxisomes and its quantification using electron microscopy or confocal laser scanning microscopy (CLSM) [86–89]. These techniques provide accurate imaging; however, they have disadvantages of being costly, laborious, and time-consuming. These drawbacks have led to the advancement of a new class of materials, "nanomaterials" for efficient detection of peroxisomes.

3.2.1. Quantum dots

Quantum dots, also known as luminescent semiconductor nanocrystals, belongs to a class of nanomaterials that have been widely used as fluorescent biosensors, thanks to their unique photophysical properties. Moreover, quantum dots are exceptionally resistant to chemical degradation and photobleaching [90], granting them advantages over conventional methods for detecting peroxisomes. However, there have been many challenges in delivering these quantum dots to cell organelles. To overcome these challenges, several methods such as cationic liposomes, translocation of peptides, dendrimers, electroporation, or microinjection have been utilized [91]. Among these methods, quantum dots coupled with polyethylene glycol peptides (QD-PEG) using localized sequences were reported to successfully target mitochondria [91]. It further holds promise in targeting other subcellular compartments like peroxisome, endoplasmic reticulum, nucleus, and other sequences [91]. Moreover, streptavidin-coated fluorescent quantum dots (cadmium selenide-based material), paired with peroxisomal membrane protein (ABCD3) antibody, were used to detect peroxisomes in the fibrate-treated liver section of cynomolgus monkeys [92]. The peroxisomes were detected in formalin-fixed fibrate-treated rat liver tissues and human liver tissues [89,92]. This method is advantageous as it can be used for peroxisome detection from different species using the same chemicals and reaction conditions.

4. Nanomaterials based fabrication of peroxisomes

4.1. Artificial organelles

Artificial organelles (AOs) such as artificial peroxisomes (APs) are the microcompartments encasing several natural or synthetic enzymes capable of governing specific cellular encapsulated

catalytic reactions [19,20], malfunctioning of which might lead to various metabolic disorders [93].

4.1.1. Liposomes

Liposomes or polymersomes provide one of the most important platforms to fabricate AOs by capturing channel proteins in the hydrophobic region and natural enzymes in the cavity; however, their poor stability limits their further development [94]. Therefore, Chen et al. reported a new channel protein-independent microcompartment by cross-linking zwitterionic vesicles (cZVs) with the carboxylic acid saturated cavity [95]. The monolayer architecture of cZVs assisted them with the intrinsic permeability, whereas the cavities made cZVs competent for the *in-situ* synthesis of different artificial enzymes. In this study, cerium oxide (CeO2) and platinum (Pt) NPs based nanozymes were synthesized *in situ* to mimic the peroxisome environment and to detoxify the ROS [95]. The resulting CeO2/Pt@cZVs showed a pH-dependent zeta potential, allowing them to resist non-specific protein adsorption and improve endocytosis property under inflammatory conditions. Under *in vitro* milieu, preincubation of cell monolayers with CeO2/Pt@ cZVs depicted a protective effect of around 40% against the PQ-mediated oxidative damage. The most promising feature of CeO2/Pt@cZVs in BALB/c mice in case of ROS-induced ear inflammation was the downregulation of inflammatory cytokines and alleviation in the inflammatory cell infiltration symptoms. This specific channel protein-independent microcompartment of cZVs opens new avenues in the fabrication of diverse arrays of AOs to treat various cell disorders [95].

4.1.2. Nanoreactors

Nanoreactors are one of the potential candidates for making such artificial cell organelles. They are made of polymers and have compartment-like structures that can house enzymes and proteins [96]. Tanner et al. reported the fabrication of an antioxidant nanoreactor to scavenge ROS, mimicking peroxisomes. The novel nanoreactor contained two antioxidant enzymes in the polymer vesicle cavity and channel proteins in the vesicle membrane [97]. The novel artificial peroxisome was considered to be a cell implant, which could be used as a therapeutic model for treating various cellular disorders. Furthermore, the feasibility of changing the type of enzymes in the polymer compartment will provide a new direction to personalized therapies.

4.1.3. Nanozymes

Nanozymes are a class of nanomaterials known to internally possess enzyme-like catalytic activities [98]. Peroxisomes are essential eukaryotic organelles that contain several enzymes regulating reactive oxygen species (ROS) and uric acid [99]. Abnormally high levels of uric acid and ROS may cause urethritis and damage to nervous tissues, respectively. Hence, the idea of artificial peroxisome was proposed as an alternative approach for such diseases. In a study by Xi et al. [100], a nanozyme based artificial peroxisome was constructed. This novel artificial peroxisome (or *pero*-nanozysome) comprised of a hollow carbon nanozyme whose surface was doped with iron and nitrogen. The structural stability was provided by iron in the center and iron-nitrogen ($Fe-N_4$) as the prosthetic group. The iron-nitrogen combination formed a metal-organic framework, mimicking the multi-enzymatic feature of peroxisome discharging functions of superoxide dismutase (SOD), peroxidase (POD), oxidase (OXD), and catalase (CAT). In *in vivo* ischaemic condition, high level of free radicals are generated (superoxide ion, hydrogen peroxide, hydroxyl ion etc), which damages neurons and underlying nervous tissues affecting normal functioning of the brain (Fig. 4). The administration of these artificial peroxisomes were shown to scavenge ROS (O_2^-, $\cdot OH$ and H_2O_2) and alleviate the degradation of uric acid, thus acting as a therapeutic agent for diseases like ischemic stroke [100]. A schematic representation showing the potential of nanozymes in restoring neuronal cell damage generated from Ischaemic Stroke is presented in Fig. 4. SOD converts superoxide ions into water and oxygen whereas CAT acts on hydrogen peroxide and transforms them into non-poisonous molecules (water and oxygen). Additionally, oxidation of uric acid to allantoin and the production of more oxygen demonstrates the OXD and POD like activity of nanozymes.

One of the biggest challenges has been the stability of these artificial peroxisomes along with parameters like size, morphology, specific delivery, hydrophilicity/ hydrophobicity, surface charge and thickness [101,102]. Therefore, many modifications have been made to the basic metal-nitrogen-based nanozyme structure to enhance the stability, biocompatibility, and targeted drug delivery [103,104]. In a study by Zhang et al. [105], porous carbon-reduced graphene nanozyme was doped with nitrogen and functionalized with palladium nanoparticles (PdNPs/N-PC-rGO), which then mimicked natural peroxisome exhibiting oxidase, peroxidase, and catalase-like activities. Because of the high electrocatalytic activity, PdNPs/N-PC-rGO was also reported to detect reduced glutathione (GSH) [105]. Another research study demonstrated the development of a peptide-metal hybrid by utilizing arginine-rich peptides and platinum nanoparticle clusters (ARP-PtNC). The novel structure mimicked the superoxide dismutase/catalase and uricase/catalase cascade system of peroxisome. The multiple enzyme-like activities provided by ten arginine residues of ARPs make them a potential candidate for gout and hyperuricemia therapy [106].

5. Intracellular drug delivery agents

White matter injury triggering cerebral palsy is one of the significant causes of disability and death in infants [107]. Some of the proposed mechanisms underlying this phenomenon include depletion of oligodendrocytes due to LPS induced peroxisomal dysfunction [108] and the activation of microglial cells via infection or inflammation [109,110]. LPS induced peroxisomal dysfunction has been known to deteriorate β-oxidation leading to the accumulation of very-long-chain fatty acids and imbalance in ROS production and degradation [111]. Whereas infection or inflammation activates microglial cells in fetal brain, which then kills normal brain cells. N-acetyl cysteine (NAC) has both antioxidant and anti-inflammatory properties making it one of the most studied drugs for attenuating inflammation in various diseases [112–114], including LPS induced white matter injury [115,116]. However, intracellular delivery of drugs still poses a significant challenge. For example, oral NAC bioavailability has been reported to be between 6 and 10% [117]. Therefore, there is an urgent requirement for efficient drug delivery vehicles to successfully deliver drugs to intracellular organelles.

5.1. Dendrimers

Dendrimers are highly branched, monodispersed polymers with high density of functional groups that can be tailored for specific therapeutic application [118–121]. In conjugation with drugs, dendrimers have been utilized as intracellular drug delivery agents [122,123]. Wang et al. [124] reported the potency of anionic polyamidoamine dendrimer-N-acetyl cysteine ($PAMAM-(COOH)_{46}-(NAC)_{18}$) for treating the neuroinflammatory disorder by controlling intracellular drug delivery. Lipopolysaccharide (LPS) induced peroxisomal dysfunction leads to abnormal production of ROS [117]. It was shown that $PAMAM-(COOH)_{46}-(NAC)_{18}$ could significantly reduce the level of ROS generation [124]. Furthermore, the conjugation of

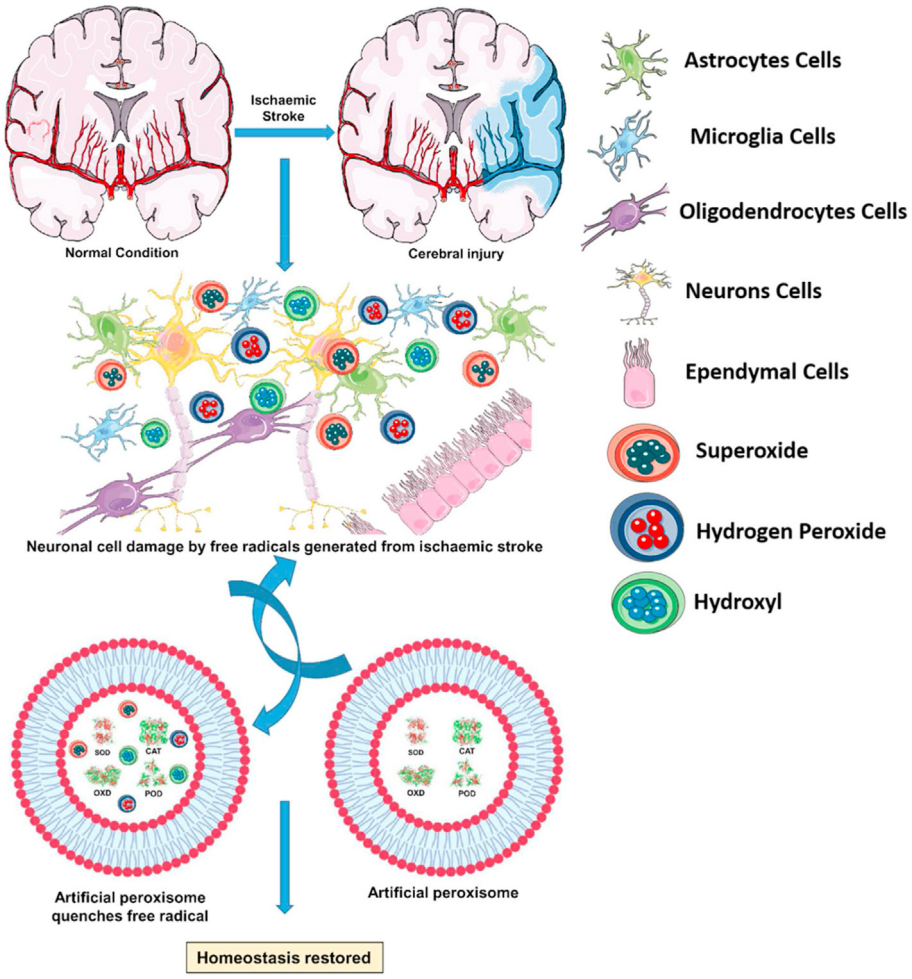

Fig. 4. Schematic representation of artificial peroxisome (nanozyme) and their potential in restoring neuronal cell damage generated from Ischaemic Stroke. Superoxide Dismutase (SOD); Catalase (CAT); Oxidase (OXD); Peroxidase (POD).

NAC with dendrimer (PAMAM–$(COOH)_{46}$–$(NAC)_{18}$) demonstrated superior antioxidant and anti-inflammatory properties compared to NAC [124]. This implies that dendrimers have the capability to be used as efficient intracellular drug delivery platforms.

6. Nanoparticles based non-invasive methods for interaction with target proteins or cells

6.1. Nanoparticles

Magnetic nanoparticle-based probes have been used to identify target proteins in native physiological conditions. This aids in understanding several biological processes, including protein-protein or protein-drug or organelle-organelle interaction. Lee et al. [125] reported a functionalized magnetic nanoparticle mimicking peroxisomal protein (PTS1-MNP) and imported into the HepG2 cells (human hepatoma). PTS1 is one of the peroxisomes targeting protein signals recognized by the Pex5p receptor, which shuttles back to the cytoplasm after releasing the protein inside the peroxisome. The functionalized magnetic nanoparticle conjugated with PTS1 was shown to interact successfully with Pex5p (PTS1-receptor protein) inside the HepG cells, showing transient interaction, recruitment of nanomaterials, and peroxisomal translocation inside the cells [125]. Such methods provide valuable platforms for studying the interaction between biomolecules or organelles (peroxisomes, mitochondria, endoplasmic reticulum, vesicles, ribosomes, endosome, lysosome) through non-invasive methods.

6.2. Nanodiamonds

Various types of nanoparticles have been utilized over decades in several applications ranging from sensing and imaging to drug delivery [126,127]. With this advancement, researchers started focusing on how these different nanoparticles accumulate or interact with cells or proteins [128–130]. The various factors affecting cellular uptake include nanoparticle size, shape, charge, functionality, concentration, time, temperature, and cell type [131–133]. However, the exact mechanism underlying the intracellular fate of these nanoparticles is still not clear. Nanodiamonds are a potential candidate for live cell tracking and for understanding intracellular fate as they have stable fluorescence and pH, generate low ROS, and are biocompatible with cells [134,135]. Schrand et al. reported nanodiamonds tagged with TAMRA dye and then used them for neuronal cell tracking. The nanodiamonds show high brightness that might be attributed to their high refractive index [136]. In another study, the fluorescent nanodiamond-gold nanoparticles (FND-Au) were used as imaging probes due to their multimodal imaging characteristics. The FND-Au nanoparticles were used to study the subcellular distribution at nanometres level

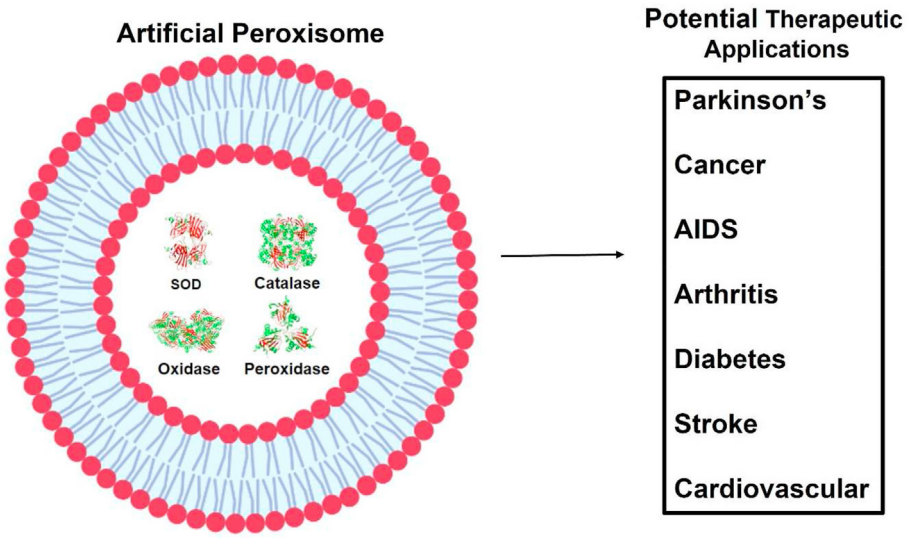

Fig. 5. Schematic representation of artificial peroxisome containing various enzymes and some of its potential therapeutic applications.

inside the cell. It was observed that the emission produced FND is not quenched by gold nanoparticles, which facilitates the optical imaging of individual FND-Au nanohybrid (nanodiamonds). The unique property of FND is associated with the negatively charged nitrogen-vacancy centers, which are present in high density and impart magnetic and optical properties [137]. Although, nanodiamonds offers these lucrative features, none of the studies so far have shown how these nanodiamonds can be used for sensing or imaging peroxisomes or for intracellular drug delivery to peroxisomes. Nevertheless, these nanodiamonds or nanoparticles can offer an interesting platform for future studies targeted at exploring peroxisomes.

7. Conclusion and future perspective

Nanomaterials-based detection and fabrication of peroxisomes holds great promise in understanding and modulating several metabolic disorders-related diseases. With the advancement in nanotechnology, nanomaterials with advanced optical and mechanical properties having specific functionality can now be easily fabricated. This implies that nanomaterials-based detection of peroxisomes in the physiological environment can be achieved in the near future by choosing the right nanomaterials or the combination of these nanomaterials with efficient delivery platforms. An example of this is the fabrication of nanoclusters, which have effectively been used to detect intracellular ROS. Moreover, the fabrication of artificial peroxisomes (AP's) can open new avenues for controlling the production of reactive oxygen species in the cells, which has implications in several biological processes. One of the examples for its future use can be to provide control over aging pathologies. Peroxisome contains enzymes responsible for the balanced regulation of hydrogen peroxide production and degradation [138]. The disruption in this balance can lead to the accumulation of hydrogen peroxide and downstream reactive oxygen species. With aging, the operational capability of the enzyme (catalase) present in peroxisome is affected, and it is unable to operate at 100% efficiency, which is one of the reasons causing disruption. Therefore, the fabrication of AP's can provide an efficient platform for maintaining the efficiency of enzymes, thus providing the balance between the production and degradation of hydrogen peroxide, which might then be used for treating aging pathologies such as parkinson's, cancer, AIDS, and arthritis (Fig. 5).

Another important strategy is to deliver a specific enzyme or protein to the peroxisome using intracellular protein delivery. This strategy can be beneficial in treating life-threatening cardiovascular events by explicitly targeting and treating high inflammatory regions. Song et al. [139] reported macrophage targeted theranostic nanodrug (MMR-Lobe-Cy) to detect inflammatory activity and deliver peroxisome proliferator-activated receptor gamma (PPARγ) activator in high-risk plaques. They demonstrated that by using this strategy, they could stabilize inflamed plaques in coronary size artery. The integration of nanomaterials with this strategy can open new pathways in the future to resolve high-risk coronary plaques.

As peroxisomes remain one of the least explored subcellular organelles in eukaryotic cells, nanomaterials can prove to be an excellent platform for continued exploration that would likely reveal additional and valuable information in the future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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