

铜基纳米酶的特性及其生物医学应用

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摘要: 天然酶对维持生物体生命活动的正常运行具有重要意义, 但天然酶固有的缺点诸如不稳定、反应条件苛刻和提纯成本高等限制了其广泛应用。与天然酶相比, 具有高稳定性、低成本、便于结构调控与改性等优点的纳米酶吸引了科学家们的关注。纳米酶类天然酶活性和选择性使其在生物医学、环境治理、工业生产等领域得到广泛应用。铜作为人体内必需元素和天然酶活性中心金属之一, 铜基纳米酶受到了人们广泛的关注和研究。本综述重点介绍了铜基纳米酶的分类, 包括铜纳米酶、氧化铜纳米酶、碲化铜纳米酶、铜单原子纳米酶和铜基金属有机框架材料纳米酶等, 并阐述了铜基纳米酶的酶学特性和催化机理, 总结了铜基纳米酶在生物传感、伤口愈合、急性肾损伤和肿瘤治疗等方面的应用, 最后对铜基纳米酶面临的挑战和未来的发展方向进行了总结和展望。

关键词: Cu; 纳米酶; 类酶活性; 生物医学应用; 综述

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Copper-based Nanozymes: Properties and Applications in Biomedicine

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Abstract: Natural enzymes play an important role in maintaining normal life activities, but suffer in their inherent instability, harsh reaction conditions and high purification costs, which limit their wide applications *in vitro*. Compared to natural enzymes, nanozymes with high stability, low cost, and ease of structural regulation and modification attract the great interests and are widely applied to biomedicine, environmental control, industrial production and other fields due to their enzyme-like activities and selectivity. As an essential element and one of the active central metals of natural enzymes in the human body, copper-based (Cu-based) nanozymes have received extensive attentions and researches. This review focused on the classification of Cu-based nanozymes, such as Cu nanozymes, Cu oxide nanozymes, Cu telluride nanozymes, Cu single-atom nanozymes, and Cu-based metal organic framework nanozymes. Then this review described the enzyme-like activities and catalytic mechanisms of Cu-based nanozymes, and also

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summarized the applications of Cu-based nanozymes, including biosensing, wound healing, acute kidney injury, and tumors. The challenges and future development direction of Cu-based nanozymes were proposed.

Key words: Cu; nanozyme; enzyme-like activity; biomedical application; review

天然酶是生物体内具有超高催化活性和选择性的生物分子,对维持生物体生命活动的正常运行具有重要意义。但由于天然酶的不稳定性、催化反应条件苛刻、反应后回收困难以及提纯成本高的缺点,天然酶在体外催化反应中的应用受到限制。因此,科学家们致力于寻找能够替代天然酶的材料。随着纳米科学技术和交叉学科的发展,2007年阎锡蕴团队^[1]首次报道了无机纳米材料的酶学特性,随后大量金属基纳米材料也被探索到具有类酶活性^[2-5],如金属氧化物纳米材料 V_2O_5 ^[6-8]、 Mn_3O_4 ^[9-10]、 CeO_2 ^[11-12]、 Co_3O_4 ^[13-14]、 CuO ^[15-16]等,贵金属基纳米材料如 Au ^[17-20]、 Ag ^[21-23]、 Pt ^[24-26]、 Pd ^[27-30]等纳米颗粒,以及金属有机框架材料(MOF)如 $Fe-MOF$ ^[31-32]、 $Cu-MOF$ ^[33-34]、 $Co-MOF$ ^[35]等。这些具有酶学特性的金属基纳米材料被称为金属基纳米酶。与天然酶相比,金属基纳米酶因其催化反应条件温和、稳定性高、成本低、易于制备、便于结构调控与改性等优势,在生物传感、免疫检测、肿瘤诊疗、神经保护、环境治理等领域具有广泛应用^[36-40]。

与贵金属 Au 、 Ag 等元素相比, Cu 是生物体内的必需元素,也是天然酶活性中心金属元素之一。它在自然界含量丰富、价格低廉,因此铜基纳米酶得到了研究者的广泛关注和研究。研究发现无论是二价铜离子、铜纳米颗粒、氧化铜、铜的金属有机框架材料还是铜单原子催化剂等都表现出了优异的酶学特性^[41-43]。2011年,Chen等^[44]发现市售的粒径在 30 nm 左右的 CuO 纳米颗粒具有类过氧化物酶(Peroxidase, POD)活性。随后人们又相继发现 CuS ^[45-46]、 $MOF-199$ ^[47]、 $Cu NPs$ ^[48-49]等均表现出优异的类 POD 活性,但这些材料均只表现出单一的类酶活性。Peng等^[50]以谷胱甘肽为配体合成的超小铜团簇($Cu NCs$)表现出了多重类酶活性——过氧化氢酶(CAT)、超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GPx)活性。随着大量无机纳米酶的发现,科学家逐渐不满足于纳米酶的随机合成。受细胞色素 P450 的轴向配体配位血红素的启发, Huang等^[51]构建了以 FeN_5 为活性中心的单原子纳米酶。而后人们以氮化碳材料、金属氧化物、金属有机框架材料等为基底制备出一系列铜基单原子纳米酶,进一步拓宽了铜基纳米酶的范围^[52-55]。

本文阐述了铜基纳米酶的分类、酶学特性,及其在生物医学中的应用。

1 铜基纳米酶的分类

1.1 铜纳米酶

金属纳米颗粒因尺寸小、表面积大而具有较大的表面自由能。高的表面自由能使得纳米颗粒不稳定,因此往往需要引入配体来防止金属核自发团聚^[56-57]。人们以氨基酸、蛋白质、聚合物等为配体制备了一系列铜基纳米酶。Ma等^[56]合成了自组装的铜-半胱氨酸硫醇纳米颗粒($Cu-Cys NPs$)(图 1(a)),通过原位活化谷胱甘肽(GSH)并提高过氧化氢(H_2O_2)的浓度,实现对乳腺癌的化学动力学治疗。Liu等^[58]通过简单水热法制备了磷酸盐玻璃(PBG)基铜纳米酶($Cu-PBG$)。该纳米酶在酸性环境中具有类 POD 活性,展现出在抗菌领域的应用前景。Wang等^[59]以半胱氨酸-组氨酸二肽(CH)为配体合成了具有类漆酶活性的 $CH-Cu$ 纳米酶。该纳米酶在含酚类化合物的环境治理中具有潜在的应用,同时还能通过智能手机实现对肾上腺素的精准定量。

近年来,金属纳米团簇以其超小的尺寸和量子尺寸效应,具有优异的稳定性、良好的生物相容性和可调控的结构等优势,吸引了科学家们的关注^[60-61]。Liu等^[62]以半胱氨酸(Cys)为配体制备了尺寸在 2.5 nm 左右的 $Cu NCs$ 。该 $Cu NCs$ 具有类抗坏血酸氧化酶(AAO)活性,基于此活性他们还开发了一种新型荧光抗坏血酸传感器。Liu等^[63]在原子水平上对原子精度的 Au_{25} 团簇进行单原子取代,得到 $Au_{24}Cu_1$ 团簇。单原子取代的 $Au_{24}Cu_1$ 团簇极大地提高了 Au_{25} 团簇的抗氧化活性,其抗氧化能力是天然抗氧化剂 Trolox 的 137 倍。此外, Meng等^[64]还在亚纳米尺度上实现了 Cu_3 团簇的精准合成。他们通过将 Cu_3 团簇固定在富缺陷的金刚石-石墨烯载体表面,使活性中心完全暴露,因此增强了 Cu_3 团簇的类氧化酶(OXD)活性,进一步有效提升了 Cu_3 团簇的抗菌活性。

1.2 氧族元素与 Cu 的化合物纳米酶

自 2011 年 Chen等^[44]发现 CuO 具有类 POD 活性后,人们就对铜的氧化物纳米颗粒进行了大量的

研究(图 1(b))。Lin 等^[65]在碱性条件下通过 H_2O_2 与 Cu^{2+} 的配位制备得到过氧亚铜(CP)纳米点。在肿瘤内部的微酸介质中, CP 纳米点发生自分解反应供给 H_2O_2 和 Cu^{2+} , 而后 Cu^{2+} 发生芬顿(Fenton)反应产生羟自由基($\cdot OH$)损伤肿瘤细胞, 实现抗肿瘤效果。Li 等^[66]报道碳点保护的 Cu_4O_3 纳米复合材料作为刺激响应型纳米酶, 其类 POD 和 OXD 活性受光照、温度、pH 和氧化剂的影响, 在生物传感、环境处理以及有机合成中具有潜在应用。除氧化铜纳米颗粒以外, 硫化铜、碲化铜等也表现出优异的一类酶活性。Yang 等^[46]通过简单的共沉淀法制备了 CuS 纳米颗粒, 并利用 CuS 纳米颗粒的类 POD 活性构建了一种新型的无标记化学发光免疫传感器, 用于检测肿瘤标志物甲胎蛋白的含量。Wen 等^[67-70]制备了 Cu^+ 和 Cu^{2+} 并存的 $Cu_{2-x}Te$ 纳米颗粒(图 1(c)), 利用其类谷胱甘肽氧化酶(GSHOx)和 POD 活性催化肿瘤内的级联反应, 提高肿瘤内氧化应激水平来诱导肿瘤细胞的死亡。同时氧化应激的持续升高能够激活免疫系统, 产生抗肿瘤免疫记忆, 有利于抑制肿瘤复发和转移, 实现肿瘤的免疫疗法。

1.3 铜基金属有机框架材料纳米酶

金属有机框架材料是由无机金属离子和有机配体通过自组装相互连接, 形成的具有周期性网格结构的有机-无机杂化材料。MOF 材料的高比表面积、高孔隙率以及优异的理化稳定性, 使其具有巨大的发展潜力^[71-73]。Wang 等^[74]制备了超薄二维金属有机框架材料铜-四(4-羧基苯基)卟啉(Cu-tetrakis(4-carboxyphenyl)porphyrin, Cu-TCPP)纳米片。Cu-TCPP 纳米片的类 POD 活性和 Cu^{2+} 催化的 Russell 机制将肿瘤内的 H_2O_2 转化为对肿瘤细胞有害的单

线态氧(1O_2), 同时借助 Cu^+ 和 Cu^{2+} 的循环氧化机制持续消耗肿瘤内部的 GSH。这双重机制并行最终实现肿瘤治疗。纳米材料的活性中心会随其尺寸和形貌的改变而改变, 因此纳米材料的催化活性极大程度依赖于它的尺寸^[75]。Zhang 等^[34]制备的 Cu-TCPP MOF 纳米点(CTMDs)表现出与二维 Cu-TCPP 纳米片相反的活性(图 1(d))。CTMDs 表现出类 SOD 和 GPx 活性, 能够显著降低氧化应激水平, 并对脂多糖(Lipopolysaccharide, LPS)诱导的急性肾损伤具有治疗作用。Wu 等^[76]制备了 Cu、Zr 双金属的 MOF-818, 通过 Cu、Zr 双金属协同作用模拟天然酶 Cu/Zn-SOD 的 Cu-Zn 双金属活性中心。发现其类 SOD 活性比以往报道的单金属 Cu-MOF 的活性提高了 3.4~8.0 倍。经密度泛函理论计算, 发现 Zr 降低了 Cu^+ 与 Cu^{2+} 之间电子转移循环的能级势垒, 因而赋予其更高的类 SOD 活性。

1.4 铜基单原子纳米酶

单原子纳米酶相较于纳米颗粒, 具有更高的原子利用率和更多的活性位点, 这为提高纳米酶的催化活性提供了新思路^[77-78](图 1(e, f))。Zhang 等^[79]以 ZIF-8 为前驱体制备了 Fe-Cu- N_6 的双金属单原子纳米酶。Fe-Cu- N_6 独特的双金属结构有利于其在催化过程中进行氧分子的双边吸附, 进而降低反应势垒, 表现出优异的一类 POD、SOD 和 CAT 活性。与天然酶相比, Fe-Cu- N_6 单原子纳米酶在数月后仍具有超高的活性和稳定性。Wu 等^[80]在氮掺杂的碳纳米片(NPC)表面制备了 Cu 单原子纳米酶(Cu SASs/NPC)。在类酶催化和肿瘤光热疗法的协同增强作用下, Cu SASs/NPC 实现了对大肠杆菌和金黄色葡萄球菌 100% 的抗菌效果。Chang 等^[81]设计了一种负载有钠-葡萄糖协同转运蛋白抑制剂 licogliflozin (LIK066) 的多孔 Cu 单原子纳米酶(Cu SAzyme)。Cu SAzyme 通过 LIK066 减少肿瘤细胞对葡萄糖的摄取从而阻断能量来源而减少肿瘤细胞内的热休克蛋白(HSPs)的合成。同时利用 Cu SAzyme 产生多种活性氧(ROS)的特性使肿瘤内已有的 HSPs 失活, 双管齐下增强肿瘤的光热疗法效果。

2 铜基纳米酶的酶学特性

2.1 铜基纳米酶的一类抗氧化酶活性和清除自由基能力

当机体受到脑外伤等外界刺激时, 体内产生过多的自由基, 单纯靠自身的氧化还原系统不能维持自身稳态, 继而自由基就会对线粒体、蛋白质、DNA

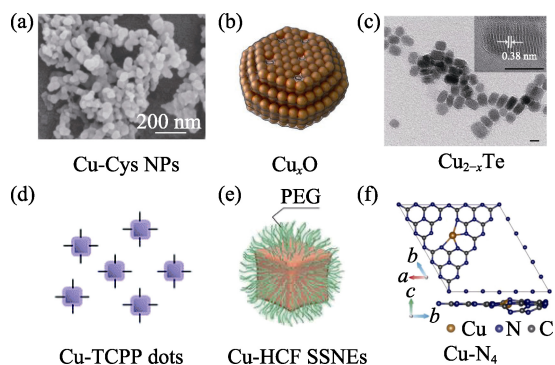


图 1 不同种类的铜基纳米酶^[34,56,67-70]

Fig. 1 Different types of Cu-based nanozymes^[34,56,67-70]

(a) TEM image of Cu-Cys NPs^[56]; (b) Schematic illustration of Cu_4O_3 ^[68]; (c) TEM image of $Cu_{2-x}Te$ ^[67]; (d~f) Schematic illustration of (d) Cu-TCPP Dots^[34], (e) Cu-HCF SSNEs^[69] and (f) $Cu-N_4$ ^[70]
NPs: Nano particles; Cu-TCPP: Cu-tetrakis(4-carboxyphenyl)porphyrin; Cu-HCF: Cu hexacyanoferrate; SSNEs: Single-site nanozymes

等产生毒害作用。因此需要引入具有抗氧化活性的纳米酶来降低氧化应激,实现疾病治疗和机体保护作用^[82-85]。纳米酶具有类天然酶的活性,对其酶学活性的研究是至关重要的。Hao 等^[68]以苯丙氨酸为配体合成了手性 Cu_xO 纳米颗粒。该纳米颗粒在 $30 \mu\text{g/mL}$ 的浓度时能够清除 90% 的超氧阴离子自由基($\text{O}_2^{\cdot-}$),展现出优异的类 SOD 活性(图 2(a))。 Cu_xO 还表现出超高的类 GPx 活性, Cu_xO 对 H_2O_2 底物的最大反应速率(V_m)为 $295 \mu\text{mol}/(\text{L}\cdot\text{min})$,比天然 GPx($14.1 \mu\text{mol}/(\text{L}\cdot\text{min})$)高出 20 倍(图 2(b))。此外 Cu_xO 还表现出一定的类 POD 活性,能够有效清除 H_2O_2 (图 2(c))。与其他金属氧化物纳米酶相比,由于 Cu_xO 同时具有类 SOD、GPx 和 POD 的三类酶活性,它在治疗 ROS 引起的疾病治疗方面具有更大的优势。

正如前文提及的一样,纳米酶的活性极大地受尺寸影响。Liu 等^[86]制备了超小 $\text{Cu}_{5,4}\text{O}$ 纳米颗粒($\text{Cu}_{5,4}\text{O}$ USNPs),尺寸在 4 nm 左右。 $\text{Cu}_{5,4}\text{O}$ USNPs

结合了 Cu 和 Cu_2O 纳米晶的优势,实现了类酶活性数量级的提高。它在 200 ng/mL 的浓度时对 H_2O_2 的清除率达到 80%,同时对 H_2O_2 表现出比天然 CAT(米氏常数 $K_m = 0.134 \text{ mmol/L}$)更高的亲和力($K_m = 0.065 \text{ mmol/L}$)。 $\text{Cu}_{5,4}\text{O}$ USNPs 具有极强的类 SOD 活性,相比于天然酶 SOD 对 $\text{O}_2^{\cdot-}$ 的清除率为 50%时的浓度(EC_{50})为 41.6 ng/mL ,而 $\text{Cu}_{5,4}\text{O}$ USNPs 的 EC_{50} 为 191.4 ng/mL ,这表明类 SOD 活性达到天然酶的 21.7%。 $\text{Cu}_{5,4}\text{O}$ USNPs 还表现出浓度依赖的清除自由基的活性。 150 ng/mL 的 $\text{Cu}_{5,4}\text{O}$ USNPs 对 H_2O_2 、 $\cdot\text{OH}$ 和 $\text{ABTS}^{\cdot+}$ (表征总抗氧化能力的一种自由基)的清除率分别达到 76%、80%和 89%(图 2(d-f)),表现出超高的清除 ROS 的活性。Chen 等^[70]以石墨化的氮化碳(CN)为基底制备得到了活性中心为 CuN_4 的铜单原子纳米酶(Cu SAs/CN),并报道 Cu SAs/CN 具有类抗坏血酸过氧化物酶(APX)活性。APX 是植物体内重要的抗氧化酶,能够以抗坏血酸(Ascorbic acid, AA)为电子供体催化分解叶绿体内的

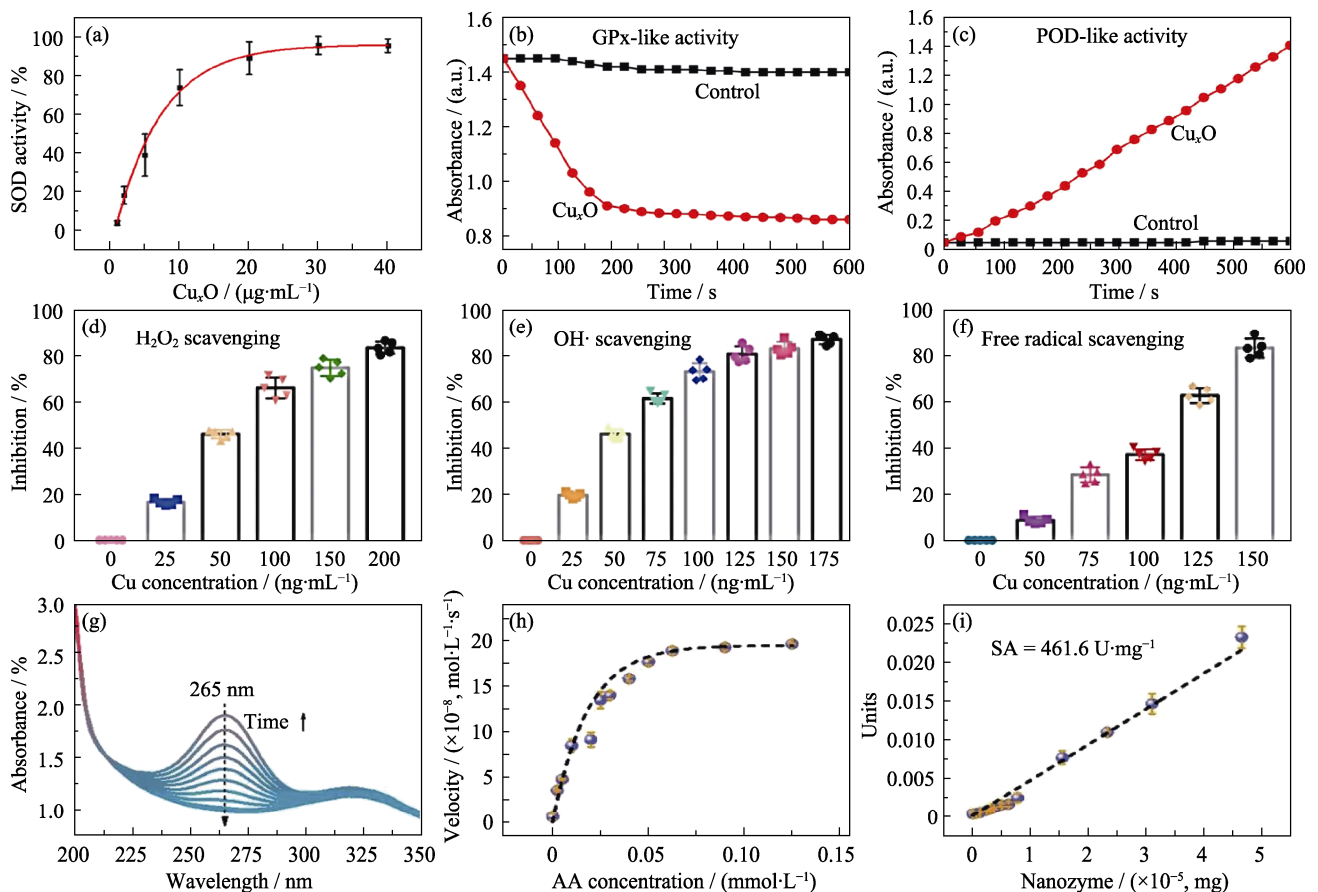


图 2 铜基纳米酶的类抗氧化酶活性和清除 ROS 能力^[68,70,86]

Fig. 2 Antioxidant-like enzyme and ROS scavenging activities of Cu-based nanozymes^[68,70,86]

(a-c) SOD-like (a), GPx-like (b), and POD-like (c) activities of Cu_xO ^[68]; (d-f) H_2O_2 (d), $\text{O}_2^{\cdot-}$ (e) and free radical (f) scavenging activity of $\text{Cu}_{5,4}\text{O}$ USNPs^[86]; (g) UV-Vis spectra of the reaction solution in the presence of Cu SAs/CN, ascorbic acid and H_2O_2 over time;

(h) Michaelis-Menton curves obtained with different concentrations of substrate AA under the fixed concentration of Cu SAs/CN and H_2O_2 ;

(i) Quantification for APX-like activities of Cu SAs/CN^[70]

ROS: Reactive oxygen species; SOD: Superoxide dismutase; SA: Specific activities; AA: Ascorbic acid

H_2O_2 。AA 在 265 nm 处的特征峰降低表明 Cu SAs/CN 具有类 APX 活性(图 2(g))。动力学实验结果显示 Cu SAs/CN 对 AA 底物的 K_m 和 V_m 分别为 0.01 mmol/L 和 11.49 $\mu\text{mol}/(\text{L}\cdot\text{min})$ (图 2(h)), Cu SAs/CN 对 AA 的亲合力比天然 APX($K_m = 1 \text{ mmol/L}$)高 100 倍。随后对其类 APX 活性进行定量, 酶活力为 461.6 U/mg(图 2(i))。以上结果均表明 Cu SAs/CN 具有优异类 APX 活性, 能够有效清除 H_2O_2 , 进一步缓解氧化应激。

2.2 铜基纳米酶类氧化酶活性和产生自由基能力

缓解氧化应激是治疗由 ROS 引起的疾病的关键, 而加强氧化应激是干预细菌感染和肿瘤的最终目标^[87-90]。近年来, 也报道了许多具有抗菌和干预肿瘤功能的铜基纳米酶^[91-92]。Wang 等^[69]制备出具有 Cu-N₆ 活性位点的六氰合铁酸铜(Cu-HCF)单原子纳米酶(SSNEs), 该活性位点是通过 Cu 与 $[\text{Fe}^{\text{II}}(\text{CN})_6]$ 的 N 端配位所形成的。借助 SSNEs 的类 GSHOx 和 POD 活性的级联酶促反应实现对肿瘤细胞的化学动力学疗法(CDT)治疗。SSNEs 能有效清除 GSH(图 3(a)), 图 3(b)显示 SSNEs 与 GSH 反应后产物 SSNEs-G 能够增强类 GSHOx 活性, 同时相比于 SSNEs, 类 POD 和 $\cdot\text{OH}$ 生成实验同样证明 SSNEs-G

具有更高的类 POD 活性和更强的 $\cdot\text{OH}$ 产生能力(图 3(c, d))。这一过程完美诠释了 SSNEs 能够持续高效清除肿瘤细胞的机理。Wang 等^[74]制备的二维金属有机框架 Cu-TCPP 纳米片, 利用相似的原理消耗肿瘤内过量的 GSH 并产生活性氧 $^1\text{O}_2$ 来实现肿瘤治疗。

Chang 等^[81]制备的铜单原子纳米酶(Cu SAzyme)能够诱导多种 ROS 生成并且应用于肿瘤治疗。Cu SAzyme 具有类 CAT 活性, 能够催化 H_2O_2 生成 O_2 , 缓解了肿瘤内部的缺氧氛围, 有利于 ROS 的产生。1,3-二苯基异苯并呋喃(DPBF)在 420 nm 处的特征吸收峰降低, 表明 Cu SAzyme 的类 OXD 活性催化 O_2 转化为 $\text{O}_2^{\cdot-}$, 而后 $\text{O}_2^{\cdot-}$ 在微酸介质中与 H^+ 反应进一步转化为 $^1\text{O}_2$ (图 3(e))。电子自旋共振测试进一步证实 Cu SAzyme 可以诱导产生 $\text{O}_2^{\cdot-}$ 、 $^1\text{O}_2$ 和 $\cdot\text{OH}$ (图 3(f))。ROS 不仅对肿瘤细胞有毒害作用, 同时还可使肿瘤细胞内的热休克蛋白失活, 实现对肿瘤细胞的化学动力学治疗和光热治疗。Liu 等^[58]制备的 Cu-PBG 纳米酶具有优异的抗菌效果, 在微酸介质中具有自发降解性和类 POD 活性, 能够释放 Cu^{2+} 并在伤口处产生大量 ROS, 对革兰氏阳性和革兰氏阴性细菌展现出优异的杀伤效果, 进而促进伤口愈合。

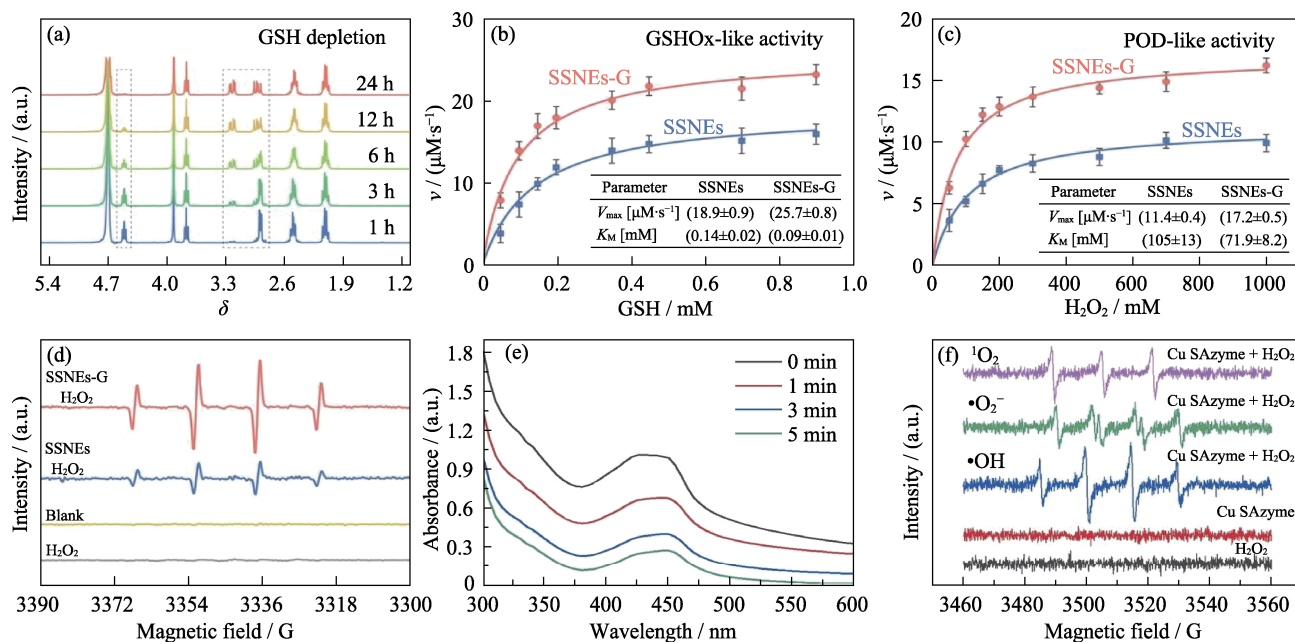


图3 铜基纳米酶类氧化酶活性和产生 ROS 能力^[69,81]

Fig. 3 Oxidase-like and ROS generating activity of Cu-based nanozymes^[69,81]

(a) ^1H NMR spectra of GSH at different points during reaction with SSNEs; (b, c) Kinetics of GSHOx-like (b) and POD-like (c) activities of SSNEs and SSNEs-G; (d) $\cdot\text{OH}$ generating activity of SSNEs and SSNEs-G^[69]; (e) $^1\text{O}_2$ generating activity of Cu SAzyme with DPBF serving as the indicator; (f) $^1\text{O}_2$, $\text{O}_2^{\cdot-}$ and $\cdot\text{OH}$ generating activity of Cu SAzyme with TEMP, BMPO and DMPO as trapping agents in the presence of H_2O_2 ^[81]

GSH: Glutathione; SSNEs: Single-site nanozyme; GSHOx: Glutathione oxidase; POD: Peroxidase; DPBF: 1,3-diphenyl isobenzofuran; mM: mmol/L; μM : $\mu\text{mol/L}$

3 铜基纳米酶在生物医学中的应用

3.1 铜基纳米酶在生物传感中的应用

由于天然酶的检测平台受天然酶固有缺陷的限制, 科学家们基于纳米酶超高的类天然酶活性和高稳定性的优势构建了用于生物分析、环境治理、疾病诊断等领域的传感器。目前铜基纳米酶的生物传感应用主要集中于对 H_2O_2 、生物分子和酶等的检

测^[62,93]。Cheng 等^[94]利用介孔 CuO 空心球的类 POD 活性构建纸质传感器, 实现 H_2O_2 的直接检测(图 4(a))。以抗坏血酸、多种氨基酸和多种金属离子等作为干扰物来检测该传感器的选择性, 作者发现其对 H_2O_2 具有超高选择性, 对 H_2O_2 的检测下限达到 $2.4 \mu\text{mol/L}$, 这能够满足生物分析等领域的需求(图 4(b))。Zhu 等^[95]基于 CuO/NiO 纳米管(NTs)的类 POD 活性和异烟肼的酰肼基团抑制 3,3',5,5'-四甲基联苯胺(TMB)氧化的原理, 实现对异烟肼的间接比色检

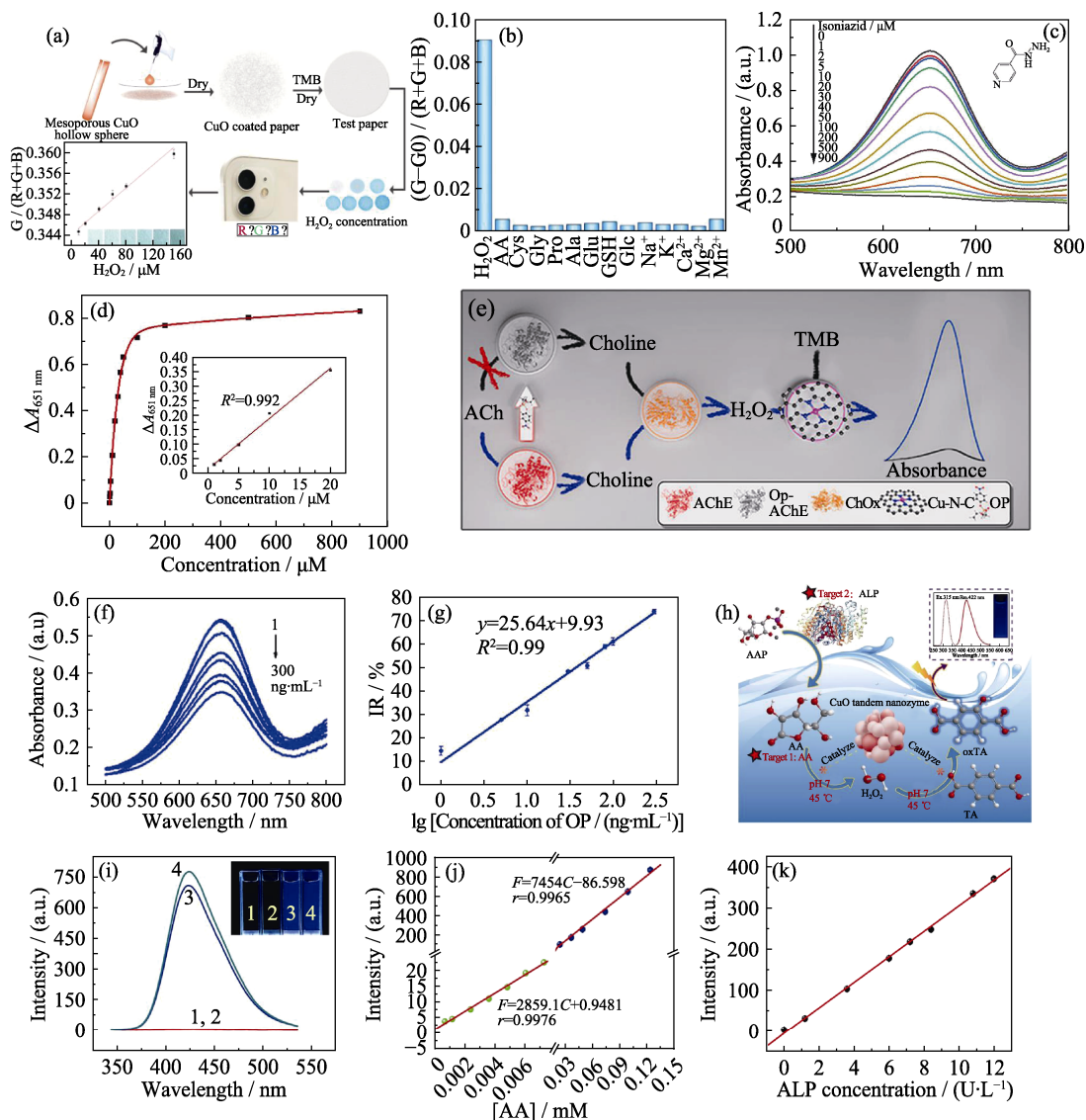


图 4 铜基纳米酶在生物传感中的应用^[94-97]

Fig. 4 Cu-based nanozymes for biosensing^[94-97]

(a) Schematic diagram of a paper sensor for H_2O_2 detection based on mesoporous CuO hollow sphere nanozymes; (b) Effects of different substrates H_2O_2 , ascorbic acid, Cys, Gly, Pro, Ala, Glu, GSH, Glc, Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and Mn^{2+} on the sensing performance of the paper sensor^[94]; (c) UV-Vis spectra of the mixed reaction system with CuO/NiO NTs, TMB, H_2O_2 and different concentrations of isoniazid; (d) Dose response curve of sensing isoniazid^[95]; (e) Schematic illustration of the three-enzyme system (ACC) containing acetylcholinesterase (AChE), choline oxidase (ChOx), and Cu-N-C single atom enzymes (SAzymes) for the organophosphorus pesticide (OP) detection; (f) Change of the absorbance at 652 nm of the ACC system with the addition of OP from 1 to 300 ng/mL ; (g) Linear relationship between the inhibition rate (IR) of AChE and the logarithm of OP concentration^[96]; (h) Schematic illustration of CuO NPs for ascorbic acid and ALP detection; (i) Emission spectra of different detection systems, with 1-4 indicating AAP-TA-CuO NPs, ALP-TA-CuO NPs, AAP-ALP-TA-CuO NPs, and TA-AA-CuO NPs, respectively; (j) Linear relationship between emission intensity and concentration of ascorbic acid; (k) Calibration plot for ALP determination with different concentrations^[97]

TMB: 3,3',5,5'-tetramethylbenzidine; GSH: Glutathione; AChE: Acetylcholinesterase; OP: Organophosphorus pesticide; AAP: L-ascorbate-2-trisodium phosphate; TA: Terephthalic acid; ALP: Alkaline phosphatase; μM : $\mu\text{mol/L}$; mM : mmol/L

测。随着异烟肼浓度的增高,氧化型 TMB(oxTMB) 在 652 nm 处的特征吸收值逐渐降低(图 4(c))。CuO/NiO NTs 对异烟肼的线性检测范围为 1~20 $\mu\text{mol/L}$, 检测限为 0.4 $\mu\text{mol/L}$ (图 4(d))。

利用铜基纳米酶类 POD 活性还可以检测能产生 H_2O_2 的生物分子。Wu 等^[96]基于乙酰胆碱酯酶(AChE)、胆碱氧化酶(ChOx)和铜单原子纳米酶(Cu-N-C single atom enzymes, Cu-N-C SAzymes)构建了一个三酶级联反应体系(ACC), 实现了对有机磷农药(OP)的检测。当体系中不存在 OP 时, 乙酰胆碱(Ach)经 AChE 和 ChOx 酶催化产生 H_2O_2 , 而后在 H_2O_2 的存在下, Cu-N-C SAzymes 氧化 TMB 显色, 完成检测过程。但当 OP 存在时, OP 使 AChE 失活, 阻断了上述过程, 通过测定 OP 对 TMB 氧化的抑制程度, 实现对 OP 的定量检测(图 4(e))。随着 OP 浓度增大, ACC 在 652 nm 处的吸收逐渐降低(图 4(f))。该检测体系对 OP 有着较高的灵敏度, 能够实现对 1~300 ng/mL 范围内 OP 的线性精准检测(图 4(g)), 其检测限为 0.6 ng/mL。除上述用于比色法检测的铜基纳米酶以外, He 等^[97]利用 CuO NPs 自身的类抗坏血酸氧化酶(Aascorbic Acid Oxidase, AAO)和 POD 活性的级联反应构建了一种荧光检测传感器。CuO NPs 能够催化 AA 生成 H_2O_2 , 进而转化为 $\cdot\text{OH}$, $\cdot\text{OH}$ 氧化对苯二甲酸(Terephthalic Acid, TA)生成具有荧光的产物, 最终通过荧光强度的增强实现对 AA 的定量检测。碱性磷酸酶(Alkaline Phosphatase, ALP)能催化 L-抗坏血酸-2-磷酸三钠盐(L-ascorbate-2-trisodium phosphate, AAP)转化为 AA, 因而还能将 CuO NPs 应用于 ALP 定量(图 4(h, i))。该检测平台表现出对 AA 和 ALP 的超高灵敏度, 对两个目标分子的检测限分别为 2.92×10^{-8} mol/L 和 0.058 U/L(图 4(j, k))。与传统的酶标化学免疫法相比, 基于纳米酶的检测方法不仅保证了检测灵敏度, 同时还大大降低了检测成本。

3.2 铜基纳米酶在伤口愈合中的应用

伤口处的炎症积累往往会引起继发性的组织损伤, 阻碍血管生成、胶原蛋白沉积和再上皮化, 进而导致伤口愈合速度变慢和组织恢复功能变差, 因此降低伤口处炎症水平是伤口愈合的关键策略。Jin 等^[98]将具有类 SOD 和 CAT 活性的 Ni_4Cu_2 纳米球与温敏型 F127 水凝胶结合用于伤口愈合(图 5(a))。利用 F127 在 20 $^\circ\text{C}$ 为液体而在 30 $^\circ\text{C}$ 时呈半固体的特性, $\text{Ni}_4\text{Cu}_2/\text{F127}$ 能够紧密贴合伤口表面, 在伤口愈合过程中隔离外部污染以免二次感染。创伤后 7 d $\text{Ni}_4\text{Cu}_2/\text{F127}$ 组小鼠伤口几乎完全愈合(图 5(b)), 同时 $\text{Ni}_4\text{Cu}_2/\text{F127}$ 组观察到了分化良好的上皮、致密

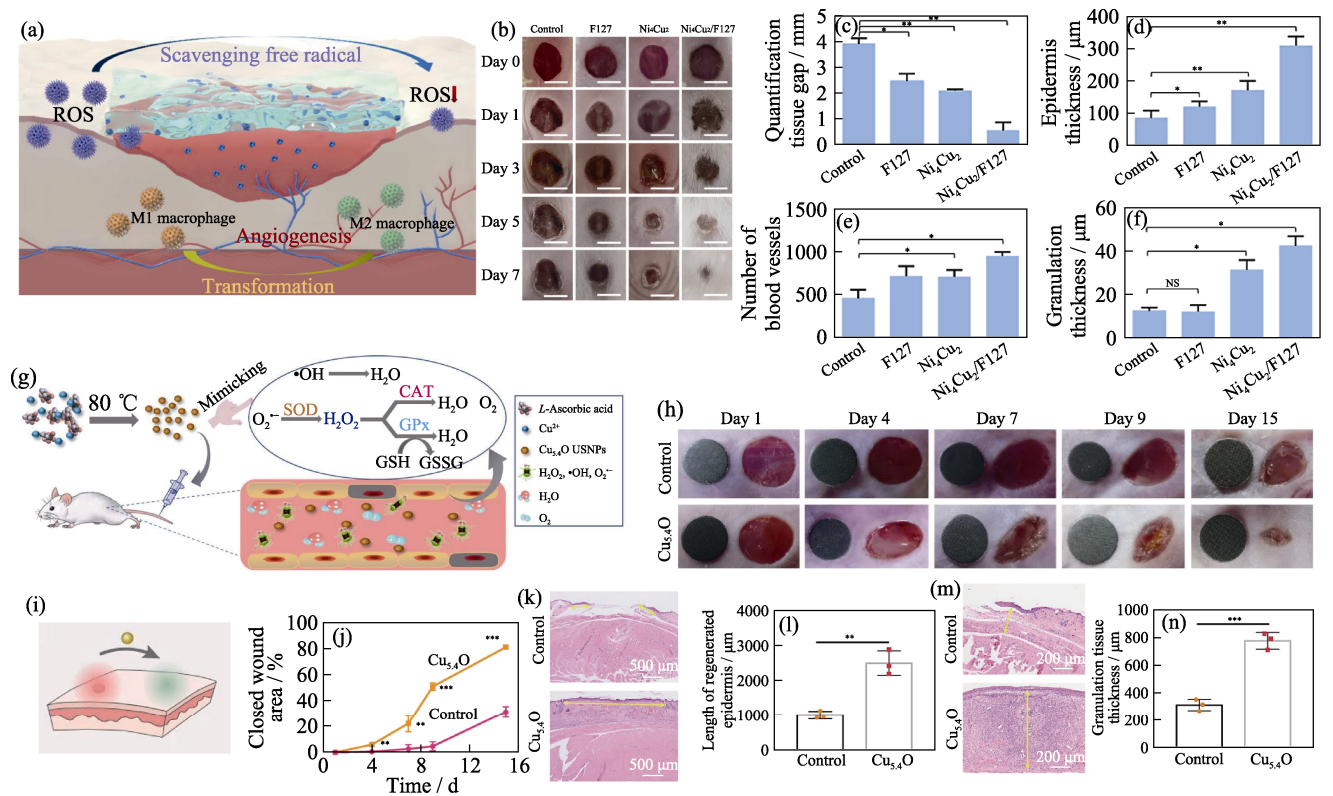
的肉芽组织、充分沉积且有序的胶原蛋白、伤口处毛囊的发育和血管的生成(图 5(c~f))。这些结果都表明 $\text{Ni}_4\text{Cu}_2/\text{F127}$ 能够减少伤口的炎症并加速伤口愈合。Xu 等^[99]构建的富铜普鲁士蓝纳米酶(HPP@Cu NZs)能够降低氧化应激水平, 实现抗炎效果, 并促进细胞增殖和血管生成, 提高感染伤口的闭合率。Zhang 等^[79]基于 Fe-Cu-N6 单原子纳米酶制备的缝合线也能够促进血管内皮生长因子的分泌, 加速由脑外伤引起的头皮创伤的愈合。

糖尿病等慢性炎症疾病往往受高氧化应激水平的影 响, 会引起伤口愈合速度变慢, 因此开发能用于糖尿病模型伤口愈合的纳米酶具有重要意义。Liu 等^[86]利用 $\text{Cu}_{5.4}\text{O}$ USNPs 多重类酶活性, 将其成功应用于糖尿病引起的伤口损伤的治疗中(图 5(g, i))。作者发现, 经 $\text{Cu}_{5.4}\text{O}$ USNPs 给药后 15 d 小鼠伤口达到 80% 的愈合率(图 5(h, j))。经 $\text{Cu}_{5.4}\text{O}$ USNPs 给药组的表皮再生长度和肉芽组织厚度均显著大于对照组(图 5(k~n)), 说明 $\text{Cu}_{5.4}\text{O}$ USNPs 能够加速糖尿病伤口愈合过程。Peng 等^[100]将 $\text{Cu}_{5.4}\text{O}$ 超小纳米酶负载于功能化的星形聚乙二醇(starPEG)和肝素中得到复合水凝胶($\text{Cu}_{5.4}\text{O}@\text{Hep-PEG}$), 该水凝胶能够在伤口部位释放 $\text{Cu}_{5.4}\text{O}$ 超小纳米酶, 清除伤口部位过量的 ROS, 促进急性伤口和糖尿病伤口的愈合。

3.3 铜基纳米酶在急性肾损伤中的应用

急性肾损伤(Acute Kidney Injury, AKI)会导致毒素和代谢产物在体内积累, 引起身体机能紊乱, 而氧化还原失衡和炎症因子是引起急性肾损伤的重要因素, 因此利用铜基纳米酶调节体内的氧化还原水平和炎症因子水平来达到治疗效果是一种潜在可行的策略。Liu 等^[86]将具有类 SOD、GPx、CAT 三重类酶活性的 $\text{Cu}_{5.4}\text{O}$ USNPs 应用于 LPS 诱导的 AKI 模型中(图 6(a))。AKI 小鼠在两周后存活率为零, 而经 $\text{Cu}_{5.4}\text{O}$ USNPs 治疗的 AKI 小鼠 14 d 后存活率为 100%(图 6(b))。同时检测血清中衡量肾功能的两个重要指标肌酐(CRE)和尿素氮(BUN), 给药组的血清中 CRE 和 BUN 水平均低于 AKI 组(图 6(c, d)), 同时病理切片染色(Hematoxylin-Eosin staining, H&E)中只能观察到少量变性蛋白在肾小管中的沉积, 说明经 $\text{Cu}_{5.4}\text{O}$ USNPs 治疗后能保持肾的完整性。此外, $\text{Cu}_{5.4}\text{O}$ USNPs 组小鼠的血红素加氧酶-1 和肾损伤分子-1 两种肾损伤生物标志物的水平均明显低于 AKI 组(图 6(e, f))。这些表明 $\text{Cu}_{5.4}\text{O}$ USNPs 能调节急性肾损伤小鼠的氧化还原水平, 从而达到治疗效果。

Zhang 等^[34]将 CTMDs 应用于 LPS 诱导的 AKI 模型, 并发现 CTMDs 能有效提高小鼠的存活率(图 6(g, h))。另外, CTMDs 能通过降低血液中炎症

图 5 铜基纳米酶在伤口愈合中的应用^[86,98]Fig. 5 Cu-based nanozymes for wound healing^[86,98]

(a) Schematic illustration of the $\text{Ni}_4\text{Cu}_2/\text{F127}$ composite hydrogel dressing in wound healing; (b) Photographs of wounds with different treatments on days 0, 1, 3, 5, and 7 with scale bar representing 5 mm; (c-f) Statistical analysis of the cross-sectional length of wound (c), epidermal thickness (d), granulation tissue thickness (e), number of blood vessels (f) around wound on day 7^[98]; (g) Schematic illustration of $\text{Cu}_{5.4}\text{O}$ USNPs with multiple enzyme-like activities and broad-spectrum ROS scavenging abilities; (h) Photographs of diabetic wounds at different time points with a 6-mm-diameter standard green disc as the size reference; (i) Schematic illustration of $\text{Cu}_{5.4}\text{O}$ USNPs in diabetic wounds healing; (j) Percentage of wound closure area at different time points; (k) Representative histological images and (l) quantification for the length of regenerated epidermis on day 15 post-surgery; (m) Representative histological images and (n) quantification for the granulation tissue on day 15 post-surgery^[86]

ROS: Reactive oxygen species; GSH: Glutathione; GSSG: Oxidized glutathione

因子, 如乳酸脱氢酶(Lactate Dehydrogenase, LDH)、肿瘤坏死因子(TNF- α)、白介素 6(IL-6), 缓解由急性肾损伤引发的炎症(图 6(i)), 同时能降低 CRE 和 BUN 水平, 表明其能缓解 LPS 引起的对肾的破坏(图 6(j)), 达到对机体的保护作用。此外, H&E 结果发现 AKI 组的肾、肝、肺、肠、胃均有一定程度的形态损伤和炎症细胞浸润, 而 CTMDs 给药组小鼠的组织损伤和炎症细胞浸润得到缓解(图 6(k))。这些结果均表明 CTMDs 能缓解炎症, 从而对急性肾损伤起到治疗作用。

3.4 铜基纳米酶在肿瘤治疗中的应用

由于肿瘤细胞的无限增殖能力和逃逸免疫系统识别和攻击机制, 癌症治疗一直是难题。目前大量的铜基纳米酶通过调节肿瘤部位的氧化应激, 引起肿瘤细胞凋亡而被广泛应用到肿瘤治疗^[101-103]。Wang 等^[56]以 Cys 为配体制备的 Cu-Cys NPs 能够消耗肿瘤内部 GSH 同时将 Cu^{2+} 还原为 Cu^+ , 而后利用 Cu^+ 的类 POD 活性将 H_2O_2 转化为 $\cdot\text{OH}$, 实现对肿瘤

细胞的化学动力学治疗(图 7(a))。与抗肿瘤药物阿霉素(DOX)相比, Cu-Cys NPs 能显著减小肿瘤的尺寸、重量和体积, 同时增强肿瘤细胞的凋亡(图 7(b~e))。这证明 Cu-Cys NPs 能够通过化学动力学疗法实现高效的肿瘤杀伤效果。

Hu 等^[104]制备了聚乙二醇(PEG)包覆的钌铜纳米颗粒(P-RuCu), 利用 P-RuCu 的类酶活性和 Ru 元素的辐射增敏性将其应用于乳腺癌的协同治疗中。Zhu 等^[105]通过物理挤压将铜单原子纳米酶(SAZs)和质子泵抑制剂(PPIs)封装于血小板膜囊泡中, 得到仿生铜单原子纳米酶载药系统(PPS)。PPIs 通过调节肿瘤内 H^+ 、 H_2O_2 和 GSH 水平, 减少肿瘤内三磷酸腺苷(ATP)的生成, 阻断能量供应。同时增强 SAZs 的类 POD 活性, 在肿瘤部位产生 $\cdot\text{OH}$, 实现对肿瘤的纳米催化治疗。以上均表明铜基纳米酶凭借其在微酸介质中优异的类 POD 活性, 已成功地应用于肿瘤治疗中。

光动力疗法(PDT)目前已被批准用于临床, 是

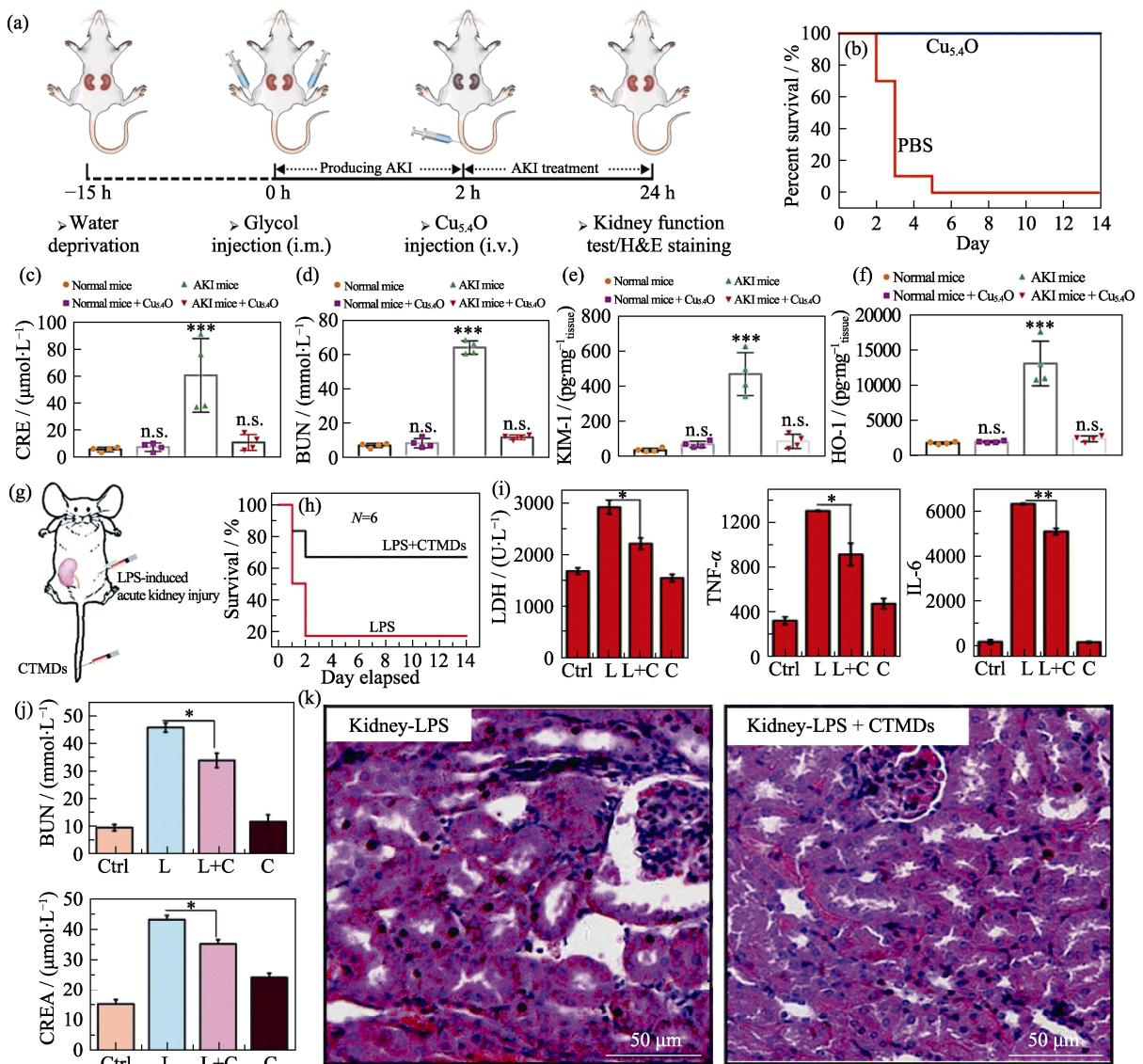


图 6 铜基纳米酶在急性肾损伤中的应用^[34,86]

Fig. 6 Cu-based nanozymes for AKI^[34,86]

(a) Schematic illustration of the establishment of AKI and $\text{Cu}_{5.4}\text{O}$ USNPs for the treatment^[86]; (b) Survival curves and the levels of (c) CRE and (d) BUN in different groups at 24 h after treatments^[86]; (e, f) The levels of (e) kidney injury molecules-1 (KIM-1) and (f) heme oxygenase-1 (HO-1) in kidney of different groups^[86]; (g) Schematic illustration of CTMDs in AKI induced by endotoxemia^[34]; (h) Survival curves, (i) levels of oxidative stress containing lactate dehydrogenase (LDH), $\text{TNF-}\alpha$, IL-6, and (j) levels of CREA and BUN^[34]; (k) H&E images of different groups^[34]
 PBS: Phosphate buffer solution; AKI: Acute kidney injury; CRE: Creatinine; BUN: Blood urea nitrogen; LPS: Lipopolysaccharide; CTMDs: Cu-tetrakis(4-carboxyphenyl)porphyrin MOF dots

一种非侵入性 ROS 介导的抗癌模式。它通过光敏剂在激光照射下介导的光化学反应产生 $^1\text{O}_2$ 发挥作用。Hou 等^[106]开发了纳米酶辅助的 PDT 平台—— $\text{HA@Cu(OH)}_2\text{-ICG}$ (nHACI, HA 为酰胺化透明质酸, ICG 为吡咯菁绿)用于肿瘤治疗。通过 nHACI 的 Fenton 反应、类 GSHOx 和类 CAT 三重活性, 下调肿瘤内 GSH 水平、改善缺氧环境并提高 ROS 水平, 从而增强基于光敏剂 ICG 的光动力治疗效率和肿瘤细胞的免疫原性细胞死亡(ICD), 实现肿瘤的有效清除(图 7(f))。动物实验结果表明 nHACI+NIR(近红外光)组具有优异的抗肿瘤效果, 能够有效抑制肿瘤的生长, 延长小鼠存活时间(图 7(g~j))。nHACI +

NIR 组小鼠肿瘤组织出现损伤过度的现象, 包括核溶解、血浆溢出和坏死区周围炎性细胞浸润。以上结果均表明 nHACI 能够有效杀伤肿瘤细胞。此外, 将 nHACI 和程序性死亡受体 1(PD-1)抗体($\alpha\text{PD-1}$)组合能够增强对肿瘤细胞 PD-1 的阻断, 根除原发性的肿瘤, 抑制远处肿瘤生长和肺转移, 并诱导肿瘤特异性免疫记忆以防止肿瘤复发。

肿瘤治疗中靶向识别肿瘤细胞, 而对正常细胞不产生伤害是关键, 因此在不影响纳米酶类酶活性的同时在其表面修饰靶向肿瘤的生物分子, 是提高纳米酶靶向性的可行策略。Meng 等^[107]设计了双嵌段 DNA(di-DNA)修饰的 CuO 纳米酶(di-DNA/ CuO),

其中 di-DNA 上的 (TC)₁₂ 片段吸附在 CuO 表面, 赋予其较强的生物相容性, 游离的羧基端(A 端)则能够靶向识别肿瘤细胞膜表面的腺苷受体, 从而赋予 di-DNA/CuO 靶向性。体内实验结果表明 di-DNA/CuO 能够通过类 POD 活性和腺苷受体介导肿瘤识别体系, 特异性地遏制肿瘤的生长, 从而杀死癌细胞。

3.5 铜基纳米酶的其他生物学应用

除了上述提到的生物学应用外, 铜基纳米酶可以清除 ROS 的能力, 还赋予了其对神经系统疾病的神经保护能力。Liu 等^[108]将 MES23.5 细胞的细胞膜(CM)修饰于 Cu_{2-x}Se-PVP-Qe(CSPQ, PVP 为聚乙烯吡咯烷酮, Qe 为槲皮素)外, 获得了能够靶向小胶质细胞的仿生纳米颗粒 CSPQ@CM, 可有效缓解帕金森小鼠的运动障碍和认知障碍, 展现出优异的治疗效果。Hao 等^[68]合成的手性 Cu_xO 纳米颗粒也成功实现了对帕金森模型小鼠的治疗。

此外, 铜基纳米酶也是优异的抗菌剂。Wang 等^[91]利用 CaP 的 pH 响应性在细菌的微酸性环境中实现 Cu₂O/Pt 纳米酶的释放, 利用 Cu₂O/Pt 的类 POD 活性产生的 ROS, 有效抑制了细菌的增长。Xi 等^[92]设计了两种铜/中空碳纳米球杂化材料——CuO-HCSs 和 Cu-HCSs, 并研究了两种不同价态铜的抗菌机制。结果发现 CuO-HCSs 通过 Cu²⁺的释放实现灭菌作用; 而 Cu-HCSs 则是利用 Cu 的类酶活性实现抗菌效果。

铜基纳米酶与多孔纳米材料的整合还能够实现药物的封装和递送^[109-110]。Wu 等^[111]构建的 Cu²⁺掺杂多孔硅纳米材料能够将双硫仑精准递送至肿瘤部位, Cu²⁺与双硫仑原位整合生成有毒的二硫代氨基甲酸酯(DTC)-铜络合物(CuET), Cu²⁺被还原产生的 Cu⁺可诱发芬顿反应产生 ROS, 这样双重作用可有效抑制肿瘤的生长。

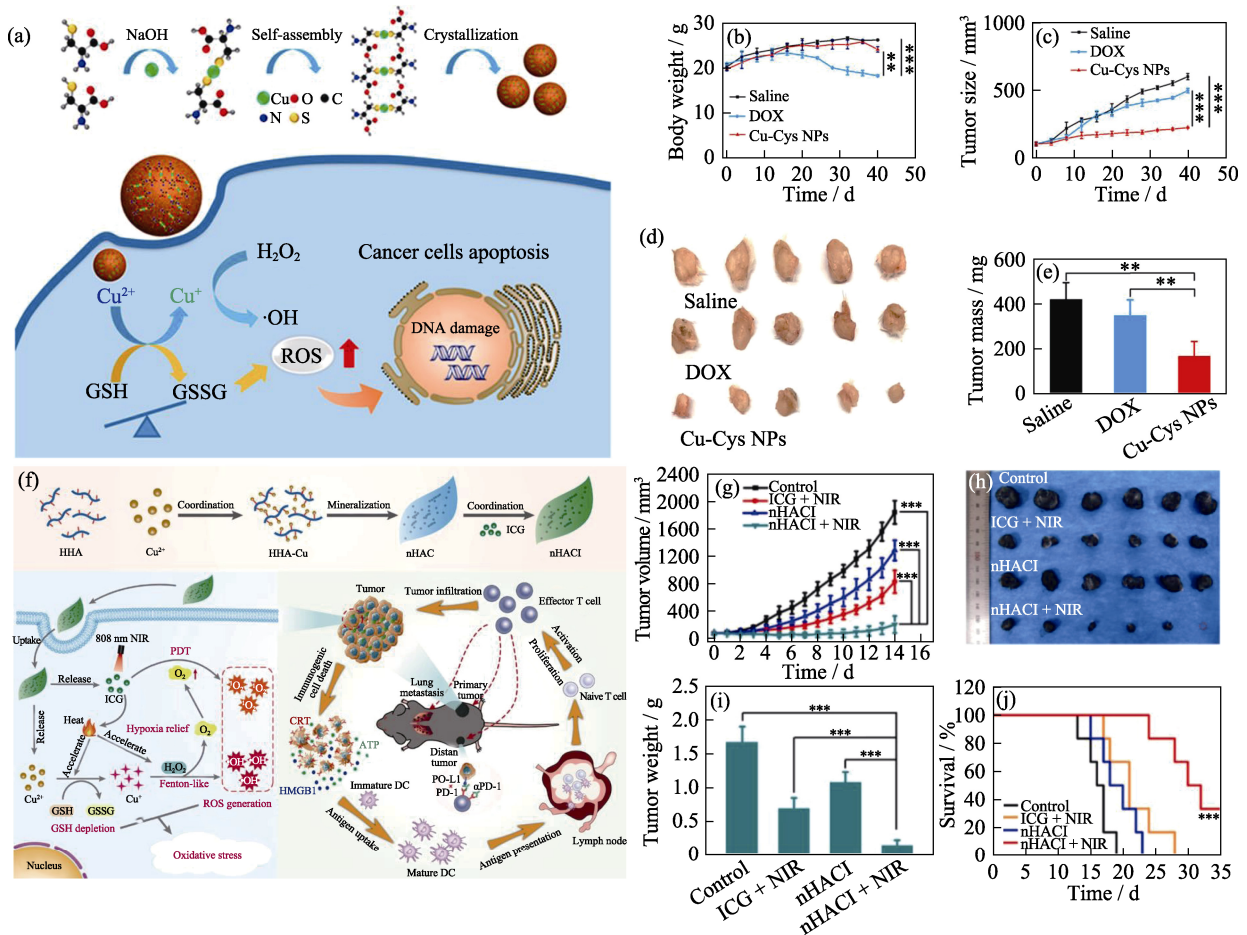


图 7 铜基纳米酶在肿瘤治疗中的应用^[56,106]

Fig. 7 Cu-based nanozymes for tumor therapy^[56,106]

(a) Schematic illustration of Cu-Cys NPs preparation and chemodynamic therapy for tumors; (b, c) Changes in body weight (b) and tumor size (c) of MCF-7R tumor-bearing mice with different treatments; (d) Photographs of tumor in different groups after 40 d of treatment; (e) Average tumor masses excised from MCF-7R tumor-bearing mice from each group^[56]; (f) Schematic illustration of the synergistic anticancer mechanism of nHACI based on PDT and PD-1 blockers; (g) Average tumor volume, (h) photographs and (i) weights of tumor in different groups; (j) Survival curves of B16F10 tumor-bearing mice in different groups^[106]

GSH: Glutathione; GSSG: Oxidized glutathione; ROS: Reactive oxygen species; DOX: Doxorubicin; HHA: Hydrated hyaluronan

4 结论与展望

近年来,铜基纳米酶具有制备简单、价格低廉、优异的类酶活性和稳定性的优势,受到科学家们的广泛关注和研究。本文主要介绍了铜基纳米酶的分类、酶学特性和生物医学应用。铜基纳米酶类 POD 活性的显色实验证实其在生物传感检测 H_2O_2 和生物分子方面具有优势;类 SOD、GPx 和 CAT 等活性赋予铜基纳米酶调节体内氧化还原水平和炎症水平的能力,对由 ROS 引起的疾病具有治疗作用;类 GSHOx、POD 活性和产生自由基的能力能够有效诱导细菌杀伤和肿瘤治疗。虽然铜基纳米酶在生物医学领域的应用研究取得了一定的进展,但其在未来的发展中仍存在一些问题和挑战。

1) 目前的铜基纳米酶局限在模拟氧化还原酶的研究中,对铜基纳米酶模拟其他类型酶如水解酶、转移酶等的研究还较少,因而构建具有新型类酶活性的铜基纳米酶对系统地研究铜基纳米酶的分类和催化机理,拓宽铜基纳米酶的应用领域具有重要意义。

2) 铜基纳米酶的催化活性是影响生物传感精确度和疾病治疗效果的决定性因素。但铜基纳米酶在与天然酶进行活性对比时,大多纳米酶只能在米氏常数 K_m 或最大反应速率 V_m 其中一个维度上超越天然酶,因此制备具有高催化活性的铜基纳米酶仍旧是之后的目标。

3) 虽然科学家们已经构建了具有 Cu-N-C 结构的单原子纳米酶,但单原子纳米酶的水溶性还是限制了其在生物医学领域的应用。而大多数铜基纳米酶的活性中心不明确,严重阻碍了研究者对其结构与催化性能之间关系的研究。因而精准设计合成具有精确结构的铜基纳米酶,并对铜基纳米酶的构效关系进行研究,仍将是我们需要关注的关键问题。

4) 虽然科学家们致力于构建与天然酶结构相似的仿生铜基纳米酶,但铜基纳米酶对底物的特异选择性较差,需要进一步提高。这是由于天然酶的结构远比铜基纳米酶复杂,因此在铜基纳米酶的构建中,我们需要关注天然酶活性中心微环境的结构特点,不仅要保证铜基纳米酶活性中心与天然酶相似,还要保证其具备类似天然酶的能够特异性吸附底物分子的三维空间结构。

5) 铜基纳米酶的体内分布实验表明,没有靶向分子的铜基纳米酶难以排出体外,主要聚集在肝脏、肺和脾脏中,因而存在安全性问题。在未来的研究中,一方面可以制备尺寸小于肾清除阈值

(5.5 nm)的超小铜基纳米酶,使其能够快速被肾脏滤出并随尿液排出体外;另一方面可以对铜基纳米酶进行化学修饰来获得靶向性,在病灶部位持续释放铜基纳米酶,达到长期有效的治疗效果。

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