Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 4
NSCBM 2024

Recent Advances of Nanozymes as Therapies for ROS-Induced Diseases

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Abstract. Recently nanozymes have received more and more attention and have been applied in many fields. Recent advances of applications of nanozymes as therapies for various reactive oxygen species (ROS)-induced diseases are summarized in this account. For each case, the rational design strategies as well as various factors regulating the multiple enzyme-like activities, are also discussed. Perspectives and Challenges are also mentioned.

Keywords: Nanozymes, therapeutics, inflammation, ROS -related diseases.

1. Introduction

Enzymes are proteins that efficiently catalyse most biological reactions thanks to their bio-operability, high activity and specificity. However, their inherent limitations such as instability, high concentration, high preparation cost, and difficulty in modification have hindered their application. Nanozymes are nanomaterials mimicking enzymes with catalytic functions of natural enzymes and the advantages of nanomaterials, including metal sulphides and oxides [1], noble metals [2], metal-organic frameworks (MOFs) and carbon materials [3]. Some nanozymes have single enzyme mimetic activity, and some have multiple enzyme mimetic activities (**Table 1**.).[4] Compared to enzymes, nanozymes offer several advantages, including simple preparation, good stability, versatile functionality, tuneable catalytic activity, and recyclability. Therefore, nanozymes have been applied in various areas including materials science, environment, biology and medicine [4]. For normal metabolism, ROS production and consumption in human bodies are necessary. However, the pathogenesis of a variety of diseases have been attributed to overproduction and mismanagement of ROS. This account summarizes the application of nanozymes as therapies for ROS-induced diseases in recent years, including Idiopathic pulmonary fibrosis, Inflammatory bowel disease and neurological diseases (**Table 1**.).

Table 1. Classification of nanozymes

No. of Activities	Nanozymes	Disease	Refs.
2	CeO_2@MMT	IBD	6
2	CeO_2@Pt, Ag,Au	IBD	7
3	$LiMn_2O_4$	IBD	8
	ZVMoNanodots	IBD	9
	Ti_3C_2NSs	IBD	10
2	Ni_3S_4	IBD	11
	Ag-AXEPS	IPF	12
	C_{60}	IPF	16
	V_4C_3NSs	IPF	17
3	PtCu NAs	PD	18
2	Pd@PEG@Bor	AD	19
6	V_2C MXenzyme	AD	20

2. Nanozymes for IBD

Millions of people suffer from IBD each year due to overproduction of ROS. For treatment of IBD, application of antioxidant therapeutics with ROS-scavenging capabilities are effective options. Fortunately, Nanozymes with a wide range of antioxidant activities are able to cope with diverse ROS environments. [5]

Rapid development of oral acid-resistant and antioxidant nanozymes for the treatment of IBD is a challenge. In 2020, the Wei group applied CeO_2 nanoparticles (NPs) containing montmorillonite (MMT) which was clinically approved for the treatment of IBD (**Figure 1**.). CeO_2 was more effective than non-catalytic antioxidants at scavenging ROS due to its' catalase (CAT)-like activity, SOD-like activity, and hydroxyl radical-eliminating activity. In addition, they were more stable than natural enzymes. MMT with negative charges could be taken orally and then specifically absorbed by inflamed colonic tissues with positive charges by electrostatic interactions. CeO_2@MMT with optimal ratio (1:9) was acid-resistant to the mice's stomach and delivered to inflamed colon by electrostatic interactions. Via ROS scavenging, inflammation was reduced as shown by in vivo relief of IBD.[6]

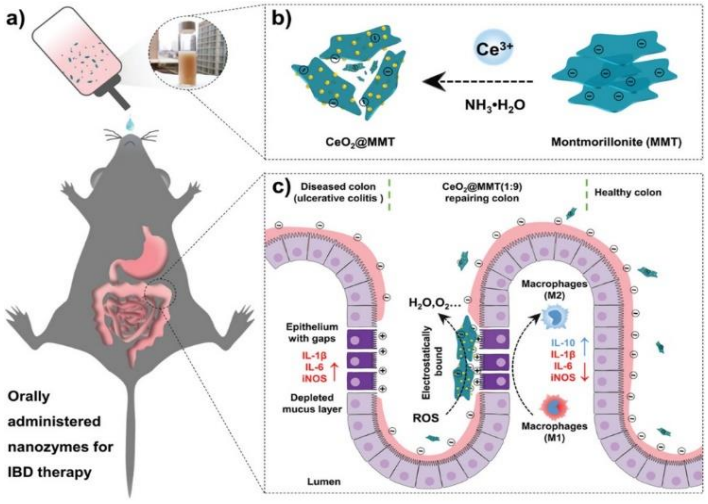

Figure 1. Design and synthesis of orally administered CeO_2@MMT for IBD. [6]

In 2022, a simple oxygen vacancy-induced metallic growth strategy was developed by the Wei group for the growth of multiple metallic NPs onto hollow CeO_2 nanospheres surface. Compared to the conventional method, neither any reducing and stabilizing agents nor pre-treatment were used. The catalytic activity was evaluated in two in vivo models of inflammation based on the composition of the oxide-loaded metals. Their multiple enzyme-like activities (CAT and SOD- like) were successful translated for the treatment of ear inflammation and IBD (**Figure 2**.). The CeO_2@Ag nanozyme demonstrated excellent therapeutic properties with a small dose of 0.5 mg/kg. [7]

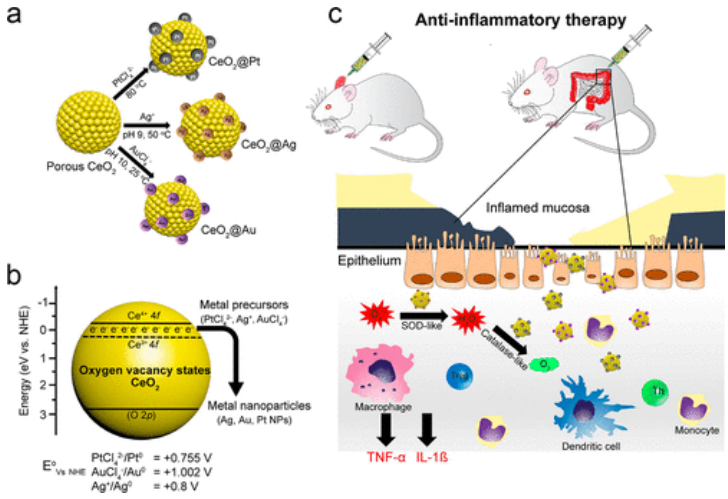

Figure 2. Synthesis of CeO_2@metal nanozymes for treating inflammatory diseases. [7]

The valence engineering strategy offers design strategy for nanozymes with multiple antioxidant activities. In 2022, the Wei group applied a model nanozyme $ZnMn_2O_4$ study the effect of Mn valence at octahedral sites with valence engineering strategy (**Figure 3**.). The multiple enzyme-like antioxidant activities which mimic SOD, CAT and glutathione peroxidase (GPx), were found to be

positively correlated with Mn^{4+}. With this strategy, the optimal nanozyme $LiMn_2O_4$ was prepared and its cytoprotective effect was proved in vitro and in an in vivo mice model of IBD.[8]

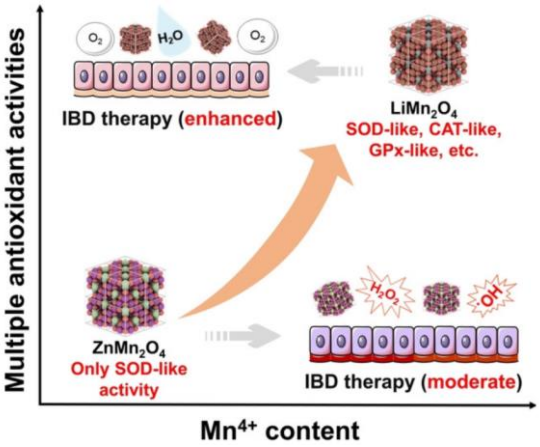

Figure 3. Design and synthesis of a nanozyme $LiMn_2O_4$ for treating IBD. [8]

In 2022, the Hu group synthesized an orally-admistrated zero-valent molybdenum nanodots (ZVMNs) with ROS- scavenging activities (**Figure 4**.). After penetration of gastric acid, these ZVMNs were absorbed by the intestine in a mice model of IBD. It was indicated that ZVMNs could decrease amount of ROS and reduce colitis without obvious side effects. Further mechanisms revealed by RNA sequencing, in which colonic tissues were protected from oxidative stress by ZVMNs via reduction of the overproduction of pro-inflammatory factors and inhibition of the nuclear factor κB signalling pathway. [9]

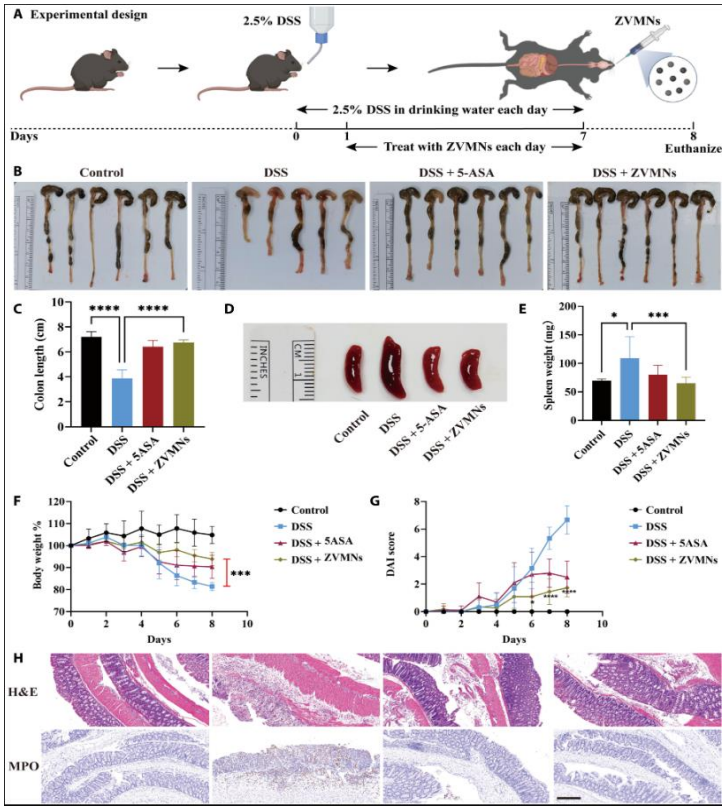

Figure 4. Therapeutic Effect of ZVMNs in the IBD in vivo model and mechanisms of ZVMNs for IBD.[9]

In 2022, based on titanium carbide MXene nanosheets (Ti_3C_2NSs) stabilized by simulated gastric fluid (SGF), the Cheng group designed an antioxidant orally administered nanozyme as IBD therapy with high biosafety (**Figure 5**.). The multiple antioxidant activities were determined by the strong reducing properties of binding Ti-C. Levels of intracellular ROS indicated that excess ROS was successfully scavenged, and oxidative stress-induced cellular damage was prevented by Ti_3C_2 NSs.

Once administrated orally, Ti_3C_2 NSs with negative charges were specifically adsorbed by inflamed colonic tissues with positive charges by electrostatic interactions, leading to effective treatment of IBD. The reduction of pro-inflammatory cytokine secretion and elimination of excess ROS, increased anti-inflammatory and effectively inhibited inflammation and cytokine secretion of M2 phenotype macrophage infiltration alleviated the symptoms of colitis. Cutaneous wound healing and functional vascularization were promoted by Ti_3C_2-based woundplast with excellent ROS scavenging properties. As a novel orally administrated nanozyme, the MXene nanoplatform could be applied for treating IBD.[10]

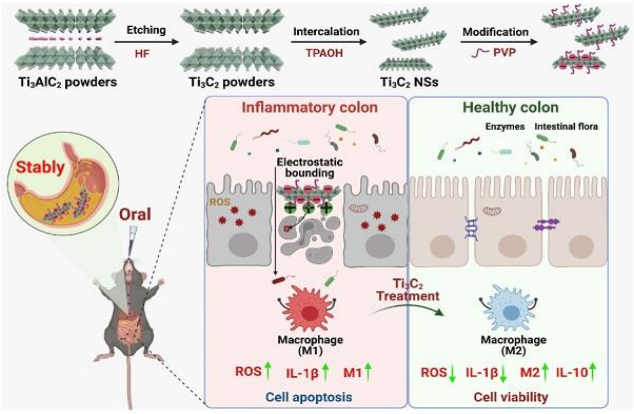

Figure 5. Design and synthesis of Ti_3C_2 NSs to for treating IBD. [10]

In 2023 the Zhu group reported a high-throughput screening strategy with nine stages to meet the multiple demanding of IBD therapy, including solubility, intrinsic stability, acid resistance radioactivity, biomimetic elements, gut microbiome toxicity, reaction energy barriers, intermediate frontier molecular orbitals and negative charges. As the best match among 146,323 candidates, Ni_3S_4 exhibited SOD-CAT-like but higher activities (3.13 and 1.80 times) than the natural enzymes (**Figure 6**.). Mice models of IBD showed that Ni_3S_4 was stable and non-toxic in the gastrointestinal tract. The diseased colon was specifically targeted, and oxidative stress was mitigated. Further mechanistic analysis by RNA and 16S rRNA sequencing indicated that Ni_3S_4 effectively inhibited pro-inflammatory cellular pathways and restored the intestinal microbiota. This research offers a new design strategy for nanozymes via a data-driven approach.[11]

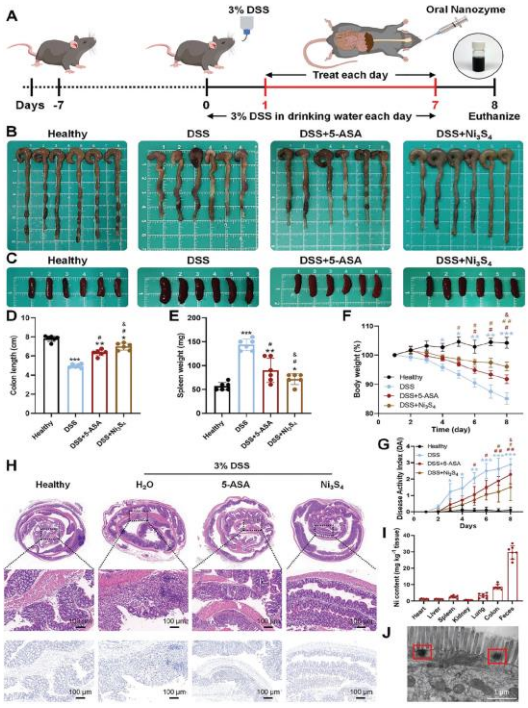

Figure 6. Therapeutic Efficacy of Ni_3S_4 in the mice model of IBD. [11]

3. Nanozymes for IPF

IPF is a form of serious fibrosing interstitial pneumonia. An oxidative/antioxidative imbalance is critical in IPF. Level of pulmonary fibrosis can be decreased by reducing oxidative stress. [12]

In 2021, the Hassan Group observed that levels of matrix metalloproteinases (MMP-2 and MMP-9), monocyte chemotactic protein-1and respiratory tumour necrosis factor-alpha were reduced in rats treated with Silver Alcaligenes xylosoxidans extracellular polysaccharides (Ag-AXEPS) (**Figure 7**.). Similarly, the mRNA levels of plasma fibrosis markers, IL-12 and BAX were also decreased. Meanwhile, the total antioxidant capacity and mRNA BCL2 were increased. As demonstrated by quantitative pathologic studies on hematoxylin-eosin-stained lung sites, level of fibrosis was also decreased. However, recovery of lung collagen as well as an increased and improved overall survival were ensured by the treatment as assessed by Masson's trichrome staining.[13]

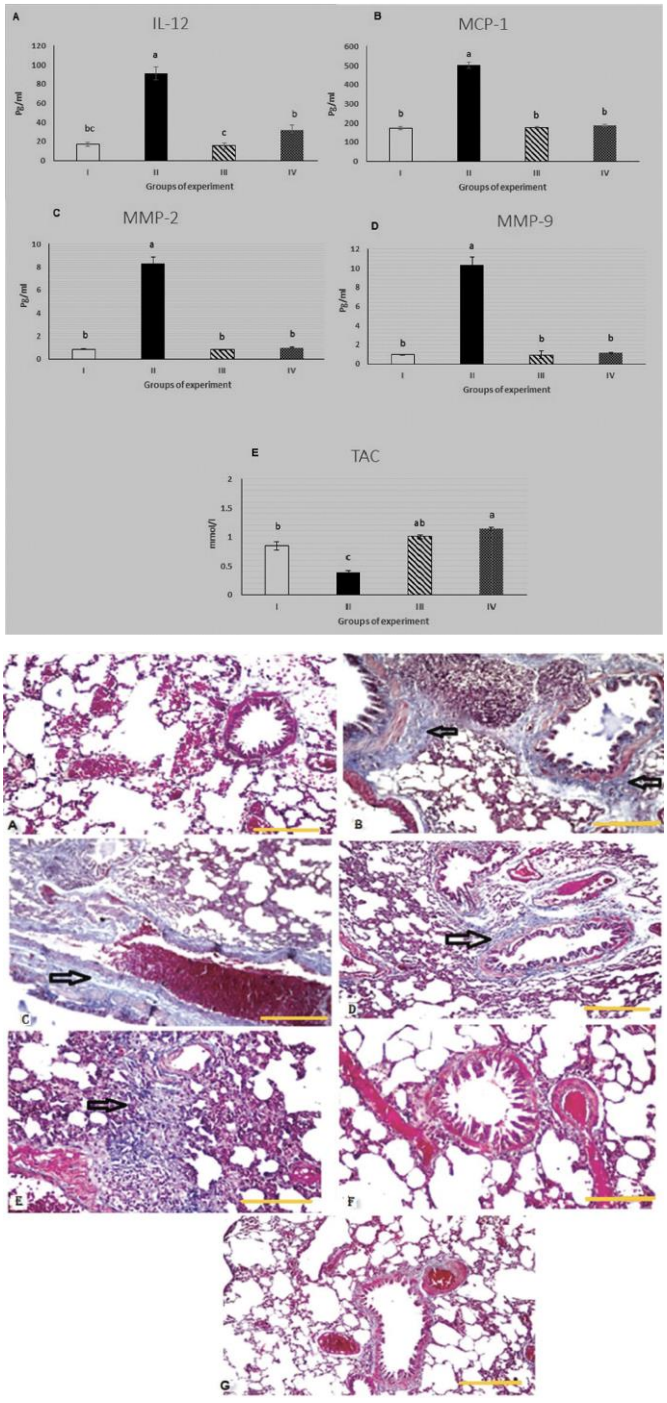

Figure 7. Therapeutic Efficacy of Ag-AXEPS in the mice model of IPF. [13]

Fullerenes have been reported as a strong free radical scavenger [14]. It was also demonstrated that at low physiological concentrations fullerene C_{60} is non-toxic and water-soluble [15]. In 2020, the Dai group reported that water-soluble $C_{60}(OH)_{22}$ showed antifibrotic activity via antioxidant effect in a mice model of bleomycin (BLM)-induced IPF (**Figure 8**.). After treatment of mice with C_{60}, the number of fibroblasts was obviously reduced, and amount of type II alveolar epithelial cells was promoted. Therefore, water-soluble C_{60} decreased the degree of BLM -induced IPF in mice due to its strong antioxidant activity. [16]

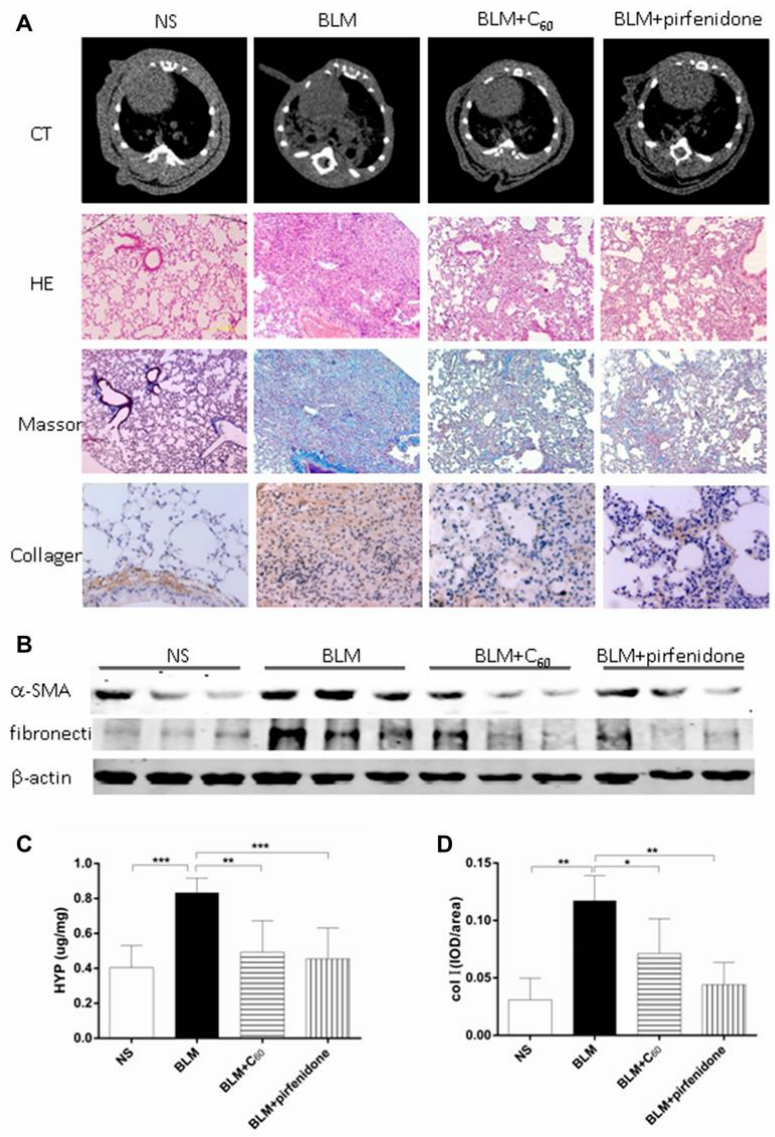

Figure 8. Therapeutic Efficacy of $C_{60}(OH)_{22}$ in the mice model of IPF. [16]

In 2023, the He group reported that V_4C_3 nanosheets (NS) can be applied as potential antioxidants for the treatment of IPF due to their ROS /RNS-scavenging capabilities (**Figure 9**.). They found that the valence composition of vanadium was adjusted, and their antioxidant behaviour could be significantly improved by subtle autoxidation. Multiple mechanisms including proton transfer, electron transfer as well as enzyme-like catalysis were triggered by valence engineering, leading to V_4C_3 NSs with broad-spectrum, efficient, and long-lasting antioxidant capacity. Myofibroblast proliferation and extracellular matrix abnormalities were significantly prevented by V_4C_3NSs due to their antioxidant properties and good biocompatibility. After treatment, anti-inflammation, alleviation of lung fibrosis, and reestablishment of antioxidant defences progression of BLM-induced IPF were observed in vivo. [17]

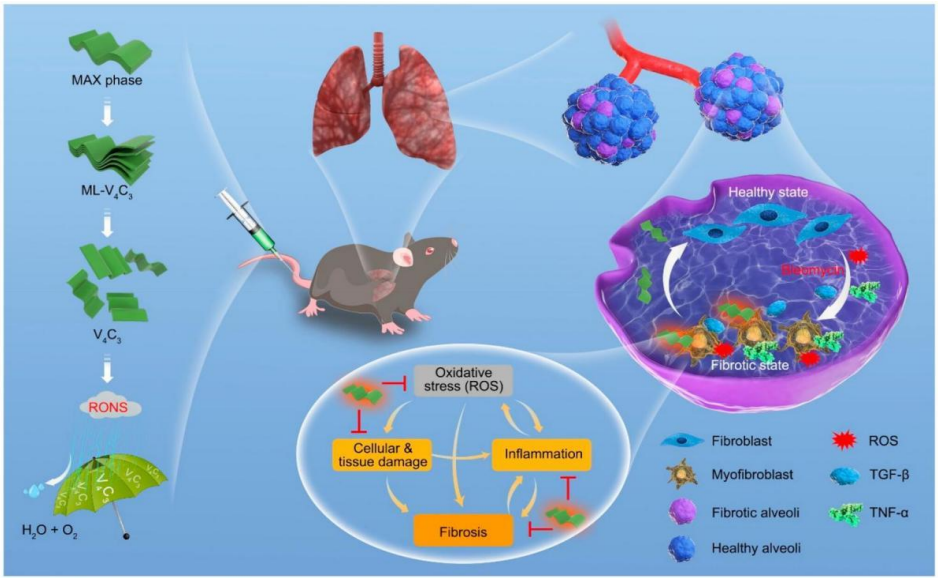

Figure 9. Design and synthesis of V_4C_3NSs for treating IPF. [17]

4. Nanozymes for neurological diseases

Parkinson's disease (PD) has been reported as one of the most frequently occurred neurological disorders. Understanding of PD has been fundamentally upended by Brack's prion-like theory. Recently pathological alpha-synuclein (α-syn) has been reported as a prion-like protein. In addition, diffusion of α-syn in the patient's brain is the important driver of PD. In 2021, the Li group reported that α-syn diffusion and cell death along with interneuronal transmission are significantly prevented by the biocompatible antioxidant nanozymes PtCu nano-alloys PtCu Nas which mimic SOD, CAT, peroxidase (POD) and DPPH-scavenging enzymes via eliminating ROS in primary neuronal cultures (**Figure 10**.). In addition, α-syn diffusion induced by intra-striatal injection of α-syn preformed protofibrils (PFF) is significantly inhibited. [18]

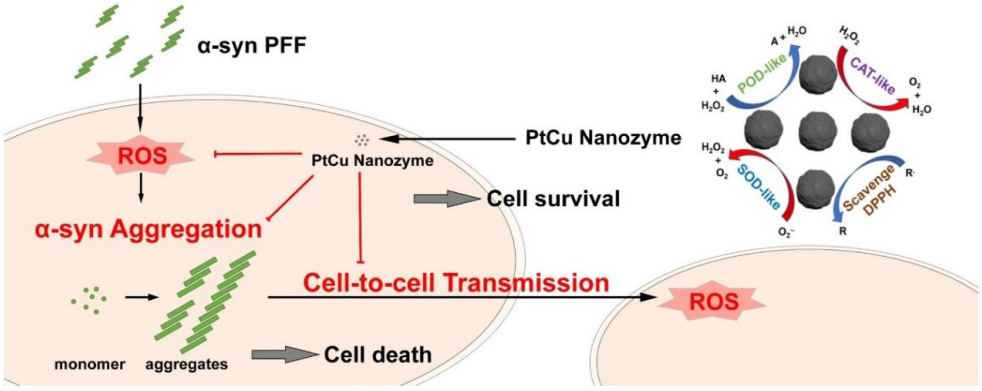

Figure 10. Illustration of PtCu NAs in the treatment of PD. [18]]

AD is the first common neurological disease that affect 50 million people worldwide. Oxidative stress has been considered as a pathological driver of AD. Mitochondrial dysfunction closely related to Aβdeposition and neurogenic fiber tangles (NFTs) is the causative agent. Octahedral palladium nanoparticles (Pd NPs) exhibit strong antioxidant capability and good biocompatibility. However, their application as therapy for AD treatment was limited by their poor permeability of blood-brain barrier (BBB). In 2021 the Jia group constructed an octahedral palladium nanozymes platform (Pd@PEG@Bor) modified with borneol (Bor) and bifunctional PEG (SH-PEG-COOH) for scavenging intracellular ROS and promoting the permeability of epithelial cells (**Figure 11**.). According to an in vitro and in vivo research, ROS and Ca^{2+} contents were effectively reduced, and mitochondrial membrane potential was maintained by Pd@PEG@Bor, with mitochondria in SH-SY5Y cells protected. In addition, concentration of Pd@PEG@Bor could be rapidly increased in the

AD mice brains and alleviate Aβ plaque deposition, neuroinflammation and neuronal loss. The AD mice's memory function and learning ability were also obviously improved. [19]

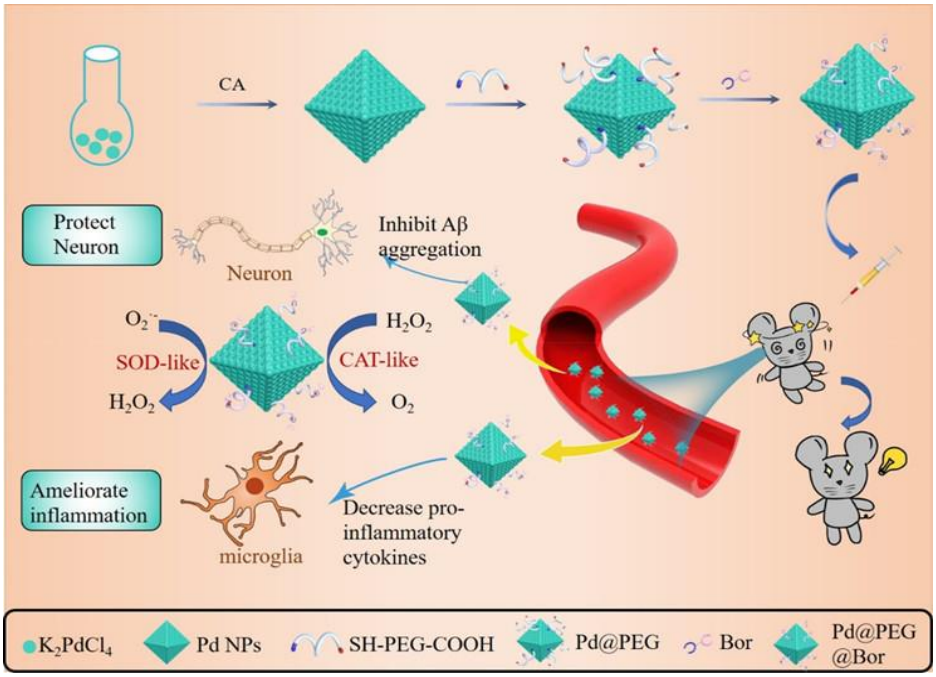

Figure 11. Design and synthesis of Pd@PEG@Bor for treating AD. [19]

5. Conclusion

In this account, recent advances in applications of nanozymes as therapies for several ROS-relateded disorders such as IBD, IPF, PD and AD are summarized. Various factors which regulate the antioxidant activities and design strategies are also discussed with specific cases. However, there are still challenges for multi-enzymic nanozymes.[4] How to modulate and balance different enzymic-like activities and prevent the interferences can only be realized with profound understanding of underlying mechanisms of 'multi-enzymatic activity'. Biosafety evaluation and polypharmacology of multi-enzymic nanozymes is also urgent because of their possible complicated and unexpected side effects.

Acknowledgments

The Author wishes to acknowledge assistance from Dr Xin Guo of Wenzhou Medical University.

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