



# Role of Nanozymes in Oral Cancer the Road Ahead

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## Abstract

Oral cancer is a result of diverse interactions in the tumor microenvironment (TME), genetic alterations along with associated risk factors such as lifestyle and microbial infections. Various modalities are employed in the diagnosis and therapeutics of oral cancer. Nanozymes which are artificial enzymes have a great potential in the diagnostic and therapeutic approach of tumors. They have enormous advantages compared to natural enzymes and possess inherent biological and physical properties. A web-based search was performed via the Google scholar, PubMed database, Web of Science with keywords nanozymes, nanoparticles in cancer and oral cancer. The other keywords used were diagnosis, therapy, TME, microbiome, molecular alterations, biosensor, targeted therapy, imaging and tissue regeneration. Original research studies, reviews, case reports published from 2012 to 2022 were included to appraise different subsections. An absolute lack of literature on nanozymes was observed in oral cancer. The present review is the first attempt to describe the role and application of nanozymes in oral cancer by correlating its outcome in tumor biology and biomedical research. Rapid development of nanotechnology has created a paradigm shift in cancer diagnosis and therapeutics. Nanozymes with novel designs can be anticipated in the future in oral cancer management.

**Keywords** Biosensing · Cancerimaging · Genetics · Microbiome · Nanoparticles · Nanozymes · Tumor microenvironment · Oral cancer · Regeneration

## 1 Introduction

Cancers are sequel of diverse interplay between the tumor cells and the tumor microenvironment (TME). An aggressive phenotype in oral malignancy is also evident to be influenced by the modulations in the TME. This comprises of cytokines interleukin (IL)-10, transforming growth factor (TGF)- $\beta$  with immunosuppressive characteristics, tumor associated inflammation, crosstalk between cancer cells and the TME through cytokine cascades, and many more. Apart from well-known causative agents like betel nut and tobacco association with bacterial, human papilloma virus (HPV) infection, genetic alterations are also attributed in

oral oncogenesis. Therapeutic resistance, recurrence, and metastasis are the major consequences of these interplays and causative agents [1–3]. Hence it is crucial to probe into novel therapeutic designs to enhance sensitivity of cancer management. Nanozymes have remarkably allured in the recent era specifically in the field of biomedical research and applications. Nanozyme is a versatile artificial nanomaterial presenting functions that mimic natural enzyme. The evolution of the nanozyme is credited to Yan and Gao who observed in Fe<sub>3</sub>O<sub>4</sub> nanoparticles an intrinsic horseradish peroxidase (HRP)-like activity. Nanozymes were primarily developed to surmount the challenges of natural enzymes like escalated cost, preparation time, denaturation rate and recycling challenges [4, 5]. Antioxidant, anti-inflammatory, biosensing are some of the inherent biological properties possessed by nanozymes apart from being fluorescent, able to image in near-infrared (NIR) and exhibit photothermal effect [5]. With these enormous advantages nanozymes are immensely advanced in various fields including tumor biology. Nanoparticles including magnetic, cerium oxide, gold and many more have displayed intrinsic catalytic activities and find application for cancer diagnosis, therapeutics and

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theranostics [1]. This review emphasises the potential applications of nanozymes in oral cancer. Despite articles published on nanoparticles in oral cancer there is no literature published on nanozymes in oral cancer. Hence this review primarily aims to correlate the application of nanozyme in cancer and biomedical research to oral cancer. The secondary aim is to inspire researchers to involve nanozymes in oral cancer studies and oncologists to employ nanozymes in oral cancer management which subsequently enhances in understanding its clinical efficacy.

## 2 Methodology of Screening Data

A web-based search was performed via the Google scholar, PubMed database, Web of Science with keywords nanozymes, nanoparticles in cancer and oral cancer. The other keywords used were diagnosis, therapy, TME, microbiome, molecular alterations, biosensor, targeted therapy, imaging and tissue regeneration. Original research studies, reviews, case reports published from 2012 to 2022 were included to appraise different subsections. A brief mention about the challenges in application of nanozymes and future prospects was performed.

## 3 Classification and Characteristics of Nanozymes

Nanomaterials are explored enormously to determine the nanozyme activities due to their catalytic similarities to catalase, peroxidase and so on. Various sources of nanozymes include the carbon-based which comprises of carbon 60, carbon dots, carbon nanodots, carbon nitride, fullerenes, graphene, graphene oxide and graphene quantum dots. Metal oxide-based like  $\text{CeO}_2$ ,  $\text{Co}_3\text{O}_4$ ,  $\text{Fe}_3\text{O}_4$ ,  $\text{MnO}_2$ ,  $\text{TiO}_2$ ,  $\text{V}_2\text{O}_5$ ; metals like silver (Ag), gold (Au), cerium (Ce), cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), palladium (Pd), platinum (Pt) is known to possess nanozyme like characteristics. Apart from these metal oxide-framework (MOF), Prussian blue, metal chalcogenides have also been explored with nanozyme like property.

Nanozymes are broadly classified into two main categories based on the mechanism of reaction namely the oxidoreductase and the hydrolase. The oxidation reaction is catalysed by the oxidoreductase which are further sub-classified based on their imitation to natural enzymes into catalase, oxidase, peroxidase and superoxide dismutase (SOD). Metal oxide nanomaterials like  $\text{ZrO}_3$ ,  $\text{CoO}_4$ , metals Pt and Pb, carbon-based nanoparticles are known to possess catalase like activities. The decomposition of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$  is known to be effectively played by catalase. Oxidase catalyse the substrate oxidation by utilising molecular oxygen.

Based on the substrate oxidised specific name is assigned like the glucose oxidase, uric acid oxidase and so on. The oxidase mimics include Au, Cu, Mo, Pt and carbon-based nanoparticles. Peroxidase catalyse substrate oxidation in the presence of peroxides. Peroxidase mimics include magnetic iron-based materials  $\text{Fe}_3\text{O}_4$ , vanadium-based, noble metals like Ag, Au, Pt, carbon-based nanoparticles and MOF-based. SOD is known to eradicate superoxides  $\text{O}_2^-$ , reactive oxygen species (ROS) naturally. SOD mimics include carbon-based, cerium-based nanoceria and melanin-based. Hydrolase catalyses the hydrolysis of bonds chemically like the nucleosidase hydrolyse nucleotide bonds, phosphate cleavage by the phosphatase enzyme. Others include esterase, protease, silicatein and carbon-based nanoparticles. To catalyse hydrolysis reactions Au nanoparticles are also widely employed as hydrolase enzymes. In addition, nanozymes are also classified based on the scavenging property on free radicals into antioxidants and pro-oxidants. Oxidative stress induced by the pro-oxidants produce free radicals in biological systems. Free radical generation reaction involves certain peroxidases or oxidases and are considered as a pro-oxidant. Antioxidant nanozymes on the contrary scavenge free radicals through catalase or SOD-like activities [6–8] (Fig. 1).

## 4 Nanozymes in Oral Cancer

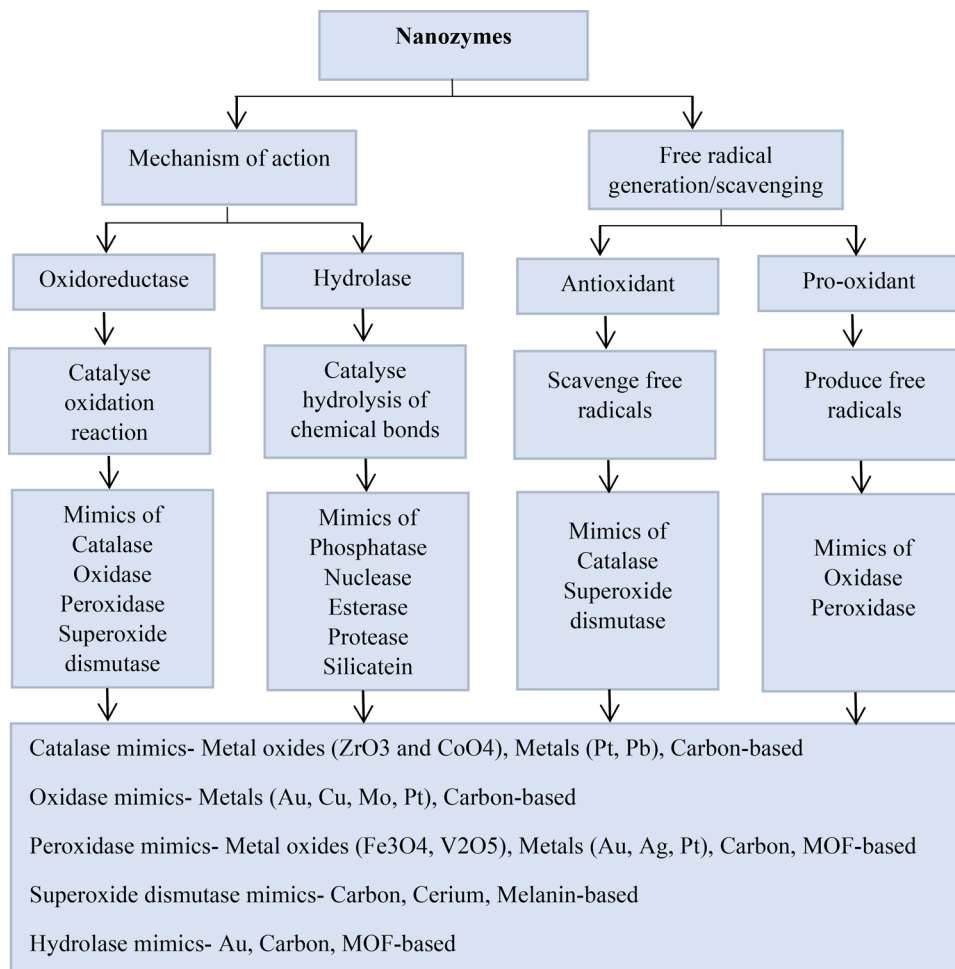
Despite oral cavity being an accessible area for investigation yet oral cancer gets obscured till it progresses to the advanced stage. Early detection and appropriate management strategies tremendously improves the survival rate, cancer related morbidity and poor quality of life in oral cancer patients. Novel technologies have a great potential in oral cancer research, diagnosing and designing the therapeutics. Nanomedicine which combines nanotechnology with medicine has created a paradigm shift in cancer diagnostics and therapeutics. In the field of nanomedicine nanoparticles are exploited as biomarkers, biosensors, for imaging, drug delivery and many others and as mentioned above many nanoparticles are known to possess nanozyme like characteristics. This section of the review discusses the role and application of nanozymes in oral cancer detection, diagnosis, therapeutics and in regeneration of tissues [9].

### 4.1 Oral Cancer TME and Nanozymes

#### 4.1.1 Oral Cancer TME

A complex milieu with dynamic network of cells which synchronise with various secretions describes the term TME. Progression, metastasis, response and resistance to treatment are majorly shaped due to the crosstalk in the TME. The complex network of cells of oral cancer TME comprises of

**Fig. 1** Classification and sources of nanozymes



endothelial cells, fibroblasts, immune cells possessing both tumor promoting and inhibitory functions and stromal cells. Regulatory T cells (Tregs), M2 macrophages, CD4 helper T cells and myeloid-derived suppressor cells (MDSCs) are pro-tumor immune cells. Anti-tumor immune cells comprise of CD4 type 1 helper T cells, CD8 + T cells, dendritic cells (DCs), M1 macrophages and natural killer cells (NK). The maintenance of the tumor milieu is attributed to various cytokines, chemokines, growth factors, cell adhesion molecules which mediate the equilibrium coupling pro-tumor and anti-tumor immune cells. Additionally, cancer associated inflammation particularly chronic inflammation produces inflammatory mediators like IL-1 $\beta$ , IL-6, cyclooxygenases and many more which are known to regulate the oral cancer TME. The TME also comprises of non-cellular components like hypoxia and ROS which play a crucial role in oral oncogenesis. Hypoxia signaling is mediated by hypoxia-inducible factors (HIFs) which comprises of HIF1 $\alpha$ , HIF2 $\alpha$ , and HIF3 $\alpha$  subunits. They configure a heterodimer with HIF1 $\beta$ . Upregulation of IL-8, vascular endothelial growth factor (VEGF), osteopontin, E-cadherin down regulation on tumor cells is known to be influenced by hypoxia. Tumor cells and

immunosuppressive cell infiltration such as MDSCs, Tregs, and tumor associated macrophages (TAMs) are observed in hypoxic regions of oral cancer. Free oxygen radicals formed due to various cell activities are termed as ROS. This includes superoxide, hydroxyl radical, singlet oxygen, and hydrogen peroxide. TME is strongly associated with elevated levels of ROS. Mitochondrial ROS is required for stabilizing HIF and head and neck cancer are known to show distinct characteristics of hypoxia. Regulation of TAMs, MDSCs, and T cells is influenced by uncontrolled ROS which results due to oxidative stress. Besides these, intrinsic factors like angiogenesis, extracellular matrix (ECM), and epithelial-mesenchymal transition (EMT) debilitates immunosurveillance in the TME. Crosstalk between these cellular and non-cellular components of the TME is one of the major contributions for immunosuppression and tumorigenesis in oral cancer [2, 10].

**4.1.2 Role and Application of Nanozymes in Cancer TME**

Nanozymes can effectively improve the TME and aid in achieving anti-tumor effects. Nanozymes have potential to

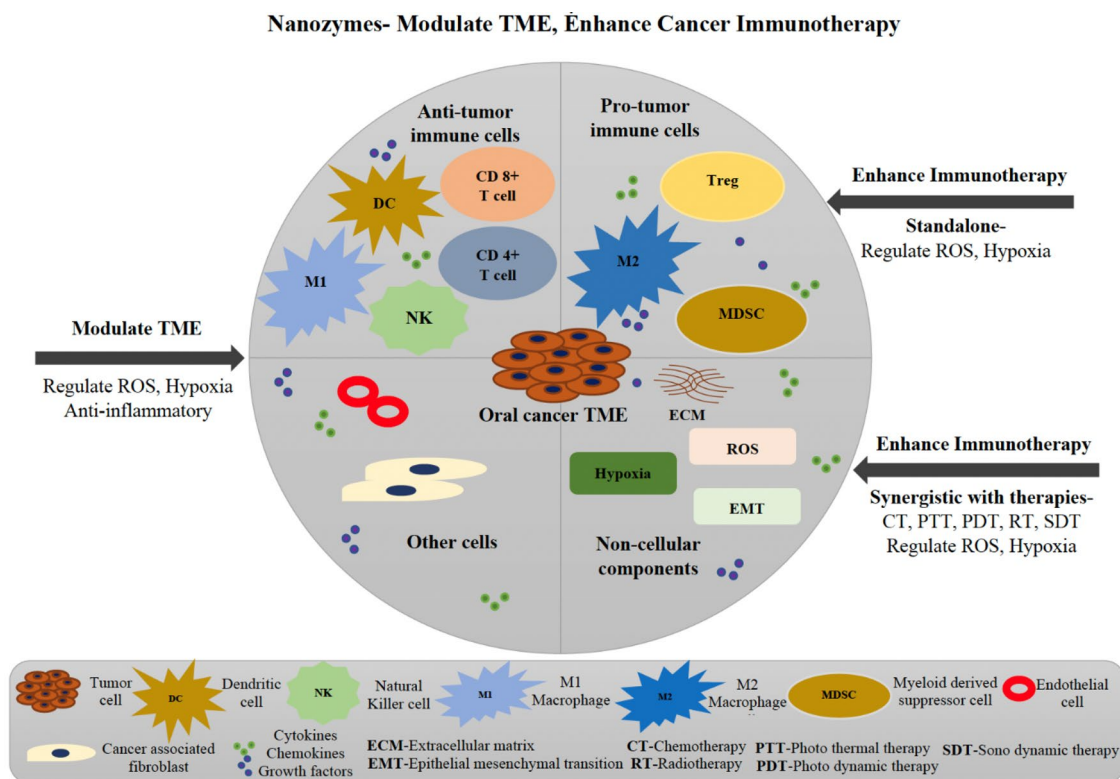
regulate hypoxia and ROS in the TME due to their intrinsic enzymatic characteristics and also exhibit anti-inflammatory effects. Catalase-like activity with production of  $O_2$  is exhibited by ROS scavenging nanozymes. Scavenging ROS nanozymes alleviate the inflammation along with the inhibition of cancer cells. Predominant catalase mimics are manganese oxide nanoparticles. MOF derivative mesoporous manganese cobalt oxide nanozyme can mimic catalase property. Attenuation of hypoxia is achieved by intracellular  $O_2$  generation and HIF-1 $\alpha$  downregulation in cancer cells by this nanozyme. Elevation of ROS level to destruct cancer cells through oxidase and peroxidase mimics have also been explored in cancer management. A predominant ability to produce ROS was observed with  $Fe_3O_4$  nanoparticles doped with other metals. Dual enzyme mimicking nanoparticles like PtFe@  $Fe_3O_4$  nanorods possessing both peroxidase and catalase like activities, Au and  $Fe_3O_4$  nanoparticles mimicking glucose oxidase and peroxidase with mesoporous  $SiO_2$  as shell is known to produce toxic ROS and have desirable ability to suppress cancer rate. Nanozymes as therapeutic enzymes that enhance cancer immunotherapy. They are utilized as standalone or synergistically with other therapies. Nanozymes particularly ROS-generating ones are used for cancer killing and they act independently. ROS scavenging nanozymes are known to synergistically enhance the avenues of anti-tumor therapy such as chemotherapy

(CT), photodynamic therapy (PDT), photothermal therapy (PTT), radiotherapy (RT), and sonodynamic therapy (SDT). These therapies depend upon the oxygen level and sensitizers mainly singlet  $O_2$  to generate ROS [1, 7, 11]. Afrasiabi et al., evaluated super paramagnetic iron oxide nanoparticles (SPIONs) effects on oral squamous cell carcinoma (OSCC) mitochondria. SPIONs act as nanozymes due to their enzyme mimetic activity. Their study demonstrated an elevated ROS level in OSCC mitochondria exposed to SPIONs and suggested it could be employed as a potential therapeutic agent [12, 13]. Chen et al., observed an enhanced intracellular ROS generation when Au-nanorod-filled mesoporous silica nanobeads were utilised in conjunction with RT in OSCC [14] (Fig. 2).

## 4.2 Oral Cancer Microbiome and Nanozymes

### 4.2.1 Oral Cancer Microbiome

The human microbiome constitutes entire genome of microorganisms in the human body. Oral microbiome represents the same in the oral cavity. Numerous studies have hypothesized the association of bacterial flora in the development of oral cancer. Poor oral hygiene, chronic periodontitis observed in oral cancer patients speculates the role of microbiome in oral oncogenesis. The population of oral



**Fig. 2** Schematic illustration of oral cancer TME and role of nanozymes in cancer TME

microbiome is affected by lifestyle factors like tobacco, betel nut and saliva. Bacterial species *Prevotella melaninogenica*, *Streptococcus mitis*, and *Capnocytophaga gingivalis* were found in high abundance in the saliva of OSCC patients. Tumor tissues in oral cancer were reported with significant number of *Capnocytophaga gingivalis*, *Eubacterium saburreum*, *Staphylococcus aureus*, *Streptococcus mitis*, *Veillonella parvula*, and many more. Hydrogen sulfide a mutagenic agent, methyl mercaptan production by *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas gingivalis* leads to chronic inflammation, tumor angiogenesis, proliferation and invasion of cells. Oral microbial species like *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus oralis*, and many others possess enzyme alcohol dehydrogenase that metabolizes alcohol to carcinogenic acetaldehyde inducing oral cancer progression. Evidence through studies also propose that potential driving force for inflammation in cancer is due to microbial infection. Oral virome studies have concentrated predominantly on HPV and Epstein Barr virus (EBV). Although the role of HPV in oral cancer is debatable HPV sub-types 16 and 18 are strongly associated with oro-pharyngeal cancer and a proportion of OSCC cases. Additionally, risk of OSCC is increased due to the colonisation of fungus *Candida albicans* [3, 15].

#### 4.2.2 Role and Application of Nanozymes in Cancer Microbiome

Nanozymes are known to possess antibacterial, antiviral and antifungal properties. In contrast to antibiotics nanozymes less possibly lead to bacterial resistance because of the inherent merit of nanomaterials like excellent membrane permeability. Additionally, bacterial biofilms are effectively eradicated due to the catalytic activities of nanozymes. Metal-based compounds, carbon-based, metal dichalcogenides and MOF-based nanomaterials have enormously evolved as antibacterial nanozymes. The mainstream mechanisms of antibacterial activity of nanozymes are ROS regulation and extracellular DNA clearance which is responsible for maintaining bacterial biofilm integrity. Bacterial cell wall, cytoplasmic membrane, nucleic acids, proteins, polysaccharides are targeted by exogenous ROS eventually leading to irreversible damage and destruction of bacteria. Prevention of formation of bacterial biofilms and destruction of mature biofilms is of paramount importance of ROS in antibacterial activity. Peroxidase and oxidase-based are the predominant nanozymes utilised for generating ROS [16, 17]. Bactericidal activity is also enhanced by nanozymes which release bactericidal ions like  $Fe_2^+$ . Photothermal effects and superparamagnetism possessed by nanomaterials also elevate the nanozyme activity [18]. Cu/Carbon hybrid, MOF/Ce-based nanoparticles, mesoporous silica supported

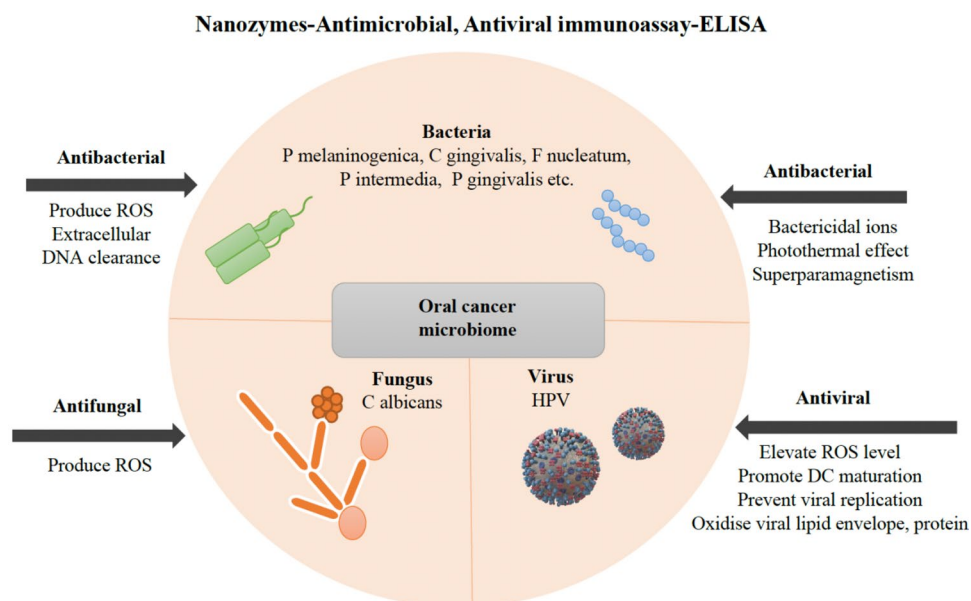
Au nanoparticles, Cu-TCPP (TCPP-tetrakis 4 carboxyphenyl porphyrin with metals Fe, Co), iron oxide nanoparticle, iron oxide nanozyme-IONzymes possess enzyme like characteristics and exhibit anti-bacterial activities [5, 17, 19]. Yu et al. utilised cerium oxide nanoparticles ( $CeO_2$ ) which exhibits nanozyme characteristics for therapeutic management of periodontitis. Their study demonstrated that  $CeO_2$  nanoparticles act as ROS scavengers in the inflammatory microenvironment and potentially be utilised to treat periodontitis [20]. Antiviral nanozymes predominantly act by elevating ROS level, promoting DCs maturation, preventing viral replication and viral lipid envelope and protein oxidation. IONzymes and Ag-TiO<sub>2</sub> single atom nanozyme were utilised in antiviral therapy of Influenza A viruses and SARS-coronavirus 2 and the application of these nanozymes could be attempted in antiviral therapy of HPV as well. Besides viral tests by nanozyme based immunoassay like enzyme-linked immunosorbent assay (ELISA) which is dependent on HRP and vaccine like chitosan with iron oxide nanozyme (CS-IONzyme) are carried out in the viral diagnostics and therapeutics [21]. Antifungal therapy using nanozymes are still in the infancy stage. Ce-based MOF exhibits multizyme like characteristics and possess antifungal properties. Disinfection of *C. albicans* were also achieved with peroxidase mimics like iodine-doped carbon dots [18]. As mentioned above bacterial flora, virus, fungus, poor oral hygiene, chronic periodontitis, are associated risk factors in oral cancer development and progression. In this regard nanozymes known to possess antimicrobial activity utilised in immunoassays could be employed in oral cancer diagnostics and therapeutics (Fig. 3).

### 4.3 Molecular Alterations in Oral Cancer and Nanozymes

#### 4.3.1 Molecular Alterations in Oral Cancer

OSCC is a heterogenic tumor and is strongly associated with mutations of genes. Most common genes pertaining to OSCC development and progression are TP53, CDKN2A, NOTCH1, HRAS, and PIK3CA. TP53 is the most frequently mutated gene and its location in the DNA binding domain of protein is associated with poor prognosis in OSCC. Apart from genetic mutations OSCC is strongly associated with epigenetic changes. The most common epigenetic changes studied in OSCC are DNA methylations and microRNA (miRNA). Hypermethylation and subsequent tumor suppressor gene silencing has been reported in OSCC [22]. Besides, numerous studies have focused on the role of miRNA in oral oncogenesis. miRNA represents small non-coding RNA which aid in regulating the gene expression by miRNA translation inhibition. miRNA is of both oncogenic and suppressive types that are up-regulated or down-regulated

**Fig. 3** Schematic illustration of oral cancer microbiome and role of nanozymes in antimicrobial activity



in oral tumorigenesis. miR-137, miR-133a, miR-503, miR-15a, miR-21, miR-24 and many others are associated with OSCC. Among these miR-21 is most frequently observed and targeted miRNA in OSCC [22, 23]. Gene alterations and miRNA serve as biomarkers and important diagnostic tools as well as potential therapeutic agents such as targeted gene therapy. Some of the drugs employed for targeted gene therapy in OSCC are epidermal growth factor receptor (EGFR) targeted drugs cetuximab, nimotuzumab, VEGF targeted drugs bevacizumab, aflibercept, programmed cell death protein-1 (PD-1) targeted drugs like pembrolizumab. Various techniques from polymerase chain reaction, microarray to recent next generation techniques is employed to detect the molecular alterations [22]. However, in routine tumor diagnosis including oral cancer immunoassays like ELISA and immunohistochemistry (IHC) are commonly employed [19].

#### 4.3.2 Role and Application of Nanozymes at Molecular Level

Nanozymes are magnificent biosensors and aid in detection of nucleic acids dsDNA, ssDNA, mutant DNA, DNA modifications like methylations as well as RNA including miRNAs [24]. Colorimetric, chemiluminescence, electrochemical, electrochemiluminescence, fluorescence, are some of the mechanisms of biosensing and enzymes play a critical role in biosensing [19, 25]. Bhattacharjee et al., have applied mesoporous iron oxide having peroxidase mimetic activity for DNA methylation detection in cancer cell lines [26]. Li et al., analysed exosomal miRNA through electrochemical biosensor by cascade primer exchange reaction. They utilised nanozyme MOF@Pt@MOF and suggested it could aid in early and accurate diagnosis of cancer [27]. Jiang et al.,

developed a Pt–Pd nanoparticle based immunochromatographic test strip with enhanced peroxidase-like catalysis for detection of p53 [28]. As mentioned above ELISA and IHC are routinely employed to detect tumor markers of diagnostic significance in cancer. For instance, Tojyo et al., employed IHC to correlate the expression between p53 and programmed cell death ligand-1 in OSCC. These immunoassays depend upon enzyme HRP and peroxidase based nanozymes could be effectively utilised [19, 29]. Besides nanozymes are explored in targeted drug delivery and gene therapy. Drug delivery systems is of two types namely passive and active. Passive targeting is dependent upon tumor tissue characteristics like vascularity, temperature and pH. Active targeting is more beneficial compared to passive targeting. Conjugation of ligands such as folate, transferrin, EGFR with nanocarriers is utilised in active targeting. This leads to ligand-receptor interaction and internalisation into tumor cells through receptor mediated endocytosis [19, 30]. Fe<sub>3</sub>O<sub>4</sub> nanoparticles with nanozyme characteristics are explored in imaging guided drug delivery and gene therapy [31]. Modification of nanozymes with folic acid can effectively target the cell surface of cancerous cells with folic acid receptors and promote cell death as oxidants. In addition, Pt nanoparticles/graphene oxide with folic acid aid in distinguishing many cancerous cells more precisely than naked-eye [1]. Essawy et al., utilised Au nanoparticles to determine their simultaneous role as both carriers and therapeutic agents in oral cancer. Their study demonstrated an enhancement in the cytotoxicity of cancerous cells with doxorubicin pH resistant Au nanoparticles besides acting as drug carriers [32]. Sürer et al., investigated a complex of EGFR targeting drug cetuximab and cisplatin-conjugated with Au nanoparticle in combination with RT in oral cancer.

They demonstrated Au nanoparticles can be used as a nanodrug carrier and exhibits radiosensitising effects [33] (Fig. 4).

#### 4.4 Oral Cancer Imaging and nanozymes

##### 4.4.1 Oral Cancer Imaging

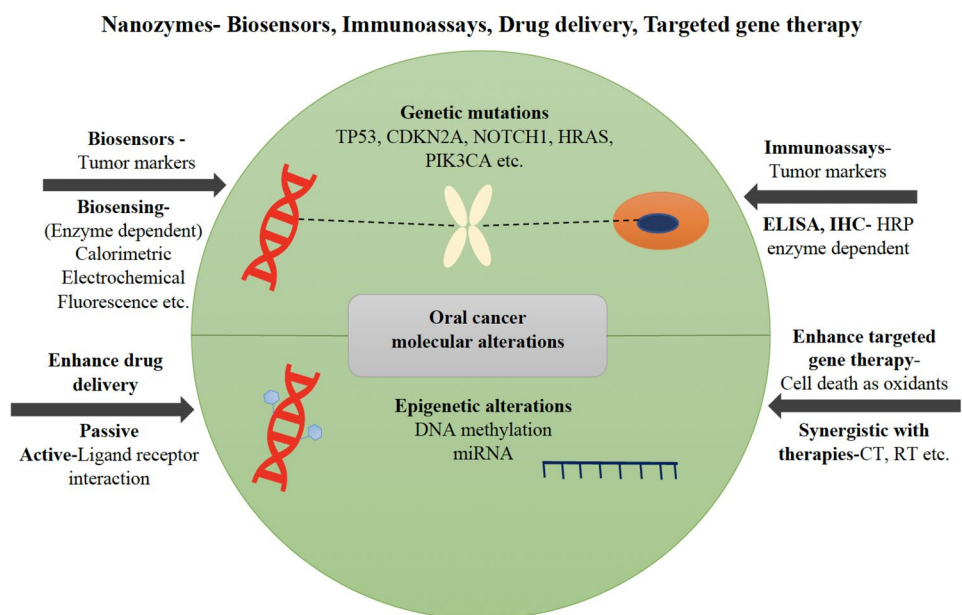
Imaging in diseases involves pathological tissue imaging through colour detection, fluorescence; live cell and organelle imaging and in vivo imaging. Imaging aids in cancer screening and surveillance and in theranostics approach involves both imaging as well as delivery of drugs and therapeutics [19, 34]. There are many imaging modalities employed in oral cancer like orthopantomography, cone beam computed tomography, single-photon emission computed tomography and others. But, the most common employed imaging techniques in oral cancer include computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET). CT helps in detecting primary tumors and local bone infiltration. Determination of boundaries of tumor precisely is achieved through multi-detector CT. MRI is a better imaging modality in comparison to CT that aids to detect minute lesions, bone marrow, soft tissues, nerves, vessels and many more. They help in evaluating the spread of tumor locally and planning of surgical resection as well. MRI study protocol involves the following sequences—T1, T2, diffusion-weighted imaging (DWI), perfusion plus or minus a contrast agent. PET is used to detect metastasis of known primary tumors as well as primary tumor site if metastasis is detected at an early stage. Ultrasonography, CT, MRI and PET are employed for assessing locoregional lymph nodes in oral cancer [35]. An

additional value for oral cancer diagnosis and treatment is achieved through fluorescence imaging. It helps in providing real-time information with an enhanced imaging sensitivity, resolution supporting decision-making clinically [36].

##### 4.4.2 Role and Application of Nanozymes in Cancer Imaging

Nanozymes aid in both in vitro and in vivo imaging for cancer diagnosis as well as theranostics. Oxidation of calorimetric substrates like 3,3',5,5'-tetra methyl benzidine (TMB) can be catalysed by peroxidase nanozymes. They produce a colour that could be utilised in tissue sections for imaging the recognised biomarkers. Magneto ferritin nanozyme (M-HFn) is employed for imaging and tumor targeting. The iron oxide nanocore of the nanozyme oxidises the colour substrate producing colour for tumor tissue detection. Cancer screening is usually done through cytological examination of exfoliated cells. Flow cytometry, cytological smear are the most common cytological detection techniques which are expensive and laborious. In this regard nanozyme based colour reaction could be effectively employed for analysing cytological features both qualitatively and quantitatively. Besides, the characteristics of nanozymes like fluorescence, paramagnetism, X-ray absorption help in pathological tissue imaging. For instance, Au nanocluster nanoprobe with fluorescence enzyme mimics targeting folate receptors aid in detection of fluorescence in tumor tissues. Nanozymes like Fe<sub>3</sub>O<sub>4</sub> conjugated with antibody are used in detection of circulating tumor cells. Nanozymes also help in real time imaging of organelles in living cells through probes. Heterogeneous Pd nanozyme aids in specific imaging of mitochondria

**Fig. 4** Schematic illustration of molecular alterations in oral cancer and role of nanozymes at molecular level



in living cells through mediating the bio-orthogonal reactions in situ. For in vivo imaging through MRI, nanozymes MnO are effectively utilised. This nanozyme possesses dual enzyme properties of SOD and catalase. They enhance MRI contrast agent and could be simultaneously used for therapy and imaging of tumor sites with increased level of superoxide radicals. Nanozyme  $\text{Fe}_3\text{O}_4$  is utilised as a highly sensitive T2-weighted MR imaging contrast agent as well as carrier for targeted drug delivery. Prussian blue nanozymes ( $\text{KFe}_3 + [\text{Fe}_2 + (\text{CN})_6]$ ) were noted of catalysing the breakdown of  $\text{H}_2\text{O}_2$ . The  $\text{O}_2$  produced were known to be utilised as an ultrasound contrast agent and  $\text{Fe}_3$  as MR imaging contrast agent. In vivo photoacoustic imaging of nasopharyngeal carcinoma was achieved through graphene quantum-dot-based nanozyme vesicle with increased peroxidase activity [19, 34]. Au, magnetic nanoparticles, carbon quantum dots are utilised in oral cancer diagnosis and therapy. Au nanoparticles can strongly scatter in NIR, magnetic nanoparticles as mentioned above are utilised in MRI, carbon and graphene quantum dots are used as nanoparticle probes when conjugated with molecular biomarkers [37]. Oxidation of colour substrates, live cell and organelle imaging through nanozymes could be attempted in oral cancer screening and diagnosis as well (Fig. 5).

## 4.5 Regeneration in Oral Cancer and Nanozymes

### 4.5.1 Regeneration in Oral Cancer

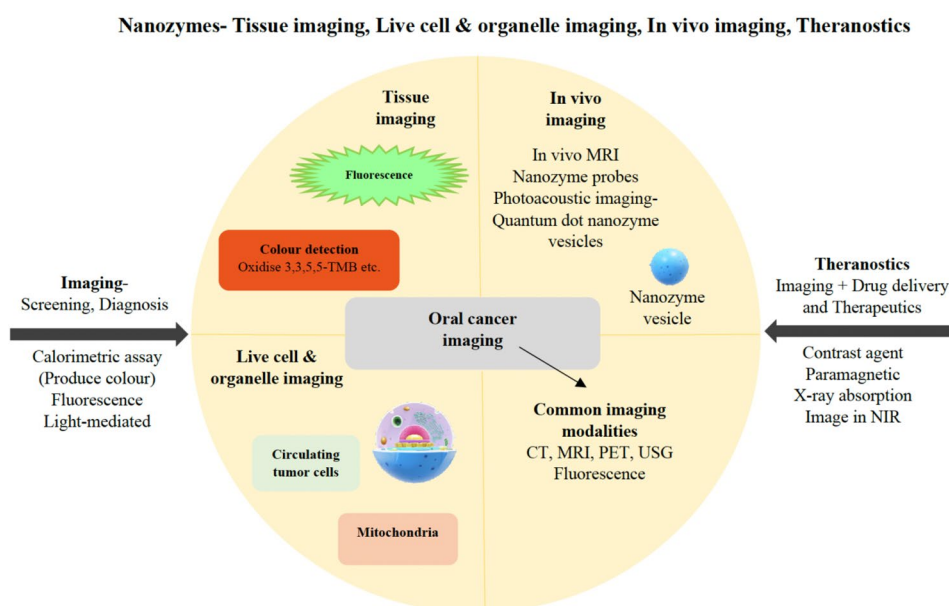
Regenerative medicine in the modern era presents as one of the most desirable reconstruction methods. Tissue engineering is used to rebuild hard and soft tissues by taking advantage of the self-repair ability of cells and tissues. The

principle of tissue engineering is based upon a triad comprising of stem cells, signaling molecules, and scaffolds or ECM. Stem cells can be embryonic or adult-derived. Differentiation into chondrocytes, osteoblasts, hematopoietic cells, neural cells and others is achieved through adult derived multipotent stem cells. Signaling molecules comprises of cytokines and numerous growth factors that are incorporated to the ECM. The signaling molecules commonly used are IL-6, TGF- $\beta$ 1, bone morphogenetic proteins, fibroblast growth factor-2, and platelet-derived growth factor. A scaffold is a porous natural or synthetic material that functions as a matrix for attachment, migration, and differentiation of progenitor cells. It provides support, feasible environment for remodeling of cells and also utilised as growth factor reservoir. Scaffolds should be biocompatible in nature. In regard to oral cancer the principles of regenerative engineering aids as a potential means to enhance maxillofacial tissue. Tissue engineering in oral cancer and maxillofacial surgery are applied for bone, cartilage, fat, muscle, nerve, salivary gland, skin, oral mucosa and vascular regeneration [38, 39].

### 4.5.2 Role and Application of Nanozymes in Tissue Regeneration

Nanozymes are potentially used in regenerative medicine for both hard and soft tissue regeneration. Nanozymes that are antioxidants enhance cell cycle protein and ion channel activation. Factors like VEGF are elevated through nanozymes and they also promote cell attachment, proliferation and differentiation. In addition, nanozymes possess antibacterial properties which could aid in achieving a sterile environment, accelerate healing and tissue regeneration. Bone regeneration depends upon enhancing proliferation,

**Fig. 5** Schematic illustration of imaging modalities in oral cancer and role of nanozymes in imaging





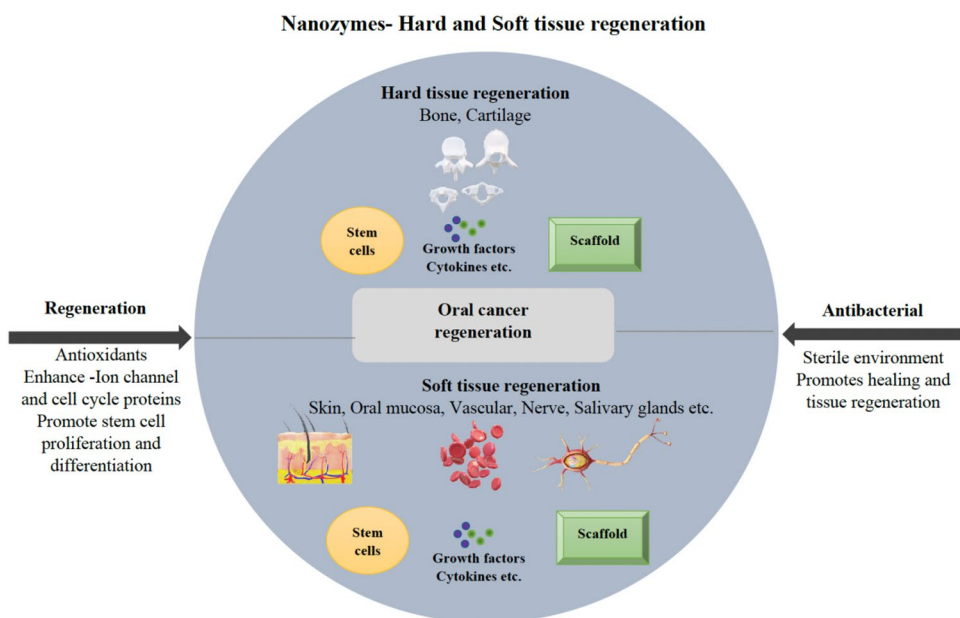
migration and osteogenic differentiation of stem cells. This is achieved through cytokines or by incorporating certain substances while synthesising biological scaffolds. Nanozymes in these biological scaffolds may be desirable. SPIONs possess peroxidase like activity help in regulation of cell cycle proteins that might lead to mesenchymal stem cells promotion. Lysosomal degradation of free Fe<sub>3</sub>O<sub>4</sub> nanozyme accelerates the cell cycle process and leads to promotion of osteogenic proliferation. Nanoceria is also widely explored in bone regeneration. Nanoceria possess antioxidant properties, and helps in achieving osteogenic differentiation of stem cells and collagen production. Variable valence Ce affects cell adhesion and proliferation. Nanoceria also promotes vascular regeneration. Activation of calcium channels of cells is achieved by bio-scaffolds containing nanoceria. Intracellular Ca<sup>2+</sup> enhances HIF-1 $\alpha$  stability, increases VEGF, and endothelial progenitor cell proliferation and differentiation. Nanozymes provides a sterile environment accelerating healing of soft tissues. Migration and proliferation of fibroblasts, keratinocytes are achieved due to reduced oxidation of cell membranes and proteins by nanozymes [5]. In regenerative medicine of oral cancer, the above mentioned nanozymes could be explored (Fig. 6).

### 4.6 Outlook and Future Prospects

Although nanozymes have versatile applications yet many significant challenges remain undetermined. These include nanozyme possibility to mimic entire activities of natural enzyme, substrate specificity, enzymatic selectivity and controlled enzymatic action in vivo [19]. In comparison to natural enzymes, it is observed that the efficiency of

nanozymes is relatively low limiting their application. Nanozymes' diverse catalytic mechanism are regulated by various factors. Basic properties of nanomaterials such as composition, shape, size determine the catalytic action of nanozymes with reaction environmental factors like pH and temperature. Nanozyme activity can be extended by altering these factors. Moreover, nanozymes demand for research on their in vivo biosafety. Most of the nanozymes applied include the inorganic nanoparticles which has the ability to penetrate into the mitochondria, nucleus or lysosome of the cells, and may irreversibly damage the cells or as a foreign agent may lead to an immune response [5, 17]. Apart from these common issues of nanozymes cancer specific issues lie in the complicated cancer TME which limits the application of nanozymes. Precisely targeting only, the cancerous cells without damaging normal cells still remains a great challenge in nanozyme-based cancer immunotherapy. Developing a hypoxia and pH responsive nanozyme systems may aid in reduction of this side effects [11]. Most of the nanozymes perform by ROS regulated mechanism in cancerous and microbial cells which limit their killing ability. To overcome this, novel nanozymes which can perform by non-ROS mechanism like esterase, nuclease, protease should be explored [18]. Recently, novel high performance nanozyme have evolved known as single-atom nanozymes (SAzymes). Integration of single atom technology with intrinsic enzyme-like catalytic active site is applied in SAzymes. They are advantageous compared to conventional nanozymes and have shown promising applications in biosensing, antibacterial and cancer therapy [25, 40].

**Fig. 6** Schematic illustration of regeneration in oral cancer and role of nanozymes in tissue regeneration



## 5 Summary

The modern era has remarkably attracted nanozymes in various fields, specifically in oncology. Nanozymes are rising as a promising concept of nano-catalytic medicine in cancer management. They aid in cancer diagnosis and therapeutics and possess antimicrobial properties, are magnificent biosensors and are employed in regenerative medicine as well. Despite the progress of nanozymes in numerous applications, there persists many challenges which needs to be focussed. This drives for exploration of novel nanozyme materials and in-depth characterisation of their catalytic properties. In addition, studies of nanozymes in oral cancer are still in the infancy stage. With accelerated progress in nano science and technology, nanozymes may pave the way for new principles in oral cancer diagnosis and therapeutics.

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