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Targeting Cancer Stem Cells Using Nanoparticles: A Review of Emerging Diagnostic and Therapeutic Strategies

Dr. Gayathri M[1], * Dr. Amuthan S[2], Dr. Narendiran N[3], Dr. Thirupurasundari N[4], Dr. Arthi Ramalingam[5], Dr. Keerthana Chandran[6]

1Junior Resident, Department Of Oral Medicine and Radiology, Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry.

2Senior Lecturer, Department Of Prosthodontics, Sri Venkateshwaraa Dental College, Affiliated To Pondicherry University, Ariyur, Puducherry.

3Senior Lecturer, Periodontology and oral Implantology, Sri venkateshwaraa dental college, Affiliated To Pondicherry University, Ariyur, Puducherry.

4Senior lecturer, Department of Prosthodontics, Indira Gandhi institute of dental sciences, Sri Balaji Vidyapeeth University, pillayarkuppam, Puducherry.

5Assistant professor, Prosthodontics and crown and bridge, Meenakshi Ammal dental college, maduravoyal, chennai.

6Assistant Professor, Conservative Dentistry and Endodontics, Dhanalakshmi Srinivasan dental college, Sirivachur Road, Perambalur.

Corresponding Author: Dr. S. Amuthan[2], Senior Lecturer, Department Of Prosthodontics, Sri Venkateshwaraa Dental College, Affiliated To Pondicherry University, Ariyur, Puducherry

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ABSTRACT:

Cancer stem cells (CSCs) constitute a small but pivotal subset of tumor cells implicated in cancer initiation, progression, therapeutic resistance, and relapse. These cells possess distinctive properties such as self-renewal, multilineage differentiation, and elevated expression of drug efflux transporters, which collectively contribute to their resistance against conventional therapies. Advances in stem cell biology including research on dental mesenchymal stem cells have elucidated the regulatory roles of key signaling pathways such as Wnt/β-catenin, Notch, and Hedgehog in maintaining CSC stemness and plasticity. In parallel, nanotechnology has emerged as a powerful tool for addressing CSC-associated challenges. Engineered nanoparticles offer targeted delivery through CSC-specific surface marker recognition, pathway inhibition, and induction of differentiation. Moreover, their ability to enhance imaging and combine therapeutic and diagnostic functions within a single platform (theranostics) has opened new avenues for personalized cancer care. This review critically examines the molecular characteristics of CSCs, outlines therapeutic vulnerabilities, and highlights recent advancements in nanoparticle-based strategies aimed at improving the diagnosis and eradication of CSCs particularly in oral and head and neck cancers.

1. Introduction

Cancer continues to be a major global health burden, with over 10 million deaths reported annually. Despite advances in early detection, surgical resection, radiotherapy, and systemic chemotherapy, long-term survival rates for many malignancies remain unsatisfactory largely due to tumor recurrence, metastasis, and resistance to conventional therapies. These clinical challenges are increasingly being attributed not merely to the genetic heterogeneity of cancer cells, but to the presence of a distinct subpopulation known as **cancer stem cells (CSCs)**. First discovered in acute myeloid leukemia and later identified in various solid tumors including breast, brain, colon, pancreatic, and notably **head and neck squamous cell carcinomas (HNSCC)** CSCs exhibit stem cell like properties such as self-renewal, multilineage differentiation, and tumor-initiating capacity.[1]

Unlike the bulk of rapidly dividing tumor cells, CSCs are inherently more resilient. They are capable of entering a

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quiescent state, possess efficient DNA repair mechanisms, overexpress ATP-binding cassette (ABC) drug efflux transporters such as ABCG2, and show elevated anti-apoptotic signaling. These adaptations enable CSCs to survive genotoxic insults and chemotherapeutic agents, contributing to **minimal residual disease (MRD)**, treatment failure, and disease relapse. Their ability to initiate tumor formation, repopulate tumors after therapy, and drive distant metastasis makes CSCs a clinically significant target that is not adequately addressed by existing treatment regimens.[2,3]

The identification and characterization of CSCs have been greatly aided by stem cell biology, particularly through research involving **mesenchymal stem cells (MSCs)** and **dental pulp stem cells (DPSCs)**. These insights have led to the recognition of key regulatory pathways involved in CSC maintenance namely **Wnt/β-catenin**, **Notch**, **Hedgehog**, and **NF-κB** signaling. These pathways coordinate the balance between stem cell proliferation, differentiation, and therapy resistance, making them attractive therapeutic targets for disrupting CSC-driven tumorigenesis.[4]

Concurrently, the field of **nanomedicine** has revolutionized the therapeutic landscape by enabling precision delivery of drugs and diagnostic agents. Nanoparticles (NPs) can be engineered for size, surface charge, and ligand specificity, allowing them to selectively home to CSCs via interaction with overexpressed surface markers such as **CD44, CD133, ALDH1**, and **EpCAM**. Beyond passive targeting, smart nanoparticles can be functionalized with antibodies, peptides, or aptamers to enhance **active targeting** of CSCs. These platforms can be loaded with chemotherapeutic agents, gene-silencing tools (such as siRNA or miRNA), or even photosensitizers for photothermal or photodynamic therapy. Importantly, some NPs are designed to perform both diagnostic imaging and therapeutic delivery—an emerging strategy known as **nanotheranostics**. [5]

In the context of **oral and head and neck cancers**, which have high rates of locoregional recurrence and poor response to conventional therapies, nanoparticle-enabled CSC targeting is especially relevant.

This review critically examines the biological underpinnings of CSCs, their role in treatment resistance and disease progression, and the recent advances in nanoparticle-based strategies for their diagnosis and elimination. By bridging CSC biology with cutting-edge nanotechnology, we aim to highlight an integrated approach that holds promise for **more durable, personalized, and effective cancer management** particularly in oral and head and neck malignancies where current modalities fall short.[6,7]

2. BIOLOGICAL FEATURES AND CLINICAL SIGNIFICANCE OF CANCER STEM CELLS

Cancer stem cells (CSCs), also referred to as tumor-initiating cells, represent a small but biologically potent subpopulation within malignant tumors. These cells possess the hallmark abilities of **self-renewal**, **multilineage differentiation**, and **tumor propagation**, traits reminiscent of normal stem cells but aberrantly regulated in the context of cancer. Initially identified in hematological malignancies, CSCs have since been characterized in a wide range of solid tumors including breast, brain, colon, and more recently, **head and neck squamous cell carcinomas (HNSCC)** with particular significance in **oral squamous cell carcinoma (OSCC)**.

What sets CSCs apart from the bulk of the tumor is not just their capacity to regenerate the tumor mass, but also their ability to resist standard therapeutic regimens, survive in adverse conditions, and remain dormant before reinitiating tumor growth. In OSCC, these characteristics directly contribute to **therapeutic failure, frequent locoregional recurrence**, and **metastasis**, making CSCs critical targets in modern cancer research.[8]

2.1. Self-Renewal and Differentiation in Head and Neck CSCs

The defining feature of CSCs is their ability to undergo self-renewal, maintaining a stable pool of stem-like cells, while simultaneously giving rise to more differentiated progeny that form the tumor bulk. In oral cancers, CSCs have been identified through **functional assays** and **surface marker expression**, notably **CD44**, **CD133**, and **ALDH1**. These markers are not merely phenotypic; they are linked to essential biological programs that regulate cell survival, motility, and differentiation.

Transcription factors such as **SOX2**, **OCT4**, and **NANOG** commonly expressed in embryonic stem cells are found to be upregulated in oral CSCs, where they maintain the pluripotency-like phenotype. The perivascular niche in oral tumors, rich in hypoxia and pro-inflammatory mediators, provides a sanctuary that supports CSC survival via **HIF-1α signaling**, **VEGF**, and **Notch pathway activation**. Hypoxia in these niches not only promotes CSC maintenance but also confers resistance to therapy through adaptive metabolic changes.

Functional studies using **tumorsphere cultures** derived from OSCC cell lines have confirmed that CSC-enriched populations exhibit enhanced **clonogenicity**, **chemoresistance**, and **tumorigenic potential** in

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immunocompromised animal models. These findings validate the stem-like and aggressive nature of CSCs in head and neck cancers.

2.2. Mechanisms of Therapy Resistance in Oral CSCs

One of the most clinically relevant attributes of CSCs is their robust resistance to chemotherapy and radiotherapy, which allows them to persist post-treatment and drive disease relapse. Several mechanisms underlie this resistance:

- **Efflux of Chemotherapeutics:**

  Overexpression of ATP-binding cassette (ABC) transporters, particularly **ABCG2** and **ABCB1**, allows CSCs to actively pump out cytotoxic drugs, reducing intracellular concentrations and blunting therapeutic efficacy.

- **Cellular Quiescence:** CSCs often reside in a **slow-cycling or dormant state**, making them inherently less susceptible to therapies targeting proliferating cells. This is particularly relevant in **cisplatin-based regimens**, where cell cycle phase specific toxicity is a limiting factor.

- **Enhanced DNA Repair:** Elevated expression of DNA repair proteins, including **Chk1, ATM, ATR**, and **RAD51**, enables CSCs to efficiently repair DNA damage induced by radiation or alkylating agents.

- **Antioxidant Systems:** CSCs in head and neck cancers have heightened antioxidant defenses, including increased levels of **glutathione (GSH)**, **superoxide dismutase (SOD)**, and **catalase**, reducing reactive oxygen species (ROS) induced apoptosis.

- **Anti-Apoptotic Signaling:** Proteins such as **Bcl-2**, **Survivin**, and **XIAP** are commonly overexpressed in CSCs and inhibit both intrinsic and extrinsic apoptotic pathways, allowing survival under hostile conditions.

Together, these mechanisms confer a survival advantage that underpins **residual disease** and necessitates therapeutic strategies that go beyond conventional cytotoxicity to include **targeted CSC elimination**.

2.3. Role of CSCs in Recurrence and Metastasis of Oral Cancer

Tumor recurrence and metastasis remain the most formidable barriers to successful cancer treatment, particularly in OSCC. CSCs are increasingly recognized as the principal drivers of these phenomena. After initial tumor debulking by surgery or cytotoxic therapy, surviving CSCs can reinitiate tumor growth often with increased aggressiveness and therapy resistance.

- **Epithelial-Mesenchymal Transition (EMT):** CSCs undergo EMT, a process that enhances motility and invasiveness. EMT-related transcription factors such as **Snail, Slug, Twist**, and **ZEB1/2** are upregulated in oral CSCs, enabling escape from the primary tumor and invasion into adjacent tissues or circulation.

- **Circulating Tumor Cells (CTCs):** CSC-like CTCs have been identified in the bloodstream of OSCC patients, often co-expressing EMT and stemness markers. These cells are capable of forming **secondary metastases**, especially in lymph nodes and distant organs.

- **Colonization of Secondary Niches:** CSCs are adept at surviving in foreign microenvironments by interacting with stromal cells, immune cells, and extracellular matrix components. In the **tongue, floor of mouth**, and **gingivobuccal sulcus**, where anatomical constraints hinder clear surgical margins, this leads to **frequent local relapse** despite apparently complete excision.

2.4. Clinical Significance and Prognostic Implications

The clinical implications of cancer stem cells (CSCs) in oral squamous cell carcinoma (OSCC) are profound, influencing tumor behavior, treatment outcomes, and long-term prognosis.

One of the most studied CSC markers in OSCC is **CD44**, a cell surface glycoprotein involved in cell-cell interaction, adhesion, and migration. CD44^high^ cell populations in oral tumors have been shown to exhibit enhanced clonogenicity and tumorigenicity. Clinically, overexpression of CD44 has been linked to:

- **Higher histological grade**
- **Perineural and vascular invasion**
- **Increased resistance to radiotherapy**
- **Shortened overall survival (OS)** and **progression-free survival (PFS)**

Importantly, CD44 expression also correlates with **poor locoregional control**, making it a strong independent prognostic indicator, particularly in tumors of the tongue and buccal mucosa.

Similarly, **aldehyde dehydrogenase 1 (ALDH1)** is a cytosolic enzyme involved in detoxifying aldehydes and regulating cell differentiation. ALDH1 activity is a

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hallmark of stemness in various cancers, including OSCC. High ALDH1 expression has been correlated with:

- **Lymph node involvement**
- **Tumor recurrence within 12–18 months post-surgery**
- **Resistance to cisplatin and 5-fluorouracil**
- **Decreased disease-specific survival**

Studies using immunohistochemistry and flow cytometry have demonstrated that ALDH1^+^ CSC populations are significantly enriched following chemotherapy or radiotherapy, suggesting their survival advantage and role in treatment failure.

Another marker of increasing clinical interest is **CD133 (Prominin-1)**, a pentaspan transmembrane glycoprotein associated with tumor-initiating cells. In OSCC and other head and neck cancers, CD133 expression has been observed more frequently in poorly differentiated tumors and is associated with:

- **Higher metastatic potential**
- **Reduced radiosensitivity**
- **Poor response to neoadjuvant therapy**
- **Inferior disease-free survival rates**

Beyond serving as **static prognostic markers**, these CSC-associated proteins are being actively explored for **therapeutic and diagnostic purposes**. Functionalizing nanoparticles with **anti-CD44, anti-ALDH1, or anti-CD133 ligands** enables the selective delivery of drugs or imaging agents to CSC-enriched tumor zones. This approach improves target specificity, minimizes off-target toxicity, and enhances the visualization of residual or dormant CSC populations through **non-invasive imaging modalities** like MRI, PET, or fluorescence imaging.

For instance, **CD44-targeted hyaluronic acid–coated nanoparticles** have shown improved drug accumulation in CSC niches, while **ALDH1-sensitive nanoprobes** allow for early detection of resistant cell populations before clinical recurrence is evident. **CD133-targeted quantum dots and liposomes** are under investigation for CSC visualization and ablation in xenograft models.

In summary, CSC markers such as CD44, ALDH1, and CD133 not only reflect the biological aggressiveness of oral tumors but also represent promising **theranostic targets**. Their dual role in prognosis and precision therapy underscores the critical need to incorporate CSC profiling into routine diagnostic and treatment planning—especially in high-risk OSCC patients where recurrence and metastasis are frequent.[9]

3. SURFACE MARKERS AND SIGNALING PATHWAYS OF HEAD AND NECK CANCER STEM CELLS

A detailed understanding of the molecular identity and regulatory circuitry of cancer stem cells (CSCs) is fundamental to designing precise and effective diagnostic and therapeutic tools. In head and neck squamous cell carcinoma (HNSCC), particularly oral squamous cell carcinoma (OSCC), CSCs are characterized by the expression of specific **surface markers** and sustained activation of **developmental signaling pathways**. These molecular signatures not only sustain stemness and therapeutic resistance but also serve as actionable targets for **nanoparticle-based detection and treatment strategies**.

3.1. CSC Surface Markers in Oral and Head & Neck Cancer

Surface markers are pivotal for isolating, identifying, and targeting CSC populations in both experimental and clinical settings. In oral cancers, several markers have been established as consistent indicators of CSC phenotype and functional behavior:

- **CD44**: This hyaluronic acid receptor is the most extensively studied CSC marker in OSCC. CD44^+^ cells display enhanced sphere-forming ability, tumor-initiating potential, and chemoresistance. Clinically, CD44 overexpression correlates with poor differentiation, lymph node metastasis, and unfavorable prognosis. Functionalized nanoparticles especially those coated with **hyaluronic acid** have been shown to selectively bind CD44^+^ CSCs, enabling targeted drug delivery and imaging.
- **ALDH1 (Aldehyde Dehydrogenase 1)**: ALDH1 is an intracellular enzyme involved in cellular detoxification and oxidative stress resistance. ALDH1^+^ CSCs in OSCC are highly tumorigenic, demonstrate superior resistance to radio- and chemotherapy, and are associated with early disease relapse. Imaging probes targeting ALDH activity (e.g., ALDEFLUOR) and **ALDH-responsive nanocarriers** are being explored for real-time CSC tracking and therapy.
- **CD133 (Prominin-1)**: A pentaspan transmembrane glycoprotein widely recognized as a CSC marker across multiple tumor types, including glioblastoma and colon cancer. In OSCC, CD133^+^ cells exhibit robust colony formation, resistance to cisplatin, and the ability to initiate tumors in xenograft models.

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CD133-targeted nanoparticles, particularly immunoliposomes and antibody-conjugated carriers, have shown promising preclinical efficacy in reducing tumor load.

- **EpCAM (Epithelial Cell Adhesion Molecule)**: While its role in OSCC remains under investigation, EpCAM is commonly used in isolating epithelial CSCs. Its overexpression is linked to enhanced cell proliferation and motility. EpCAM-directed nanoparticle systems, already validated in breast and colon cancer models, may be repurposed for OSCC targeting pending further validation.
- **c-Met (Hepatocyte Growth Factor Receptor)**: Frequently overexpressed in oral CSCs, c-Met regulates proliferation, motility, and epithelial-mesenchymal transition (EMT). It is particularly associated with invasive and metastatic OSCC phenotypes. **c-Met antibody–functionalized nanoparticles** have demonstrated CSC-specific uptake and inhibition of metastatic spread in HNSCC models.

The combination of multiple markers for instance, **CD44^+/ALDH1^+** or **CD44^+/CD133^+** enhances specificity and sensitivity in CSC identification. These dual-marker populations exhibit higher tumorigenic potential and are more resistant to standard therapies compared to single-marker subtypes.

3.2. Key Signaling Pathways in CSC Maintenance

Beyond surface antigen expression, CSCs in head and neck cancers are maintained by several **evolutionarily conserved signaling cascades**, which regulate cell fate decisions, survival, and plasticity. Dysregulation of these pathways confers therapeutic resistance and supports the maintenance of the CSC phenotype.

- **Wnt/β-Catenin Pathway**: Activation of the Wnt pathway promotes β-catenin accumulation, nuclear translocation, and transcription of genes involved in self-renewal. In OSCC, aberrant Wnt signaling supports CSC proliferation, EMT, and therapy evasion. Nanocarriers loaded with **Wnt inhibitors** or **β-catenin siRNA** are under development to selectively suppress this axis in CSCs.
- **Notch Signaling**: The Notch pathway influences stemness and differentiation through cell-cell interactions. In HNSCC, **Notch1** may act as a context-dependent oncogene, with its activation linked to CSC maintenance and radioresistance. Nanoparticle-mediated delivery of **γ-secretase inhibitors** or **Notch-targeting siRNA** is a promising avenue to modulate this pathway in CSCs.
- **Hedgehog (Hh) Pathway**: The Hh-GLI signaling axis is essential for embryonic development and is aberrantly reactivated in many cancers. In oral CSCs, Hedgehog pathway activation correlates with stemness, survival under hypoxia, and resistance to therapy. **Smoothened (SMO) inhibitors**, such as vismodegib, are being encapsulated into polymeric nanoparticles to improve CSC targeting and systemic bioavailability.
- **NF-κB Pathway**: Chronic inflammation mediated by nuclear factor-κB (NF-κB) enhances CSC survival, EMT, and immunosuppression. NF-κB is constitutively active in OSCC CSCs and contributes to their resistance to genotoxic therapies. Nanoparticles delivering **NF-κB inhibitors**, such as **curcumin or bortezomib**, have shown promising CSC-sensitizing effects in preclinical models.
- **PI3K/Akt/mTOR Pathway**: This pathway regulates cell growth, metabolism, and survival. Its hyperactivation in CSCs promotes stemness and resistance to apoptosis. Nanocarrier-based delivery of **dual PI3K/mTOR inhibitors** or **Akt-targeting siRNA** is under investigation in CSC-enriched tumor models.
- **EMT-Associated Transcription Factors**: EMT promotes CSC plasticity and metastasis through transcription factors such as **Snail**, **Slug**, **ZEB1/2**, and **Twist**. These regulators allow non-CSC tumor cells to revert to a stem-like state under environmental stress. Targeting EMT pathways using **multifunctional nanoparticles** may reduce CSC adaptability and invasiveness.

3.3. Crosstalk between Pathways and the Tumor Microenvironment

Importantly, these signaling pathways do not function in isolation. **Crosstalk** between Wnt, Notch, Hedgehog, and PI3K/Akt axes allows CSCs to dynamically adapt to environmental changes, evade therapy, and maintain their stem-like state. This redundancy poses a major challenge for single-pathway interventions and supports the rationale for **combinatorial or multifunctional nanotherapeutics**.

The **tumor microenvironment (TME)** including hypoxic regions, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and extracellular matrix (ECM) components plays a crucial role in CSC sustenance. Hypoxia, for instance, activates **HIF-1α**, which further stimulates Notch and Wnt signaling, reinforcing the CSC phenotype. Nanoparticles designed to co-target **CSC pathways and the TME**, such as **dual-delivery systems** carrying anti-VEGF and

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Wnt inhibitors, are emerging as effective strategies to disrupt CSC-niche interactions.

Moreover, **immunosuppressive signals** within the TME such as TGF-β and IL-6 protect CSCs from immune clearance. **Nanoparticle-based immune modulators**, including **immune checkpoint inhibitors** or **TLR agonists**, may help reverse this immunoediting and expose CSCs to host defenses.[10]

Table 1: Surface Markers of CSCs in Oral and Head & Neck Cancers

Marker	Function	Role in CSCs	Clinical Relevance
CD44	Cell adhesion and migration	Tumor initiation, metastasis	Correlates with poor prognosis
ALDH1	Oxidative stress resistance	Detoxification, therapy resistance	Associated with early relapse
CD133	Cell membrane glycoprotein	Enhanced clonogenicity	Indicator of radioresistance
EpCAM	Epithelial adhesion	CSC identification	Emerging marker in OSCC
c-Met	Tyrosine kinase receptor	EMT, invasiveness	Target for nanoparticle therapies

4. NANOPARTICLES IN THE DIAGNOSIS AND IMAGING OF CANCER STEM CELLS IN ORAL AND HEAD & NECK CANCERS

The early and precise identification of cancer stem cells (CSCs) within tumor tissues and circulation is essential for improving clinical outcomes, particularly in aggressive malignancies like oral and head and neck squamous cell carcinoma (HNSCC). CSCs are implicated in therapy resistance, disease relapse, and metastasis. However, traditional methods for CSC detection such as flow cytometry, immunohistochemistry (IHC), and tumor biopsy often lack the sensitivity, specificity, and real-time applicability needed for dynamic monitoring. Additionally, these approaches are invasive and limited to isolated time points, offering a restricted view of CSC biology.

Advancements in nanotechnology have paved the way for the development of **nanoparticle-based diagnostic platforms** that offer high specificity, sensitivity, and multiplexing capabilities. These nanoparticles (NPs) can be engineered to recognize CSC-specific markers, enhance signal detection in imaging modalities, and even allow for simultaneous diagnosis and therapeutic intervention (theranostics).

4.1. Nanoparticle-Enhanced Imaging Techniques

Nanoparticles have revolutionized imaging by serving as contrast enhancers and carrier vehicles for targeted imaging agents. In CSC detection within oral and head and neck cancers, several imaging platforms have benefitted from nanoparticle integration:

- **Magnetic Resonance Imaging (MRI)**: Superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with monoclonal antibodies targeting CSC markers like **CD44** or **CD133** have demonstrated excellent contrast enhancement. These NPs accumulate in CSC-rich regions, providing sensitive, non-invasive imaging of primary tumors and potential metastatic niches in HNSCC.
- **Fluorescence Imaging**: Quantum dots (QDs) and near-infrared fluorescent dyes encapsulated in liposomes or polymeric nanoparticles have been used for real-time CSC tracking. In OSCC, **hyaluronic acid-modified QDs** targeting CD44^+^ cells have enabled precise visualization of CSC populations in vivo, with superior resolution compared to conventional fluorophores.
- **Photoacoustic Imaging**: Gold nanoparticles (AuNPs), particularly **gold nanorods or nanoshells**, possess strong optical absorption in the near-infrared range. When functionalized with antibodies against **CD133** or **EpCAM**, these particles provide dual benefits of imaging depth and target specificity in oropharyngeal and hypopharyngeal tumors.
- **Surface-Enhanced Raman Spectroscopy (SERS)**: SERS-active nanoparticles functionalized with peptides or aptamers for CSC surface markers offer ultra-sensitive, label-free detection. Their ability to distinguish CSCs from bulk tumor cells in biopsy specimens represents a leap forward in diagnostic precision.

These imaging tools offer the potential for early CSC detection, intraoperative tumor margin visualization, and non-invasive post-treatment monitoring.[11]

4.2. Nanobiosensors for CSC Marker Detection

Beyond imaging, **nanosensor platforms** have been developed for sensitive biomarker detection in saliva, blood, and other body fluids offering minimally invasive alternatives for CSC identification:

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- **Electrochemical Biosensors**: Gold nanoparticles, carbon nanotubes, and graphene oxide have been used to fabricate sensors capable of detecting CSC-associated molecules such as **CD44**, **ALDH1**, or specific microRNAs (e.g., miR-21, miR-34a). These biosensors demonstrate excellent sensitivity and specificity, and some have been successfully tested using saliva samples from OSCC patients.
- **Colorimetric Nanosensors**: These platforms utilize enzyme conjugated nanoparticles that change color upon binding to CSC markers. Portable and user-friendly, they offer the possibility of rapid point of care screening for CSC biomarkers particularly useful in resource-limited settings.
- **Field-Effect Transistor (FET)-Based Nanosensors**: Semiconductor nanodevices functionalized with aptamers or antibodies show promise in detecting femtomolar concentrations of CSC-related markers. In OSCC, salivary detection of CSC-related miRNAs using FET nanosensors is under exploration as a non-invasive diagnostic strategy.

These biosensor technologies open the door to **real-time, dynamic monitoring** of CSC activity, therapy response, and risk of recurrence.

4.3. Circulating CSC Detection via Nanoparticles

The detection and characterization of **circulating CSCs (cCSCs)** a subpopulation of circulating tumor cells (CTCs) with stem-like properties holds promise for monitoring **minimal residual disease (MRD)** and predicting recurrence. This is particularly relevant in oral cancers, where local control remains challenging.

- **Magnetic Nanoparticles**: Iron oxide NPs functionalized with ligands against **EpCAM**, **CD44**, or **CD133** have been integrated into magnetic separation assays to selectively isolate cCSCs from peripheral blood samples. These systems have demonstrated potential for high-purity enrichment and downstream genetic or proteomic profiling.
- **Microfluidic Platforms**: Nanoparticle-enhanced microfluidic devices offer precise control over cell sorting, allowing for the isolation of cCSCs based on both physical and molecular characteristics. These approaches are being adapted for **liquid biopsies** in HNSCC patients.

  Applications include:

  - Early detection of recurrence
  - Tracking response to therapy
  - Stratifying patients based on CSC burden and aggressiveness

4.4. Theranostic Nanoparticles for Dual Imaging and Therapy

Theranostic nanoparticles represent a convergence of diagnosis and therapy within a single nanoplatform. By integrating targeting ligands, imaging agents, and therapeutic payloads, these systems enable **real-time monitoring of drug delivery, biodistribution, and therapeutic efficacy**.

In oral cancers, several nanotheranostic systems have shown promise:

- **Gold Nanoshells**: Conjugated to **CD44 antibodies**, these NPs enable both **photoacoustic imaging** and **photothermal ablation** of CSCs in OSCC xenografts.
- **SPION-Based Systems**: Dual-loaded with iron oxide cores (for MRI) and chemotherapeutics (e.g., doxorubicin), these nanoparticles provide multimodal imaging and selective drug release in CSC niches.
- **Fluorescent Polymeric NPs**: Deliver both siRNA and imaging dyes, allowing visualization of **gene silencing effects** in CSC-rich tumors.

Advantages of these systems include:

- CSC-specific drug accumulation
- Minimal off-target toxicity
- Visualization of treatment response in real time

Nanotechnology has dramatically transformed the landscape of CSC detection and imaging in oral and head and neck cancers. Through innovative nanoparticle designs ranging from targeted imaging probes to biosensors and theranostic platforms clinicians and researchers can now **visualize, quantify, and monitor CSCs with unprecedented precision**. These advancements pave the way for personalized, responsive cancer management strategies that could significantly reduce relapse and improve long-term outcomes in OSCC and related malignancies.[12]

Table 2: Nanoparticles in CSC Imaging and Diagnosis

Nanoparticle Type	Target Biomarker	Imaging Modality	Application
SPIONs	CD44, CD133	MRI	Detection of CSC-rich regions

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Quantum dots	CD44	Fluorescence	In vivo CSC imaging
Gold nanorods/shells	EpCAM, CD133	Photoacoustic	Deep tissue visualization
Graphene-based biosensor	ALDH1, CD44	Electrochemical	Saliva/blood marker detection

5. NANOPARTICLES IN THE THERAPY AND ERADICATION OF CANCER STEM CELLS

Oral and head and neck squamous cell carcinoma (HNSCC) continues to exhibit high rates of therapeutic failure and disease recurrence, despite advances in multimodal treatments. A significant contributor to this failure is the persistence of cancer stem cells (CSCs), which are inherently resistant to conventional therapies and capable of initiating tumor regrowth. CSCs exploit multiple survival mechanisms, including drug efflux, quiescence, enhanced DNA repair, and anti-apoptotic signaling, making their eradication particularly challenging.

Nanoparticles (NPs) have emerged as a promising tool to selectively eliminate CSCs while sparing healthy tissues. These nano-sized platforms enable **precise delivery**, **sustained release**, **multimodal targeting**, and **co-loading of therapeutic agents**. They also allow integration with imaging modalities, creating opportunities for simultaneous diagnosis and therapy theranostics.

This section highlights the state-of-the-art nanoparticle-based strategies designed to target and eradicate CSCs in oral and head and neck cancers.

5.1. CSC-Targeted Drug Delivery Systems

Nanoparticles can be functionalized to target CSC surface markers such as CD44, CD133, and ALDH1, thereby facilitating the selective delivery of chemotherapeutic agents to CSC populations within the tumor mass.

- **Polymeric Nanoparticles**: Biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and chitosan are frequently used to formulate nanoparticles loaded with standard chemotherapeutics (e.g., doxorubicin, cisplatin). Ligand conjugation such as hyaluronic acid (HA) for CD44 targeting enables improved retention in CSC niches. These systems enhance drug uptake in CSCs and reduce systemic toxicity.
- **Liposomal Nanocarriers**: HA-functionalized liposomes encapsulating curcumin, paclitaxel, or salinomycin have demonstrated selective toxicity against CD44^+^ CSCs in OSCC models. These carriers also inhibit tumorsphere formation and enhance apoptosis.
- **Gold Nanoparticles (AuNPs)**: AuNPs conjugated with anti-CD133 aptamers or antibodies serve dual roles: as drug delivery vehicles and agents for photothermal therapy. They enable site-specific accumulation and, upon light activation, induce localized thermal ablation.
- **Mesoporous Silica Nanoparticles (MSNs)**: MSNs offer high drug loading capacity and tunable release profiles. When functionalized with peptides or antibodies targeting CSC markers, they facilitate the delivery of both hydrophobic drugs and nucleic acids.

These delivery systems improve drug bioavailability, overcome resistance mechanisms, and enhance cytotoxicity within CSC-rich tumor regions.

5.2. Gene and RNAi-Based Nanodelivery Systems

Targeting the molecular pathways that sustain CSCs offers another level of therapeutic specificity. Nanocarriers enable safe and effective delivery of genetic material including siRNA, miRNA, and CRISPR components into CSCs.

- **siRNA Delivery**: Polymeric and lipid nanoparticles have been used to deliver siRNAs targeting β-catenin (Wnt pathway), Notch1, and Gli1 (Hedgehog pathway). In oral CSCs, such interventions reduce expression of stemness markers (e.g., SOX2, NANOG), inhibit self-renewal, and sensitize cells to chemotherapy.
- **miRNA Therapy**: Tumor-suppressive miRNAs like miR-34a and miR-200c, which inhibit epithelial-mesenchymal transition (EMT) and CSC plasticity, are being delivered using nanocarriers to suppress CSC proliferation and invasion.
- **CRISPR/Cas9 Systems**: Although still in early development, nanoparticle-mediated delivery of CRISPR-Cas9 targeting CSC-maintaining genes (e.g., OCT4, CD44) has shown potential in disrupting tumor-initiating capacity in HNSCC preclinical models.

These strategies offer a route toward **molecular reprogramming of CSCs**, pushing them toward differentiation or apoptosis.

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5.3. Photothermal and Photodynamic Therapies

Photothermal therapy (PTT) and photodynamic therapy (PDT) use light-activated nanoparticles to destroy CSCs with high precision and minimal damage to normal tissues.

- **Gold Nanorods and Nanoshells**: These structures accumulate in CSC niches and generate localized heat when exposed to near-infrared (NIR) light. CD44-targeted gold nanoshells in OSCC models have led to significant CSC depletion and tumor shrinkage.
- **Photosensitizer-Loaded NPs**: Nanoparticles encapsulating agents like chlorin e6, IR780, or methylene blue can generate reactive oxygen species (ROS) upon NIR irradiation, inducing selective apoptosis in CSCs.
- **Synergy with Immunotherapy**: PDT and PTT can stimulate **immunogenic cell death (ICD)**, potentially transforming the immunosuppressive CSC niche into an immune-responsive site, enhancing the efficacy of checkpoint inhibitors or dendritic cell vaccines.

These light-activated systems are particularly attractive for accessible tumors such as those in the oral cavity.

5.4. Combination Therapy Using Multifunctional Nanoparticles

Given the multifactorial survival strategies of CSCs, combination therapy is often required. Nanoparticles can co-deliver multiple therapeutic agents, enabling synergistic interactions.

- **Dual Drug Systems**: Liposomes co-loaded with **cisplatin and salinomycin** (a selective CSC inhibitor) have shown improved tumor regression and reduced CSC markers in HNSCC xenografts.
- **Chemo-Photothermal Composites**: CD44-targeted gold nanoparticles carrying doxorubicin can be activated by NIR light, combining cytotoxic chemotherapy with PTT for robust CSC ablation.
- **Stimuli-Responsive Nanocarriers**: Smart nanoparticles sensitive to **pH**, **redox potential**, or **enzymes** ensure the release of therapeutic payloads specifically in the CSC microenvironment. For instance, pH-sensitive micelles disintegrate under acidic conditions prevalent in tumor niches, maximizing intracellular drug delivery.

These approaches address CSC heterogeneity and adaptive resistance mechanisms, improving therapeutic durability.

5.5. Preclinical and Translational Advances

Preclinical studies using OSCC and HNSCC cell lines (e.g., SCC-9, CAL27, FaDu) and **patient-derived xenograft (PDX) models** have demonstrated the efficacy of nanoparticle-based CSC therapies. Key outcomes include:

- Reduction in tumorsphere formation
- Decreased expression of CSC markers (e.g., CD44, ALDH1)
- Delayed tumor initiation in immunodeficient mice
- Enhanced radiosensitivity and chemosensitivity
- Decreased recurrence rates post-treatment

While most nanoparticle-based CSC therapies are currently in the experimental or early translational phase, several platforms have progressed to **early-phase clinical trials**, particularly in breast and pancreatic cancers. With further validation, similar approaches can be tailored for oral and head and neck cancers, especially given the accessibility of these sites for localized nanoparticle delivery and light-based therapies.

Nanoparticle-mediated therapeutic strategies offer a **paradigm shift in the management of CSCs**, especially in oral and head and neck cancers. Their capacity to target CSCs at the molecular and phenotypic level through drug delivery, gene silencing, phototherapy, or combination regimens holds tremendous potential for preventing relapse and improving survival. As research advances toward clinical translation, CSC-targeted nanomedicine is poised to become a cornerstone of precision oncology.[13-15]

Table 3: Nanoparticle-Based Therapeutic Approaches for CSC Eradication

NP Platform	Therapeutic Cargo	Target/ Mechanism	Outcome in HNSCC Models
Liposomes + HA	Curcumin	CD44, NF-κB inhibition	CSC depletion, reduced sphere formation
Gold NPs	Anti-CD133, chemo	CD133+ CSCs	Targeted cytotoxicity + photothermal ablation
Polymeric NPs (PLGA)	siRNA (β-catenin)	Wnt pathway inhibition	Decreased stemness, tumor regression

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pH-responsive micelles	Dual drug (cisplatin + salinomycin)	CSC micro environment	Synergistic CSC and bulk tumor killing

6. CHALLENGES, FUTURE PERSPECTIVES, AND CLINICAL TRANSLATION OF CSC-TARGETED NANOTHERAPY

Nanoparticle-based therapies targeting cancer stem cells (CSCs) have demonstrated considerable potential in preclinical models of oral and head and neck squamous cell carcinoma (HNSCC). These systems promise to overcome the limitations of conventional treatments by delivering drugs directly to CSCs, enhancing imaging precision, and enabling synergistic multimodal therapies. However, transitioning these innovations from laboratory success to clinical efficacy presents numerous challenges.

This section explores the biological, technical, and regulatory barriers currently limiting the widespread clinical application of CSC-targeted nanomedicine. It also discusses future directions and strategic considerations that may facilitate successful translation into routine oncologic practice.

6.1. Challenges in Clinical Translation

Tumor Heterogeneity and CSC Plasticity

One of the most significant challenges in CSC-targeting is their phenotypic plasticity. CSCs are not a fixed population; rather, they can transition between stem-like and differentiated states in response to microenvironmental cues such as hypoxia, inflammation, or treatment-induced stress. This plasticity undermines the efficacy of static targeting strategies based solely on one or two surface markers (e.g., CD44, ALDH1, CD133). Additionally, inter-patient and intra-tumoral heterogeneity in CSC marker expression in HNSCC necessitates multiplexed or adaptive targeting approaches tailored to individual tumor biology.

Limited Nanoparticle Penetration and Accumulation

The tumor microenvironment of head and neck cancers is characterized by dense extracellular matrix components, irregular vasculature, and regions of hypoxia conditions that hinder nanoparticle penetration. While the enhanced permeability and retention (EPR) effect has been a cornerstone of passive nanoparticle targeting, it is often inconsistent in oral tumors due to their unique anatomical and vascular characteristics. Moreover, nanoparticles are frequently sequestered by the mononuclear phagocyte system (especially in the liver and spleen), reducing their accumulation in CSC niches.

Immunogenicity and Safety Concerns

Despite the advantages of nanocarriers, concerns about their long-term biocompatibility and immunogenic potential remain. Metallic nanoparticles (e.g., gold, silver) and some inorganic carriers such as quantum dots may trigger immune responses or accumulate in off-target organs. Even biodegradable polymers can elicit inflammatory reactions if not properly designed. Addressing these concerns requires thorough characterization, long-term toxicology studies, and regulatory vigilance.

Inadequate Preclinical Models

Most current studies rely on two-dimensional monolayer cultures or immunodeficient murine xenograft models that fail to fully recapitulate the human tumor microenvironment. These models lack immune interactions, stromal complexity, and CSC–niche dynamics found in real tumors. Consequently, results from these systems may not translate effectively to clinical settings. More predictive platforms such as **patient-derived organoids**, **tumor on a chip models**, and **humanized xenograft systems** are urgently needed for accurate preclinical evaluation.

6.2. Future Perspectives

Personalized and Multifunctional Nanomedicine

Advancements in precision oncology are steering nanoparticle development toward personalized solutions. Multifunctional nanocarriers that integrate CSC-specific ligands, imaging agents, therapeutic payloads, and environmental sensors could dynamically adjust treatment based on individual tumor behavior. By leveraging CSC profiling through liquid biopsies or imaging-guided feedback, future nanoparticles may deliver tailored therapy with real-time monitoring.

Biomimetic and Cell Membrane Coated Nanoparticles

Biomimicry in nanomedicine has shown potential to overcome biological barriers. Nanoparticles cloaked in **cell membranes** (e.g., from leukocytes, cancer cells, stem cells, or platelets) can escape immune surveillance, extend circulation time, and preferentially accumulate in tumors. In HNSCC, platelet- or macrophage-mimetic nanoparticles may preferentially home to inflamed or fibrotic tumor niches harboring CSCs.

Stimuli-Responsive and Smart Nanoplatforms

CSCs thrive in distinct microenvironments often marked by low pH, high glutathione, hypoxia, and unique

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enzymatic profiles. Smart nanoparticles that respond to these conditions by triggering site-specific drug release or signaling pathway modulation offer precision and efficiency. For instance, **pH-responsive liposomes**, **redox-sensitive micelles**, and **enzyme-triggered silica nanocarriers** are under investigation for CSC-selective delivery in oral cancers.

Synergistic Integration with Immunotherapy

CSCs are known to evade immune recognition and promote an immunosuppressive tumor microenvironment. Nanoparticles that ablate CSCs and concurrently modulate immune responses may reprogram this suppressive niche. Combining CSC-targeting nanocarriers with **immune checkpoint inhibitors**, **cancer vaccines**, or **cytokine-loaded NPs** could activate systemic anti-tumor immunity and prevent recurrence.

6.3. Road to Clinical Translation

For nanoparticle-based CSC therapies to reach clinical practice, several critical steps must be addressed:

Rigorous Clinical Trials

Phase I/II trials focusing on safety, pharmacokinetics, biodistribution, and preliminary efficacy are urgently needed, particularly in oral and head and neck cancers. Patient selection based on CSC biomarker expression (e.g., CD44^high^ or ALDH1^high^) can enhance response predictability. Incorporating nanoparticle-based **companion diagnostics** may facilitate real-time monitoring of CSC burden and treatment efficacy.

Regulatory Harmonization and Classification

Nanomedicine currently falls between the domains of **pharmaceuticals and medical devices**, leading to regulatory ambiguities. Theranostic nanoparticles, in particular, pose classification challenges. Regulatory frameworks need to evolve to clearly define standards for safety, manufacturing, and efficacy evaluation. International harmonization of guidelines will also streamline global access and approvals.

Scalable Manufacturing and Cost Control

For wide clinical adoption, nanoparticles must be produced at scale with reproducible quality and cost-efficiency. Standardized protocols for synthesis, sterilization, storage, and functionalization are essential. Environmentally friendly or **"green" synthesis methods**, alongside simplified nanoparticle designs, may reduce production costs and broaden global accessibility, especially in low-resource settings.

While nanoparticle-based strategies offer a compelling future for CSC-directed therapy in oral and head and neck cancers, significant translational hurdles remain. Overcoming these challenges requires multidisciplinary collaboration among oncologists, material scientists, regulatory authorities, and industry stakeholders. As technology advances and our biological understanding of CSCs deepens, the clinical realization of CSC-targeted nanomedicine moves closer to becoming a transformative reality in precision oncology.[16-18]

7. CONCLUSION

Cancer stem cells are central to the pathogenesis, recurrence, and treatment resistance of head and neck cancers, particularly oral squamous cell carcinoma. While conventional therapies fail to eradicate CSCs, nanoparticle-based approaches offer new hope for selective targeting, improved diagnostics, and effective therapeutic delivery.

This review has outlined the key biological features of oral CSCs, their molecular markers and signaling pathways, and how nanotechnology is being leveraged for their detection and eradication. Despite significant progress, real-world translation will require multidisciplinary collaboration across oncology, nanotechnology, regulatory science, and clinical medicine.

As personalized medicine becomes the future standard of care, nanoparticle-enabled CSC targeting stands poised to transform the treatment paradigm for oral and head and neck cancers.

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