

Review Article

Enhancing oral cancer treatment via photodynamic therapy: Gold nanoparticle-based delivery system for 5-aminolevulinic acid (5-ALA)

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A B S T R A C T

Oral squamous cell carcinoma (OSCC) is a significant global health concern, responsible for approximately 300,000 new cases and 145,000 deaths annually, making it the sixth most common malignancy worldwide. Traditional treatments, including surgery, radiation, and chemotherapy, often lead to severe side effects such as physical disfigurement, functional loss, and systemic toxicity. These limitations have spurred the search for alternative therapies, with photodynamic therapy (PDT) gaining recognition for its reduced invasiveness, improved targeting, and better cosmetic outcomes. However, PDT faces challenges, including inadequate photosensitizer (PS) delivery, poor specificity, and degradation in physiological environments. Nanotechnology has emerged as a promising solution to enhance PDT by improving the stability, selectivity, and therapeutic efficacy of PSs. Gold nanoparticles (AuNPs) have shown the potential to enhance PDT outcomes, particularly in OSCC, by inhibiting tumor proliferation and improving diagnostic accuracy without systemic toxicity. Despite these advancements, the conventional chemical synthesis of AuNPs poses environmental concerns, high costs, and potential biocompatibility issues. This study introduces a novel biogenic synthesis approach for AuNPs, utilizing green chemistry principles to create more biocompatible and environmentally sustainable nanoparticles. The novelty of this research lies in the application of green-synthesized AuNPs to enhance PS delivery in PDT, offering a more effective and less toxic treatment option for oral cancer. This innovative approach addresses the limitations of current PDT and nanoparticle synthesis methods, contributing to the development of more sustainable and biocompatible cancer therapies. The study's findings are contextualized within the increasing scholarly and patent activity surrounding AuNPs and 5-aminolevulinic acid (5-ALA) in cancer therapies, underscoring the growing importance of this field in advancing OSCC management.

1. Background

To contextualize our research on biocompatible gold nanoparticles (AuNPs) for targeted cancer therapy, it's essential to examine the scholarly activity surrounding related keywords such as "oral cancer," "photodynamic therapy," "gold nanoparticles," "Biogenic synthesis" and "5-ALA." The graph below, sourced from [Lens.org](https://lens.org), illustrates the number of publications over time that incorporate these keywords [Fig. 1](#).

The trends illustrated by the graphs provide a clear picture of the growing scholarly and patent activity surrounding the use of AuNPs and 5-ALA in cancer therapies, particularly in the context of oral cancer and photodynamic therapy (PDT). The first graph highlights a significant increase in research publications between 2016 and 2018, signaling a period of intense academic interest and exploration in this field. This surge reflects the expanding recognition of AuNPs and 5-ALA as critical components in innovative cancer treatment strategies.

Simultaneously, the second graph shows a steady rise in patent

activity beginning around 2010, with continued growth peaking in recent years. This trend underscores the importance of intellectual property in this rapidly evolving field, as researchers and companies alike work to secure innovations that enhance the therapeutic potential of AuNPs. The increase in both granted patents and applications indicates ongoing advancements and a strong commitment to developing cutting-edge treatments.

2. Introduction

Oral squamous cell carcinoma (OSCC) poses a significant global health challenge, with approximately 300,000 new cases and 145,000 deaths annually, making it the sixth most common malignancy worldwide. OSCC accounts for about 90 % of all oral cancers, highlighting its prevalence and the urgent need for effective early detection methods [1, 2]. Traditional treatments like surgery, radiation, and chemotherapy, while somewhat effective, often lead to severe side effects, including

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Fig. 1. Publication and patent trends related to AuNPs 5-ALA in oral cancer therapies. Data sourced from [Lens.org](https://lens.org).

physical disfigurement, loss of function, and systemic toxicity. These limitations have driven the exploration of alternative treatments, including photodynamic therapy (PDT), which has gained increasing recognition as a promising approach. PDT offers advantages over conventional therapies, such as reduced invasiveness, better targeting, and improved cosmetic outcomes. However, PDT is not without its own challenges, such as inadequate delivery of photosensitizers (PS), poor specificity, and degradation in physiological environments. This has led to the integration of nanotechnology into PDT to enhance photosensitizer delivery, stability, and selectivity, improving overall therapeutic efficacy [3].

Nanotechnology holds the potential to revolutionize PDT by addressing its limitations. Nanomaterials can enhance the stability of PS, increase their selective uptake by cancer cells, and improve the generation of reactive oxygen species (ROS), which are critical for the destruction of cancer cells. Recent studies have explored the use of nanoparticles, particularly gold (AuNPs), effectively inhibits the proliferation and invasion of OSCC without systemic toxicity [4]. Meanwhile, AuNPs have been utilized to enhance diagnostic techniques, such as laser-induced fluorescence and Raman spectroscopy, improving the detection rates of OSCC by modifying emitted spectra and providing better specificity[5,6]. Overall, the integration of these nanotechnologies offers a promising avenue for improving OSCC management. However, traditional chemical synthesis methods for nanoparticles are costly and environmentally harmful, often involving toxic solvents and harsh conditions. These methods also risk producing nanoparticles that lack biocompatibility or biodegradability, posing toxicity concerns in clinical applications [7]. To limit these obstacles, this study aim is to introduce more biocompatible synthesis method for AuNPs which is green approach. These green-synthesized AuNPs enhance the precision and efficacy of PS delivery in PDT, providing a more effective and less toxic treatment option for oral cancer. By focusing on the biogenic synthesis of nanoparticles, this study seeks to overcome the limitations of current PDT and nanoparticle synthesis methods, contributing to the development of more sustainable and biocompatible cancer therapies.

2.1. Cancer

Cancer, which is defined by the abnormal and uncontrollable growth and dissemination of cells, presents a major worldwide health problem [8]. In 2022, global cancer statistics revealed nearly 20 million new

cases of cancer worldwide, including nonmelanoma skin cancers (NMSCs), along with 9.7 million cancer-related deaths, as reported by the International Agency for Research on Cancer (IARC) (Fig. 2). These alarming figures suggest that one in five individuals may develop cancer in their lifetime, while one in nine men and one in twelve women are likely to succumb to the disease. Looking ahead, demographic projections estimate that the number of new cancer cases could rise to 35 million annually by 2050. To combat this growing burden, targeted investments in cancer prevention, especially those addressing key risk factors such as smoking, obesity, and infections, are critical. Effective prevention strategies could not only reduce future cancer incidence and mortality but also yield substantial economic and societal benefits for nations globally in the decades to come [9].

2.1.1. The hallmarks of cancer

In 2000, Hanahan and Weinberg introduced the concept of the “hallmarks of cancer” delineating the essential biological alterations that define cancer cells. These hallmarks encompass the following.

- Cancer cells acquire the capacity to grow and multiply on their own, without relying on external signals for growth, resulting in uncontrolled cell division and the creation of tumors.
- Cancer cells develop resistance to growth suppressors, allowing them to continue growing even when faced with signals that would ordinarily stop their proliferation.
- Cancer cells employ strategies to evade the normal process of programmed cell death (apoptosis), enabling them to persist and multiply despite genetic damage or other factors that would normally trigger cell death.
- Cancer cells develop the capability to endure unlimited division, beyond the typical replicative senescence observed in healthy cells, thus enabling replicative immortality. One way to accomplish this is by activating telomerase, an enzyme that preserves the length of telomeres, which in turn safeguards the ends of chromosomes.
- Cancer cells stimulate angiogenesis, the formation of new blood vessels, to provide a continuous supply of oxygen and nutrients, essential for the growth and survival of tumors.
- Cancer cells acquire the ability to invade neighbouring tissues and disseminate to remote locations in the body, resulting in the development of additional tumors (metastases). The capacity of cancer cells to spread and form metastases is a key factor in the high

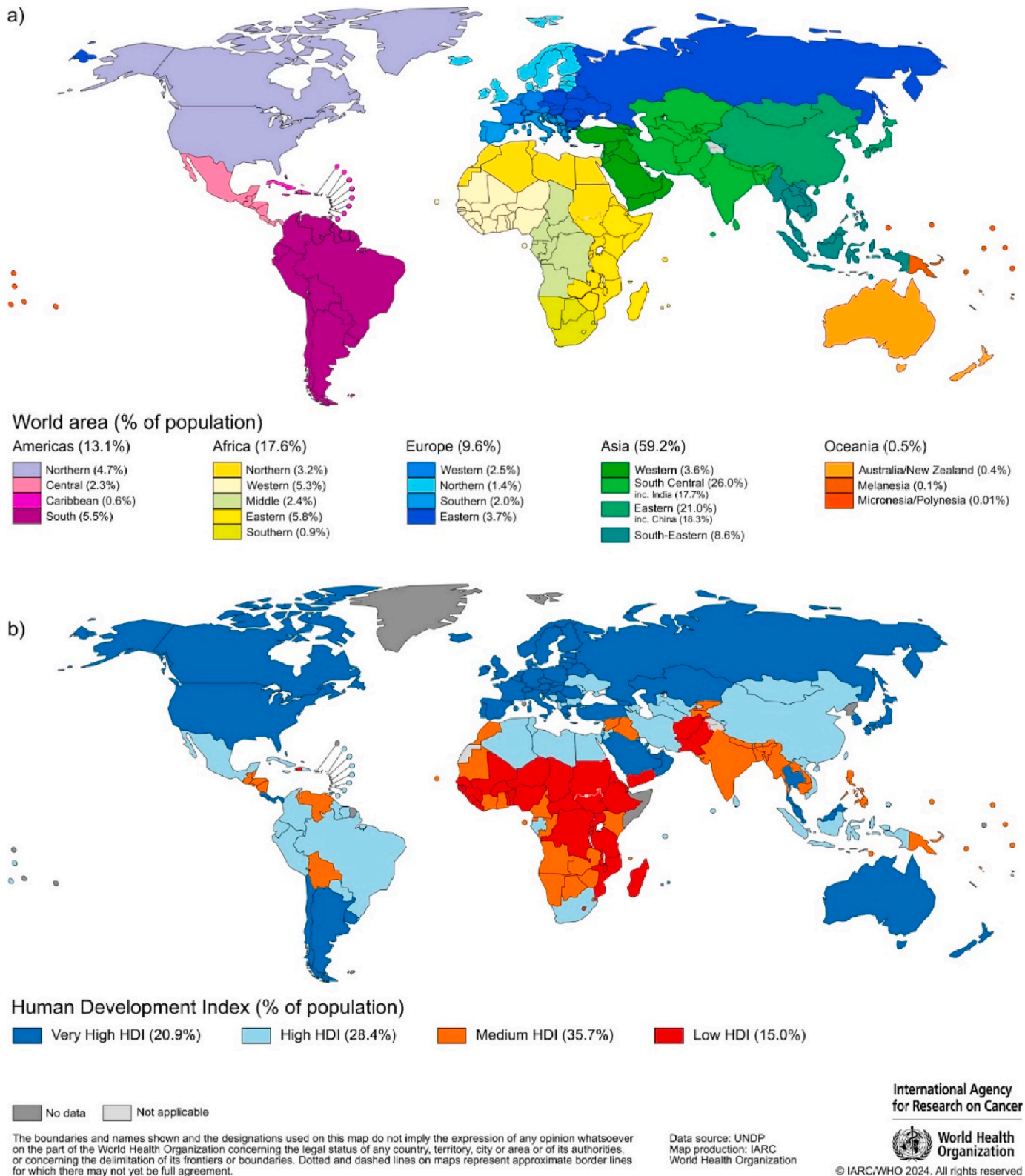


Fig. 2. Global maps display (A) the 20 regions of the world and (B) the Human Development Index (HDI) categorized into four tiers. The legend includes the population sizes for each respective area [9].

- mortality rate associated with cancer, as metastases are the main cause of death in cancer patients.
- Cancer cells employ tactics that bypass the immune system, thereby preventing their detection and elimination by immune cells.
- Cancer cells demonstrate elevated levels of genetic abnormalities, leading to increased proliferation and adaptability. This provides them with a selective advantage within the tumour microenvironment.

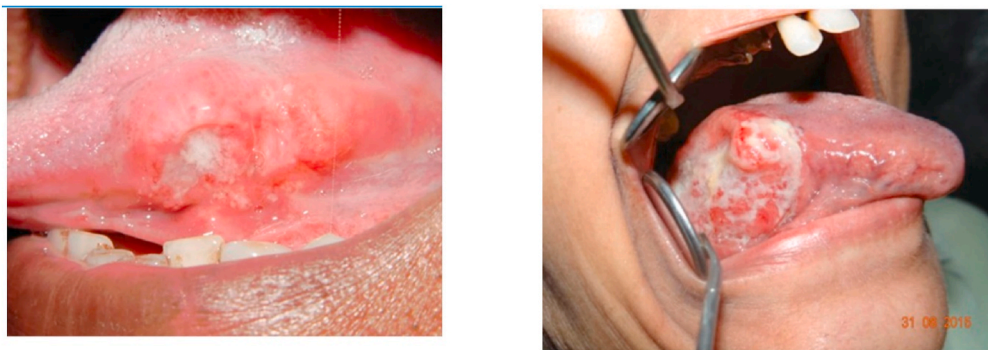


Fig. 3. Oral cancer (OSCC of the tongue) [12].

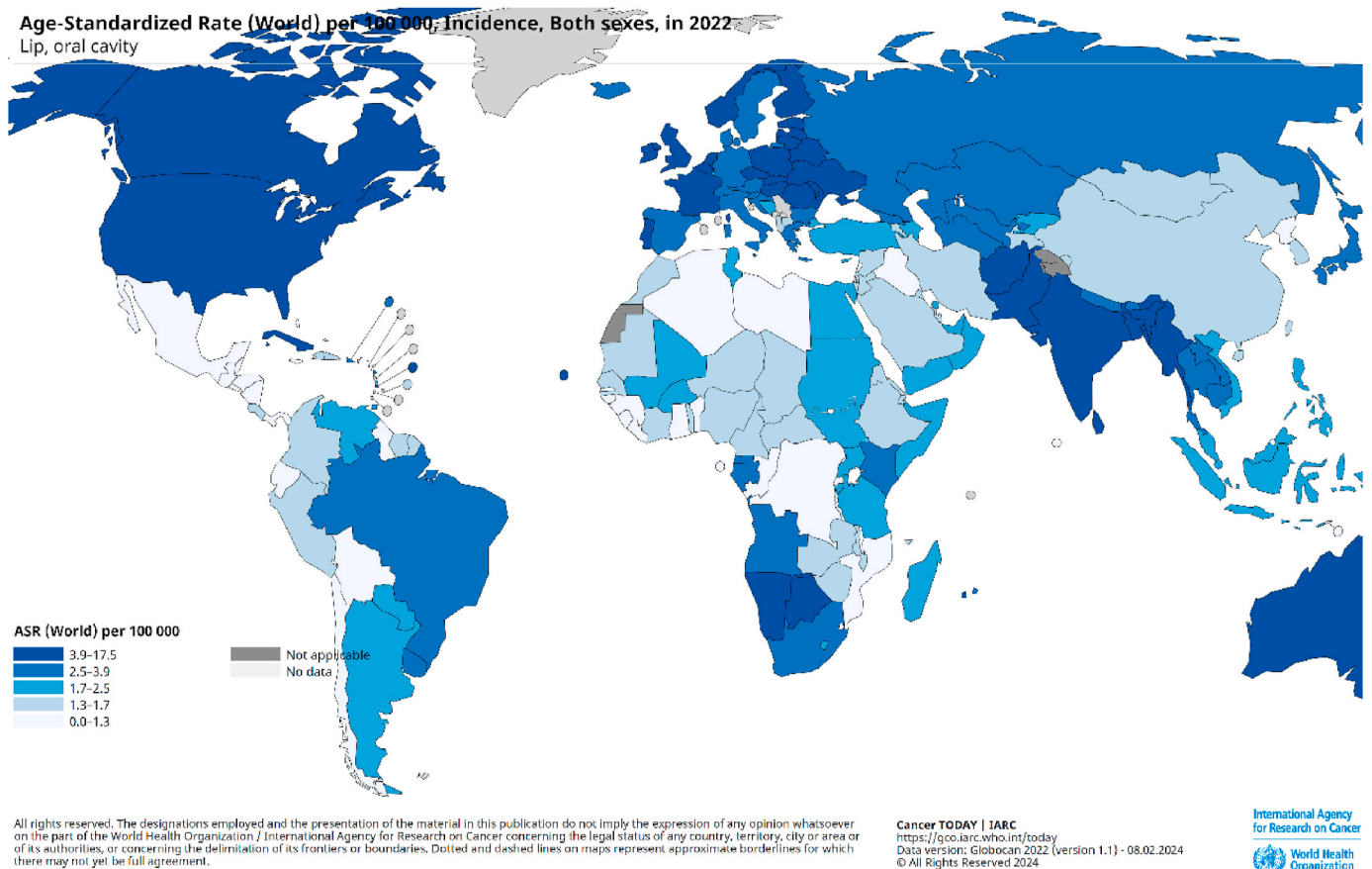


Fig. 4. Global distribution of lip and oral cavity cancer by [14].

- Tumor-promoting inflammation refers to the persistent inflammation that occurs in the microenvironment of a tumour. This inflammation has a role in the development and advancement of cancer by producing bioactive chemicals that stimulate cell growth, the formation of new blood vessels, and the spread of cancer cells to other parts of the body [10].

2.2. Oral squamous cell carcinoma (OSCC)

OSCC which arises in the oral mucosa, is a prevalent form of head and neck cancer that leads to disfigurement and functional impairments, such as difficulties in swallowing, speech, and taste, significantly affecting patients' quality of life (Fig. 3) [11].

2.3. Global distribution of lip and oral cavity cancer

The 6th most common cancer worldwide is oral cancer with nearly 355,000 new diagnoses and 177,000 death yearly. Out of which OSCC accounts for 90 % of all oral cancers [13]. An analysis of the age-standardized incidence rates for lip and oral cavity cancer in 2022, retrieved from the Global Cancer Observatory (GCO) [14], reveals significant geographical variations. Fig. 4 depicts high rates in South Central Asia, Melanesia, and Eastern Europe. Conversely, lower rates are observed in Northern America, Northern Europe, and Western Europe. These disparities underscore the importance of geographically targeted interventions and public health initiatives to effectively address the global burden of lip and oral cavity cancer.

OSCC develops in a similar way to other cancers, with several genetic and epigenetic alterations occurring in a step-by-step process. The

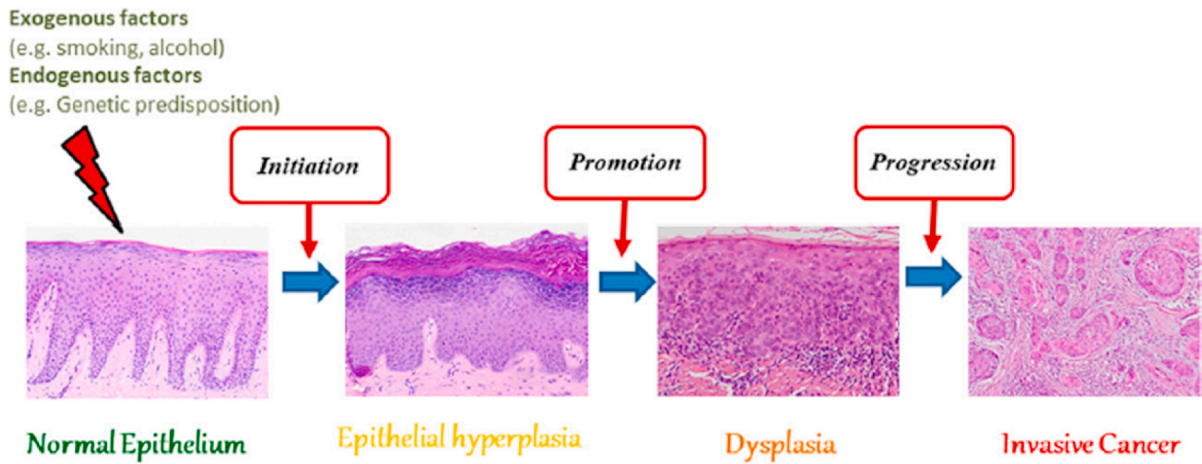


Fig. 5. Normal epithelial cells evolve into precancerous tissue and eventually develop into malignant tumors [15].

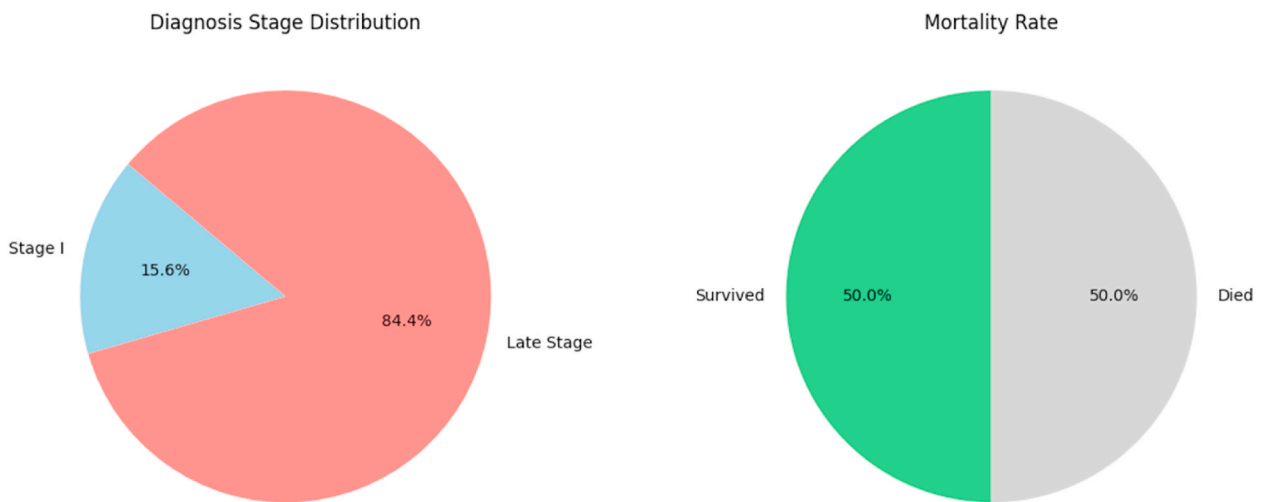


Fig. 6. Diagnosis Stage and Mortality Rate: 84.4 % of patients were diagnosed at a late stage, while 15.6 % were diagnosed at stage I. The mortality rate is evenly split, with 50.0 % surviving and 50.0 % dying [11].

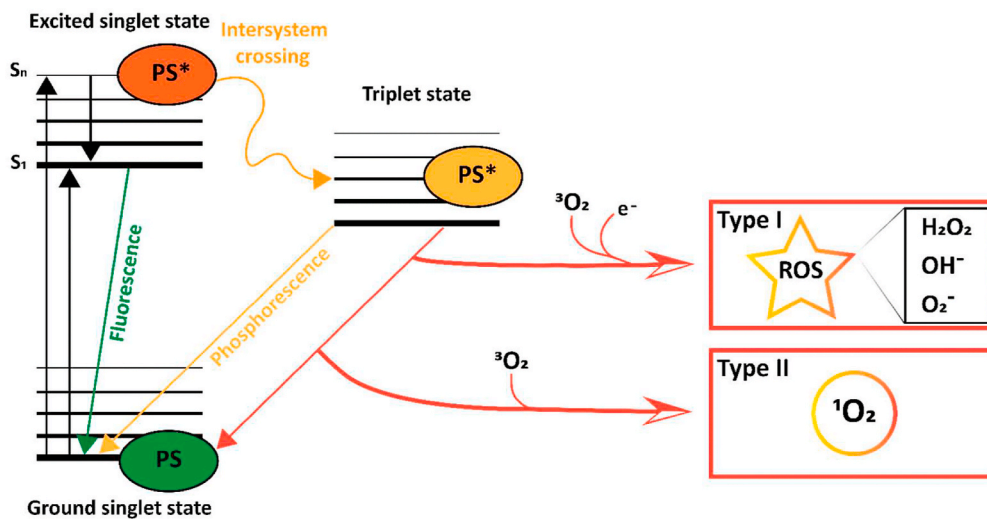


Fig. 7. Principle of Photodynamic Therapy: A photosensitizer is activated by light, and produces reactive oxygen and ROS to destroy the cancer cells [59].

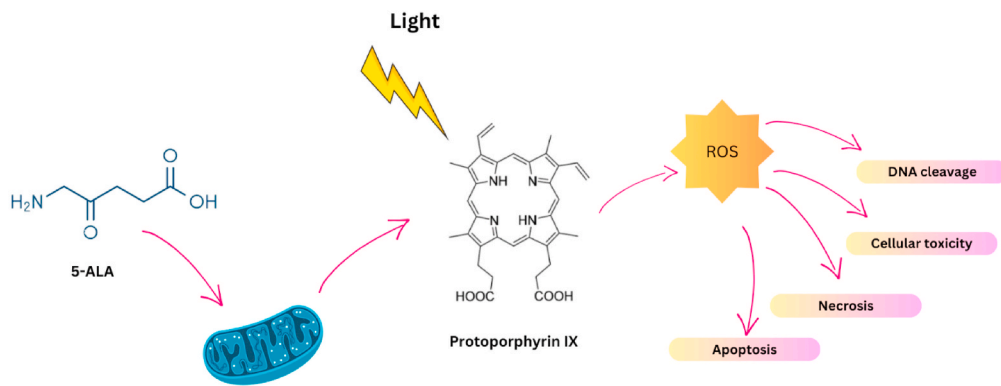


Fig. 8. Mechanism of 5-ALA-induced cell death.

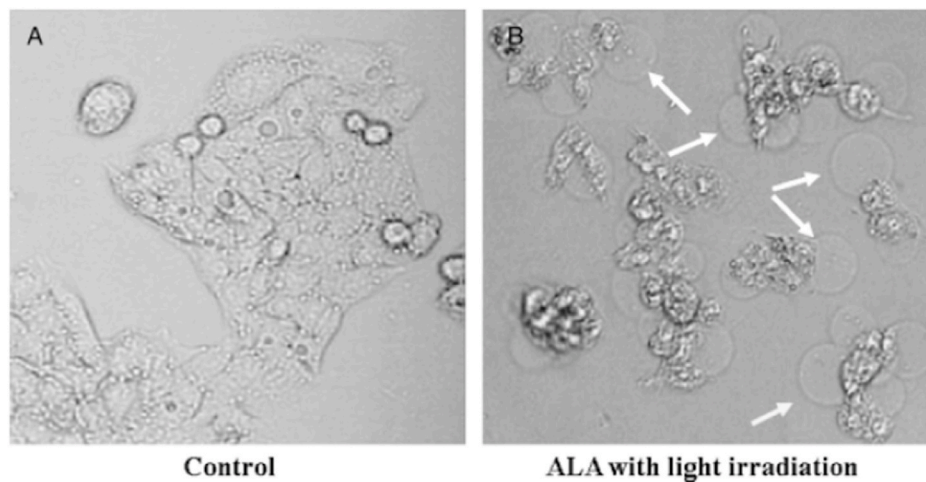


Fig. 9. Cell morphology post-photodynamic therapy with ALA at 4 hours [24].

Table 1
PDT of OSCC —preclinical studies in vitro by [25].

Cell line(s)	Photosensitizer(s)	Radiation
KB	hematoporphyrin	635 nm and 5 mW/cm ²
Tca8113	Hematoporphyrin monomethyl ether 1.25, 2.5, and 5 µg/ml	530 ± 20 nm, 0.6–3.0 J/cm ²
SCC-25	porfimer sodium 15 µg/ml	Visible light
SAS and GNM	5-Aminolevulinic acid	635 ± 5 nm at various doses
NT8e and 4451	5-Aminolevulinic acid 200 mg/ml	630 ± 20 nm, 7 mW/m ² , 8.4 kJ/m ²
Ca9-22	5-aminolevulinic acid 1 mM	580–720 nm, 100 mW/cm ² , 0–8 J/cm ²
Ca9-22, KJ-1	5-aminolevulinic acid 1 mM	633 nm
SCC4, SAS	5-aminolevulinic acid 0–0.375 mM	640 nm, 10 J/cm ²
SCC4	5-aminolevulinic acid 0–0.375 mM	640 nm, 10 J/cm ²
HSC-4	5-aminolevulinic acid 0–2 mM	600–700 nm, 9.6 J/cm ²
Ca9-22	5-aminolevulinic acid 1 mM	635 ± 5 nm, 4 J/cm ²
YD10B	hexenyl ester of ALA 5 mM	613–645 nm (peak 635 nm), 5 J/cm ² , 35 mW/cm ²
DOK, Ca9-22	methyl aminolevulinic acid 7.5 mM	Red light, 6 J/cm ² , 200 mW/cm ²
HSC-2, HSC-4	5-aminolevulinic acid 1 mM/L	630 nm, 70 mW/cm ² , 20–120 J/cm ²

development of oral cancer typically involves a steady accumulation of molecular irregularities, regardless of external influences like smoking, alcohol intake and certain types of HPV infections, as well as internal factors such as genetic predisposition and rare ailments such as Fanconi

anemia. These concerns involve genetic changes, anomalies in chromosomes and shifts in epigenetic profiles. Together, they trigger the transformation of normal epithelial cells into precancerous tissue and eventually into malignant tumors [15] Fig. 5.

2.3.1. Treatment option

Surgical excision is still considered with or without adjuvant therapy (e.g. radiotherapy or chemoradiotherapy) the most effective method for treating localized cutaneous squamous cell carcinoma (CSCC) [16]. There are several procedures to achieve this, such as excisional surgery, cryosurgery, curettage and desiccation, Mohs micrographic surgery, and micrographic surgery. Nevertheless, these methods are linked to several adverse effects, including large-scale facial defects and scar formation, radioactive bone necrosis, trismus, dysphagia, erythema or ulcerative mucosa, facial nerve palsy, and other complications [13]. Moreover, the management of widespread locally destructive or metastatic disease continues to be difficult, and the therapies available are seldom effective in curing the disease. Administering radiation therapy is the primary non-surgical intervention for malignancies located on the lip and eyelids. However, radiation therapy frequently leads to serious side effects such as nausea, vomiting, skin redness, thinning of the skin's outer layer, dilatation of small blood vessels, tissue death, and the emergence of secondary cancer induced by radiation. These adverse effects may provide a particular challenge for certain persons to tolerate. xerostomia, dermal necrosis, dysphagia, auditory impairment, and salivary gland dysfunction [17,18].

Although there are various treatment options available, the overall 5-year survival percentage for people diagnosed with OSCC is around

Table 2
Summary of studies investigating the effects of 5-ALA on various cancer types beyond oral cancer.

Photosensitizer	Combination therapy	Cancer cells used	findings	Suggestions	Reference
5-ALA	Cisplatin	Triple-negative breast cancer (TNBC) cells.	This combination induces apoptosis through mitochondrial damage, with simultaneous treatment proving more effective than sequential protocols. Furthermore, this strategy allows for reduced dosages of both cisplatin and 5-ALA, minimizing their respective side effects. However, this combination has shown toxicity in non-cancerous cells like MCF-10A	Need for advanced nanoparticle-based drug delivery systems. Such systems could enhance the therapeutic efficiency of the treatment while reducing off-target toxicity, improving selectivity for cancer cells	[26]
	Nil	Human hepatocellular carcinoma cell line (HepG2)	Research shows that 5-ALA-mediated PDT effectively induces apoptosis in HepG2 cells. After 18 h of incubation, ALA/PpIX was mainly localized in the cytoplasm, leading to 80 % cell death at a 2 mM drug dose and 2 J/cm ² light intensity	There is a call for more accurate diagnostic tools to better detect the early stages of liver disease. Advanced imaging techniques and biomarkers are suggested as potential areas for improvement.	[24]
	Nil	tongue tumor tissue	oral administration of 5-ALA at a concentration of 250–500 mg/kg achieved the highest accumulation of PpIX in tumor tissue, with peak fluorescence observed 5 h post-administration.	A key limitation of PDT lies in the effective absorption of photosensitizing (PS) drugs within tumors, particularly in internal organs. For instance, in gastric cancer, 5-ALA-based photodiagnosis (PDD) is often preferred over PDT for assessing surgical resection margins due to this issue. To overcome these limitations, future studies should prioritize the development of next-generation PS drugs that offer improved physical, chemical, and therapeutic properties, enabling more effective and targeted therapies	[23]

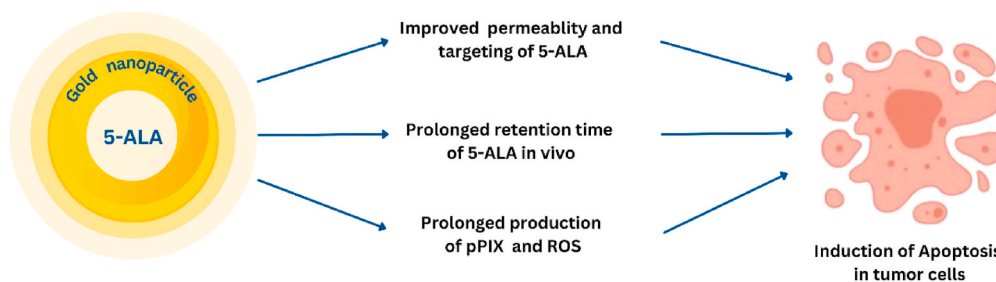


Fig. 10. 5-ALA incorporated in Gold-nanocarrier system to induce apoptosis.

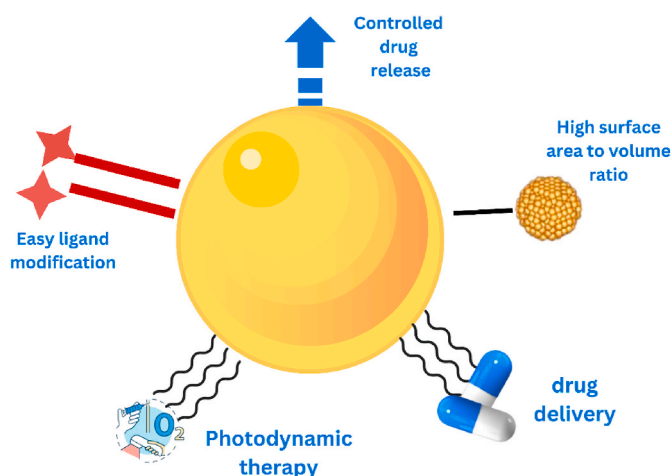


Fig. 11. Inherent properties of AuNPs.

fifty percent [19] (Fig. 6). Loco-regional recurrence is the primary reason for treatment failure in OSCC patients, affecting approximately 35 % of them and greatly reducing their chances of survival [11]. The field of oral oncology is seeing an increasing need for diagnostic and treatment methods that are less intrusive, can detect cancer at an early

stage, and are cost-effective. Nanomaterial-based technologies provide significant promise for both the diagnosis and treatment of cancer. Advances in nanotechnology have contributed significantly to the development of new techniques for quickly diagnosing and precisely treating different types of malignancies, such as oral cancer [13]. For instance, PDT is a highly successful and noninvasive form of treatment. This method utilizes a light-sensitive medication that, when activated by a particular wavelength of light, releases reactive oxygen species (ROS) that can cause cell death, thereby preventing the proliferation of cancer cells [20].

2.4. Photodynamic therapy for OSCC

PDT has been in existence for over a century and is known for its minimal invasiveness, low systemic toxicity, and insignificant medication resistance. Multiple clinical trials have demonstrated that PDT effectively preserves the oral anatomy, function, and appearance of individuals with OSCC. Additionally, PDT minimizes adverse effects without causing any long-lasting or widespread harm. Following numerous rounds of PDT treatment, OSCC patients experienced a significant decrease in morbidity compared to the group receiving conventional therapies [19].

PDT is a clinically verified, noninvasive procedure that effectively treats cancer and other malignant disorders. PDT provides several benefits, including little invasiveness, improved cosmetic results, low morbidity, and minimal functional disruptions. It is well tolerated and

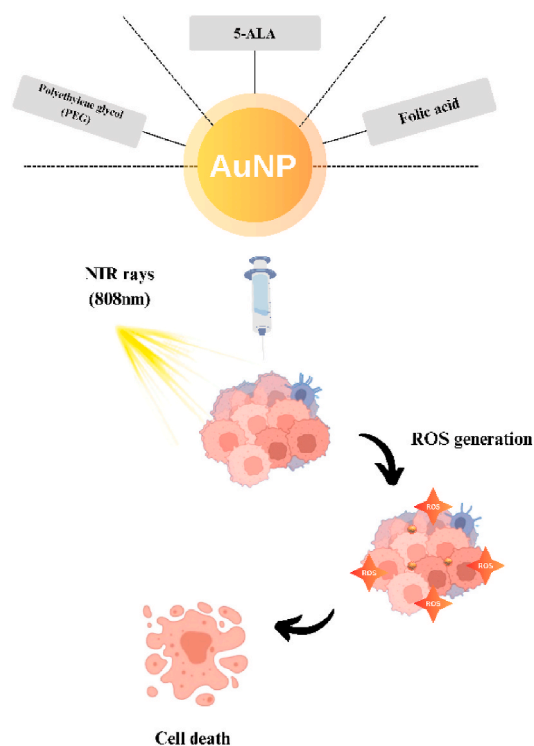


Fig. 12. 5-ALA-conjugated gold nanoparticles (AuNPs) for targeted photodynamic therapy.

can be repeatedly administered at the same location. Therefore, PDT is extensively utilized in the management of superficial skin malignancies [17,21]. PDT utilizes PSs administered systemically or locally with the subsequent use of light at the appropriate intensity and wavelength. As a result, reactive oxygen species (ROS) are formed leading to the destruction of the tumor by either cell necrosis or apoptosis.

2.4.1. Principle

PDT works through a photochemical reaction involving a PS, oxygen, and light. Here's a simpler breakdown.

- **Photosensitizer Activation:** A PS is introduced and accumulates in cancer cells. When exposed to specific light wavelengths, the PS absorbs the light energy and becomes excited.
- **Energy Transition:** The PS undergoes a shift from its lowest energy level to a higher energy state. The molecule rapidly transitions to the lowest excited singlet state, remaining there temporarily before returning to the ground state. During this process, it emits light (fluorescence) or undergoes intersystem crossing to a triplet state.
- **Triplet State (T1):** The PS in the triplet state has a longer lifespan, which increases the chances of producing reactive oxygen species (ROS).
- **ROS Production:** There are two main mechanisms:

1. The PS transfers energy to nearby molecules, forming free radicals and ROS like superoxide anion ($O_2^{\bullet-}$), leading to oxidative stress and tumor cell destruction.
2. The PS transfers energy directly to oxygen molecules, creating singlet oxygen with strong oxidative properties, which also kills cancer cells [59] Fig. 7.

2.4.2. Photosensitizer

PSs, crucial for PDT, have a tendency to selectively accumulate in tumour tissues rather than normal tissues. While the precise mechanisms responsible for this specific localization are not well comprehended, various elements have been proposed to play a role. The causes contributing to this phenomenon encompass the existence of permeable blood vessels, reduced pH levels, a high level of low-density lipoproteins, and limited removal of lymphatic fluid inside the localised tumour environment [22].

2.4.2.1. 5-aminolevulinic acid (5-ALA). 5-ALA offers advantages over other PSs in PDT. It causes minimal harm to healthy cells, is quickly eliminated from the body and efficiently converts to Protoporphyrin IX (PpIX). In cancer cells, the enzyme ferrochelatase becomes inactive due to the Warburg effect, leading to an accumulation of PpIX. This accumulation triggers photochemical reactions that increase the levels of singlet oxygen and superoxide within the cell, thereby enhancing the effectiveness of PDT as a treatment option [23] Fig. 8. Post-PDT with ALA at 4 Hours. Conducted by the [24], indicates cell shrinkage and membrane blebbing (indicated by arrows) see Fig. 8. These findings support the safety and efficacy of ALA in cancer therapy (Fig. 9).

The following Table 1 adapted from the study conducted by [25] summarizes various PDT studies on different oral cancer cell lines, highlighting the specific PSs and irradiation conditions used while Table 2 presents a summary of studies examining the effects of 5-ALA on different cancer types beyond oral cancer. The table includes the observed outcomes, specific findings, and suggestions for future research.

However, the effectiveness of 5-ALA in PDT is limited due to its water loving properties and the challenges in transporting it efficiently through cell membranes. Additionally, 5-ALA faces stability issues under certain biological conditions that reduce it. In alkaline environments, 5-ALA loses a proton and becomes negatively charged, making it polar and difficult to pass through cell membranes effectively. To overcome these obstacles, it is crucial to develop methods that improve the delivery and stability of 5-ALA for optimal clinical applications [27]. To address this limitation, several studies have demonstrated that 5-ALA-AuNP conjugates significantly enhance cell-killing efficiency compared to 5-ALA alone. The in vitro PDT efficacy of 5-ALA can be improved by conjugating it with AuNPs, which facilitates greater delivery of 5-ALA into cells [28] Fig. 10.

3. The Role of Nanotechnology in PDT

Nanotechnology has revolutionized cancer therapy. Nanotechnology is an interdisciplinary discipline that combines biology, physics, chemistry, optics, digital analysis, and materials science. It focuses on the

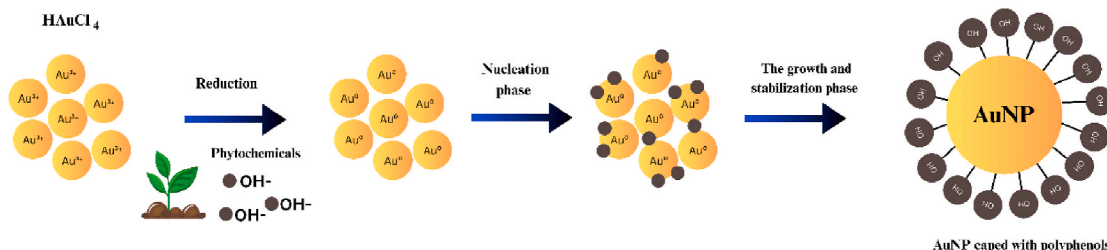


Fig. 13. General steps of AuNP synthesis by the green method.

Table 3

Previous studies have shown the anticancer activity of green-synthesized AuNP.

Plant source used	Shape	Size (nm)	Cell line used	Anti cancerous activity	Invitro assay used	References
<i>Brazilian Red Propolis</i>	Spherical	8–15	Bladder (T24) and prostate (PC-3) cancer cell line	The biogenic AuNPs showed dose-dependent cytotoxic effects. However, they were generally less toxic than the original extracts from which they were made. They still hold promise due to their potential for targeted delivery to cancer cells.	Resazurin assay	[44]
<i>Butea monosperma</i>	Spherical, rod, triangular, hexagonal	30	Normal endothelial cells (HUVEC, ECV 304) and cancer cell lines (B16F10, MCF-7, HNGC2 and A549)	These biosynthesized AuNPs were compared with chemically synthesized (c-AuNP). Interestingly, the biosynthesized AuNP did not exhibit significant cytotoxicity on normal cells. The BM leaf extract used in their synthesis showed slight cytotoxic effects, likely due to the presence of active anticancer biomolecules. Overall, the BM leaf extract proved to be an effective and eco-friendly medium for producing stable, biocompatible AuNPs.	MTT assay	[45]
<i>orchid</i>	Spherical	14–50	Breast cancer (AMJ 13) cell lines	The results show that gold nanoparticles (AuNPs) effectively inhibit the growth of AMJ-13 in a dose-dependent manner. Significant changes in cell morphology, including signs of apoptosis, were observed after 72 h of treatment.	MTT assay	[46]
<i>Argemone mexicanaL.</i>	Hexagonal	20–40	Human colon cancer cell line, HCT-15	The cytotoxicity of biosynthesized AuNPs showing significant growth inhibition in a dose- and time-dependent manner. At a concentration of 50 µg/mL, AuNPs inhibited 90.10 % of cell growth after 24 h and 96.17 % after 48 h. The treatment also caused noticeable DNA fragmentation and increased caspase-3 activity, indicating apoptosis.	MTT assay	[47]
<i>Trachyspermum ammi seed extract</i>	Spherical	16.63	HepG2 cancer cell line	The results demonstrated significant inhibition of cell proliferation, indicating that TA-AuNPs effectively interfere with cell cycle regulation and tumor progression. The increased concentration of TA-AuNPs led to a more pronounced reduction in cell viability, confirming its potential as an effective anti-cancer agent.	MTT assay	[48]
<i>Nigella sativa leaf extract</i>	Anisotropic	13–78	Hep-G2 liver cancer cell line	The results indicated that higher concentrations of gold nanoparticles led to increased cell death, with the most significant effect observed at 100 µg/ml, followed by 75 µg/ml, 50 µg/ml, and 25 µg/ml. The mechanism behind this includes the generation of reactive oxygen species (ROS) and the subsequent induction of apoptosis.	MTT assay	[49]
<i>walnut green Shell extract</i>	Spherical, triangular	10–50	MCF7 cells	The results indicated a significant decrease in cell viability in a dose-dependent manner ($p < 0.05$). The IC50 value for GNPs against MCF7 cells after 24 h was found to be 3 mM ($p \leq 0.05$).	MTT assay	[50]
<i>Crassocephalum rubens Leaf extract</i>	Spherical	20 ± 5	MCF-7 and Caco-2 cells	B-AuNPs at 125 and 250 µg/mL reduced cell viability to below 50 %, significantly compared to the vehicle control ($p < 0.05$). MCF-7 cells showed apoptotic features like cell shrinkage and membrane blebbing, while Caco-2 cells rounded up and detached. Morphological changes at 250 µg/mL B-AuNPs were similar to those with positive controls.	MTT assay	[51]
<i>Petroselinum crispum Leaf extract</i>	Spherical, semi-rod aggregates, flower-shaped nanoparticles	20–80	Human cancerous colorectal cell line	The results show a decrease in cell viability with increasing sample concentration. AuNP exhibited the highest cytotoxicity, likely due to its smaller size and greater surface area, enhancing its anticancer effect.	MTT assay	[52]

design, production, characterization, modification, and application of nanoparticles (NPs). Nanotechnology has made major strides in the field of medicine, particularly in the areas of diagnosis, medication design, drug-delivery systems, and treatment of diseases such as cancer. These achievements can be attributed to the distinctive characteristics of nanoparticles [29]. Nanotechnology has helped overcome the limitations of traditional PSs in PDT, allowing for more precise tumor targeting with minimal harm to healthy tissues.

3.1. Metal and metal oxide nanoparticles

The potential of metal and metal oxide nanoparticles (MON) in PDT

is great due to its $^1\text{O}_2$ production capabilities with potential applications in tumor inhibition. Both metal and MON exhibit effective production of $^1\text{O}_2$ that could make these materials useful in inhibiting tumor growth. Similarly, these were found to be efficient in PDT for oral cancer treatment [30]. Among other metals and MON, AuNPs used for over fifty years in various medical applications and have shown remarkable effectiveness in drug delivery and PDT [31]. Despite the fact that AuNPs are robust, they exhibit excellent therapeutic activity in many biomedical areas including drug delivery and PDT [32]. AuNPs provide notable benefits because of their stability, biocompatibility, high surface area to volume ratio and non-toxic nature that make them well-suited for PDT [33] Fig. 11. AuNPs possess strong plasmonic properties, rendering them

Table 4

Previous studies show that 5-ALA-AuNPs enhance PDT efficacy against cancer cells.

Nanocomposite	Cell line used	Findings	references
5-ALA@AuNP	HaCat (normal human keratinocytes) and A431 (human skin cancer cells).	When 5-ALA was conjugated with gold nanoparticles (forming 5-ALA-GNPs), the treatment enhanced the effects of PDT even further in A431 cells. This combination resulted in greater suppression of cell viability, higher levels of apoptosis, and increased generation of singlet oxygen compared to treatment with 5-ALA alone. Essentially, the addition of gold nanoparticles made the PDT more effective against the cancer cells.	[17]
	Skin melanoma (B16F10) and A431 cells	The results showed that pure gold nanoparticles were more cytotoxic to cells than 5-ALA or the gold-5-ALA conjugate. However, upon irradiation, the gold-5-ALA conjugate exhibited significantly higher cytotoxicity than either 5-ALA or pure gold nanoparticles, with cytotoxicity levels doubling, indicative of increased reactive oxygen species production.	[53]
	Fibrosarcoma tumor cells	The study found that 5-ALA-conjugated AuNPs significantly enhanced PDT by selectively accumulating in tumor cells, producing higher reactive oxygen species, and resulting in 50 % more cytotoxicity compared to 5-ALA alone. This method effectively targeted tumor cells with minimal damage to healthy fibroblasts, offering a promising approach for more efficient and selective cancer treatment.	[54]
	K562 (Human leukemia cell line)	The study demonstrated that 5-ALA-conjugated gold nanoparticles (5-ALA-GNPs) significantly enhance the efficiency of PDT by improving the delivery of 5-ALA to tumor cells and boosting the generation of reactive oxygen species (ROS). This enhanced delivery leads to higher levels of PpIX in the cells, which is the active photosensitizer in PDT. The study found that 5-ALA-GNPs generated	[28]

Table 4 (continued)

Nanocomposite	Cell line used	Findings	references
		twice as much PpIX in K562 cells compared to 5-ALA alone, leading to significantly increased cell-killing efficiency. GNPs playing a critical role in delivering 5-ALA more effectively to tumor cells.	
	Human cervical cancer cell line	Findings indicate that AuNPs enhance the effects of ALA by increasing cell damage, promoting apoptosis, and raising oxidative stress. This synergy enables effective cancer cell destruction with lower concentrations of ALA, which helps minimize its adverse effects. ALA–AuNPs show potential as innovative therapeutic options. However, further research is required to thoroughly investigate the chemical and biological interactions between ALA and AuNPs.	[55]
	Hela cells	The study demonstrated that 5-ALA conjugated with AuNP enhances bioavailability and allows precise control of ALA release using a 532 nm pulse laser. This controlled release improves the effectiveness of ALA, PDT in HeLa cells, suggesting a new approach for online therapy and personalized theragnostic.	[56]

well-suited for photothermal therapy (PTT) by producing substantial heat upon exposure to near-infrared (NIR) light, so effectively eradicating cancer cells. By complementing the photosensitizer 5-ALA, AuNPs greatly augment the generation of reactive oxygen species (ROS) when exposed to light, therefore enhancing the efficacy of PDT. Furthermore, AuNPs can function as nanocarriers, surpassing the constraints of 5-ALA when employed in isolation in PDT [34]. A study conducted by Kumar and colleagues in 2024 shown that 5-ALA conjugated AuNPs successfully suppressed the proliferation of oral cancer cells in a laboratory setting [35].

AuNPs can be synthesized using many techniques including physical, chemical, and biological processes. Despite their high cost and complexity, Physical methods provide great degrees of purity. Chemical technologies, although economical, provide health and environmental hazards including toxic chemicals and byproducts, rendering them less appropriate for medicinal applications. Therefore, there is an increasing fascination with ecologically sustainable biological methods called green synthesis for the production of AuNPs. These techniques employ natural raw materials such as plant extracts to produce nanoparticles without producing toxic waste, therefore improving safety and sustainability for biomedical uses [36]. Tetra-chloro-auroic acid (HAuCl4) is often utilized as a precursor in the environmentally friendly production of AuNP. Certain compounds employed in the environmentally friendly

synthesis of AuNP also function as stabilizers, inhibiting nanoparticle aggregation and preserving the colloidal system's stability. Utilized stabilizers include starch, chitosan, and polyethylene glycol (PEG). The synthesis and stability of metallic nanoparticles produced by biological methods are influenced by several factors. These parameters encompass pH values, solvent concentrations, reaction time, and temperature. The dimensions and morphology of the resultant nanoparticles can be affected by these factors, frequently manifested by a modification in the color of the solution [37]. The folate receptor (FR) (α) has been a primary focus of research for its potential as a target for cancer treatment. The overexpression of FR has been observed in various types of cancer, including clinical samples from patients with OSCC at different stages [38]. The incorporation of folic acid and PEG into AuNPs allows these nanoparticles to bypass the immune system and specifically target overexpressed FR on cancer cells [35]. Implementing this focused strategy not only enhances the results of treatment but also tackles issues associated with drug resistance and bioavailability. In summary, these results emphasize the considerable capacity of AuNPs to improve PDT for oral cancer, representing notable progress in precision medicine [39] Fig. 12.

4. Green synthesis

Concerns about energy consumption, the emission of hazardous chemicals, and the complex equipment required in conventional procedures have led to the growing popularity of the green approach. An emerging method for green synthesis involves the use of plant or fruit extracts abundant in polyphenols. Polyphenols, characterized by the presence of many phenol groups within each molecule, exhibit effective reducing properties in the synthesis of nanoparticles and also serve to inhibit the generation of detrimental byproducts [40]. To isolate polyphenols from plants, one can employ polar solvents such as alcohol or water. The properties of the nanoparticles generated are influenced by several parameters including the particular plant extract employed, the quantities of both the extract and metal salts, pH levels, temperature, reaction duration, and mixing velocity [41]. The green synthesis approach provides a direct, economical, and replicable technique for manufacturing nanoparticles with customized characteristics [42].

4.1. General overview of AuNP synthesis by green approach

The production of AuNPs through plant extracts involves a three-phase synthesis process: reduction, nucleation, and growth/stabilization. In the reduction phase, gold ions (Au^{3+}) from HAuCl_4 are reduced to gold atoms (Au^0) by bioactive compounds in the plant extracts, which serve as reducing agents. This initial reduction is crucial for initiating nanoparticle formation. Following this, the nucleation phase occurs, where the gold atoms aggregate into small clusters, forming nuclei that dictate the size and uniformity of the nanoparticles. The growth and stabilization phase involves the deposition of additional gold atoms onto these nuclei, influenced by the balance of reducing and capping agents present in the extract, which prevent aggregation and define the final size and shape of the AuNPs Fig. 13. This environmentally friendly biosynthesis method offers advantages over traditional chemical processes, enhancing the biocompatibility and stability of the nanoparticles [43].

5. Conclusion and future directions

The reviewed literature in Tables 3 and 4 underscores the significant potential of AuNPs in cancer therapy. These traditional chemical synthesis methods have demonstrated promising results, however, these methods often involve the use of hazardous chemicals that can pose risks to both human health and the environment, leading to concerns about their long-term safety and biocompatibility. While the potential of green synthesis approaches, as evidenced by [57] offers superior outcomes in

terms of safety, biocompatibility, and efficacy. The study reveals that green synthesized 5-ALA-AuNPs achieve higher ROS production and cytotoxicity in cancer cells, positioning green synthesis as a promising, yet underutilized, method in nanomedicine.

Despite its benefits, green synthesis remains less common, likely due to challenges such as reproducibility and scalability. Therefore, future research should focus on optimizing green synthesis techniques to address these limitations. By prioritizing green methods over conventional chemical synthesis, it is possible to achieve reproducible, safe, and environmentally friendly approaches for the treatment of oral cancer and potentially other malignancies. This shift in focus could pave the way for more sustainable and effective cancer therapies, aligning with the growing demand for eco-friendly medical solutions.

CRedit authorship contribution statement

Romesa Soomro: Conceptualization, Writing – Original Draft, Investigation. Romesa led the conceptualization of the research, conducted the investigation, and was the primary writer of the original draft. Che Azurahanim Che Abdullah: Supervision, Conceptualization, Writing – Review & Editing. Che Azurahanim provided guidance, shaped the conceptual framework, and reviewed and edited the manuscript. Mohamed Abdelmonem: Visualization. Mohamed contributed to the graphical design of the paper. Bachren Azra Saputra: Data Curation. Bachren was responsible for gathering and organizing data specially oral cancer.

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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