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Review Article

Recent Trends in Screening of Oral Cancer

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Abstract: This Review focuses on the pressing global healthcare concerns surrounding the high prevalence of oral carcinoma and its late-stage detection. The World Health Organization (WHO) prioritizes early diagnosis and prevention of oral cancer, emphasizing the significance of timely oral screenings for understanding disease prognosis. Detecting crucial signs and symptoms during initial oral screening significantly enhances patient survival rates. Contributing factors to elevated mortality and morbidity include socio-economic elements, insufficient public awareness, as well as basic medical shortages. While visual examination is conventionally employed to In the presence of risk factors, keep track of client survival, its clinical utility is limited. To address this, efficient screening tools are needed to differentiate between benign and malignant lesions, providing early information about oral squamous cell carcinoma (OSCC). Optical imaging techniques such as tissue-fluorescence imaging and optical coherence tomography show promise. Oral cancer ranks as the sixth most frequent cancer globally, primarily oral squamous cell carcinoma. Detection methods include comprehensive clinical examinations, costly biochemical tests, and invasive biopsies. Saliva emerges as a noninvasive, promising diagnostic fluid for early oral cancer detection. This Review emphasizes its potential, containing a variety of biomarkers (DNA, RNA, and protein indicators) that help with early diagnosis. Direct contact with oral cancer lesions improves the specificity and sensitivity of saliva for screening. Numerous salivary biomarkers have been found, including defensin-1, P53, and cells (IL-8, IL-1b, and TNF-a). However, further research is needed for clinical validation. Late-stage oral cancer diagnosis contributes to elevated mortality rates. Early detection and treatment remain crucial for improved patient outcomes. Spectroscopy, salivary proteomics, toluidine blue staining, auto fluorescence, brush biopsy, DNA analysis, and biomarkers are a few noninvasive techniques that show potential. Nanotechnology-based detection systems, utilizing nanoparticles, offer highly sensitive and specific diagnostic techniques, potentially revolutionizing oral cancer management.

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(OSCC) emerges as a predominant focus, contributing to an overwhelming majority (approximately 90%) of all oral cancer cases and profoundly affecting the lives of over 300,000 individuals each year across the globe [1]. Distinguished by its origin in the oral mucosa, OSCC paints a concerning picture with an alarming escalation in its incidence and a disconcerting five-year mortality rate of 62% [2]. The pivotal role of late-stage diagnosis in shaping the unfavorable prognosis associated with oral cancer is undisputed. Addressing this critical issue and striving to curb the elevated mortality rate necessitate the identification of dependable diagnostic markers capable of detecting cancerous alterations in the early stages. The recurrent nature of oral cancer further compounds prognosis challenges, thereby underscoring the critical importance of early detection facilitated by sensitive and specific biomarkers [3]. Oral cancer encompasses a multifaceted spectrum of malignancies originating from the lips, oral cavity, and pharynx [4]. The global impact of this formidable disease is staggering, casting a shadow over an estimated 481,000 new individuals annually across diverse regions. Notably, the United States [5] is not immune, with oral cancer ranking as the sixth most prevalent cancer within its borders. Despite the encouraging survival rate of 80 to 90% associated with the early detection of OSCC, a sobering reality remains: The World Health Organization (WHO) reports an alarming mortality ratio when juxtaposed with other malignancies, translating into a five-year death rate following diagnosis of a troubling 45% [6]. This strikingly high morbidity rate can be attributed, at least in part, to the delayed identification of the disease [7]. The absence of widespread national screening initiatives, coupled with the lack of well-defined adequate biological indicators in order to detect oral cancer early, collectively perpetuates the prevalence of late-stage diagnoses [8]. Oral squamous cell carcinomas (OSCC) firmly hold their position as the dominant presence among oral cavity cancers. The primary risk factors that shepherd individuals towards the development of these malignancies encompass tobacco usage, alcohol consumption, and the utilization of betel nuts. Interestingly, these risk factors also play a significant role in the emergence of a slew of potentially malignant lesions (PML), further accentuating the urgency of implementing effective strategies for early detection. Conventional oral examination (COE) remains the cornerstone for identifying PML and OSCC. This procedure typically involves the confirmation of clinical suspicions through biopsy, followed by comprehensive histopathological analysis. While histopathology retains its mantle as the gold standard for OSCC diagnosis, the process is notably time-intensive, entailing several days for specimen preparation and subsequent analysis. Furthermore, the accuracy of histopathological findings rests upon the proficiency of skilled pathologists. It is pertinent to highlight that while histopathology excels in identifying cellular changes, detecting molecular alterations necessitates specialized techniques, thereby limiting its broader applicability. This review further delves into both the existing and emerging adjuncts that hold the promise of enhancing the detection and diagnosis of oral cancer. In addressing the pervasive presence of oral cancers, with a pronounced focus on OSCC, a sense of urgency envelops the global healthcare landscape. Urgent and concerted efforts are imperative to prioritize early detection as a pivotal strategy for mitigating the substantial mortality rate attributed to late-stage diagnoses. The quest for effective diagnostic markers and the advancement of innovative screening strategies emerge as integral components in the ongoing battle against the devastating impact of oral cancer. As scientific research forges ahead, making remarkable strides, and innovative techniques gain momentum, a glimmer of hope permeates the horizon, promising improved prognoses and enhanced patient outcomes within the realm of oral cancer treatment and management. The path forward mandates collaborative endeavors among healthcare professionals, researchers, policymakers, and the broader community, all converging to effectively address this formidable health challenge and chart a course towards a more promising future in oral cancer care. The development of interdisciplinary efforts, encompassing early detection campaigns, innovative biomarker research, and cutting-edge diagnostic techniques, forms a pivotal cornerstone in reducing the burden of oral cancer on a global scale. By fostering awareness, advancing research, and driving policy changes, the medical community can pave the way for a brighter future, characterized by early intervention, enhanced prognoses, and ultimately improved quality of life for those affected by oral cancer.

Table 1 :Indicators of Potential Oral Carcinoma:
Red patches (erythroplasia) on the oral mucosa.
Lesions that exhibit a combination of red and white areas, or irregular white patches.
Presence of an abnormal lump or mass.
Fissuring or raised exophytic (outward-growing) margins within the ulcers.
feeling of numbness or pain in the affected area.
Abnormal blood vessels supplying a lump or lesion.
Tooth mobility or a loose tooth.
Delayed healing of an extraction socket after a tooth removal.
A solid invasion beneath the mucosal tissue is indicated by induration.
adhesion of the lesion to more deeply embedded tissues, to the outermost layer of skin or the tissue above, or both.
swelling of the lymph nodes adjacent.
Difficulties in swallowing (dysphagia).
Unexplained weight loss.

When an oral lesion persists for longer than three weeks, cancer should be considered because these signs of oral malignancy are common (8, 22). OSCC can manifest itself in a variety of ways (Table 1).

• A lump or ulcer that has become indurated and is marked by a hard infiltration underneath the mucosal tissue.
• A granular ulcer with elevated exophytic borders or fissuring.
• A lesion that manifests as white or as red and white patches. • The presence of an erythroplasia, a red lesion.
• The development of a mass that may be accompanied by unusual blood vessels.
• Post-tooth extraction, delayed healing of the extraction socket.
• Fixation of the lesion to the mucosa or deeper tissues beneath the skin.
• Extra characteristics, as listed in Table 2.
• Enlargement of the cervical lymph nodes, especially when this is accompanied by hardening or fixation of the lymph nodes. Patients with oral cancer may experience enlarged nodes as a result of infection, tumor-induced reactive hyperplasia, or metastatic disease.

Table 2. Present Diagnostic Techniques in Use.
Biopsy followed by histopathological analysis.
Application of vital staining.
Utilization of biomarkers.
Assessment of DNA ploidy (chromosomal polysomy).
Implementation of brush biopsy.
Application of optical methods.

The diagnostic procedures now used are included in Table 2 and include brush biopsy, histopathology-assisted biopsy, vital staining, biomarkers, DNA ploidy analysis, and visual methods for evaluation.

1.1 Present Diagnostic Approaches

Possibilities and Constraints the primary objectives revolve around early detection and prompt treatment [9]. However, due to the uncertain sensitivity and specificity of COE [10], there is a compelling requirement for enhanced diagnostic instruments capable of identifying initial lesions and distinguishing between potentially malignant and benign conditions. This necessity gains prominence [11], given the substantial volume of oral lesions evaluated during oral cancer screenings, constituting 5-15% of individuals undergoing screening by dentists

1.2 Biopsy and histopathological examination

specimen ought to encompass a significant size, encompassing both areas of suspicion and seemingly unaffected tissue. This comprehensive approach facilitates the pathologist's ability to formulate a diagnosis without necessitating additional samples. Given that dysplasia within the lesion is more likely to manifest in red rather than white regions, it is advisable for the biopsy to encompass these red areas. Most biopsy sites exhibit rapid healing within a matter of days. Ensuring ample sampling is crucial to enable a conclusive diagnosis, necessitating the collection of at least one substantial specimen. To preempt the potential setback and distress stemming from an unfavorable pathology report in a patient with strong suspicion of a malignant neoplasm, some practitioners opt to perform multiple biopsies during the initial visit. The adoption of an excisional biopsy should be approached with caution, as this method may fall short in removing an adequate margin of tissue in cases of malignancy. Such a decision could inadvertently restrict the options available to the surgeon or radiotherapist, and may also compromise the availability of clinical evidence regarding the lesion's location and characteristics. The diagnosis of carcinoma is ascertained through histopathological examination, which reveals specific criteria: Severe dysplasia, characterized by dysplastic changes spanning the entire epithelial thickness. Invasion of the underlying lamina propria, evident through the extension of rete pegs, indicating penetration across the basement membrane. A specimen's histological analysis is basically subjective and rife with potential pitfalls. A significant challenge when dealing with potentially malignant lesions (PML) is ensuring that the sample is performed from the region most likely to have the highest concentration of cellular changes characteristic of premalignancy , specifically dysplasia. Red spots should be chosen for biopsy over white spots in order to accomplish this. Incisional biopsies still occasionally yield false-negative results. It is interesting that research has shown that even in leukoplakia cases where dysplasia has been ruled out through incisional biopsy, these lesions, with total excision, may unexpectedly retain oral squamous cell carcinoma in up to 10% of cases [12]. Since spontaneous molecular changes suggestive of early malignant transformations can take place both inside and outside of a clinical lesion that may be malignant, this event is not surprising. Additionally, there is reported variation in pathologists' interpretations, and even one pathologist may offer different diagnoses when presented with the same specimens on subsequent occasions [13].

1.3 Vital staining

Numerous endeavors to identify potential dysplastic regions prior to biopsy have regrettably not demonstrated absolute reliability. However, such efforts may offer some assistance, particularly in cases characterized by a pervasive 'field change,' a phenomenon often observed in individuals with a heightened risk

of oral squamous cell carcinoma. Toluidine-colored staining is an easy and inexpensive diagnostic technique that highlights aberrant mucosal areas using a blue-hued dye. In order to target nuclear material within malignant lesions and possibly malignant lesions (PML), TB uses a simple metachromatic nuclear dye, conserving healthy mucosa. There are various steps in the TB staining process: The procedure involves the following steps: (a) the patient rinses their mouth for 20 seconds with a solution of 1% acetic acid, followed by two additional rinses with water for the same amount of time; (b) the mouth is then rinsed with a solution of 1% toluidine blue that contains 5-10 cc; and (c) another rinse with 1% acetic acid is performed. Oral potentially malignant lesions (PMLs) with a high risk and poor prognosis may be detected via toluidine blue (TB) staining [14]. The development of oral squamous cell carcinoma has been linked genetic modifications such allelic loss and loss of heterozygosity, which are connected to positive TB staining. It should be noted that positive TB staining may indicate these genetic alterations even in cases when tumors seem benign histologically or show modest dysplasia [15]. It's important to emphasize the preoperative value of TB staining. The efficiency of OSCC was demonstrated in a case study when pre- or malignant cells were found more than 1 cm away from the visible lesion. This finding sparked a resection that might not have been thought of by a conventional oral examination (COE) alone 16. It is important to keep in mind that even mucosal tissue that appears to be normal might have molecular alterations that are symptomatic of the earliest carcinoma.

1.4 Physiological indicators

Several molecular indicators have been studied thus far in the context of OSCC development, including the expression of the p53 protein in the TSG, chromosomal polysomy (DNA ploidy), and changes known as loss of heterozygosity (LOH) in chromosomes 3 or 9. It is thought that mutations in the TSG p^{16} gene are connected to these alterations in chromosomes 3p and 9p [17]. Incorporating these biomarkers as supplementary components to the standard histopathological assessment holds the potential to enhance prognostic insights and optimize the management of potentially malignant lesions (PMLs). However, their widespread integration continues to be hindered by factors including the financial implications and intricacy of the tests, the lack of accessible facilities in some laboratories, and the dearth of in-depth outcome studies carried up to date

1.5 DNA ploidy

DNA- ploidy refers to the quantification small DNA in nuclear cells, potentially serving as an indirect gauge of significant genetic alterations. Such a metric could serve as a substitute for specific molecular markers. The assessment of DNA ploidy is a relatively straightforward process, accomplished through automated image cytometry of nuclei derived from routinely processed tissue specimens. Proficiency in this technique is accessible across numerous pathology laboratories

1.6 Brush biopsy

(Fig 1) A leukoplakia, a white spot on the mouth's lining, is being positioned in front of the Orcellex brush, a tool used to harvest cells from the mouth. The cell collection process is intended to extract cells from the leukoplakia. The brush biopsy employs a miniature nylon brush to collect cytology specimens, which are subsequently subjected to computer scanning and analysis using Oral CDx. This process identifies and visualizes individual cells. Upon the identification of potentially suspicious cells, a pathologist evaluates them to establish a conclusive diagnosis. If the samples are determined to be malignant, the pathologist also gives the clinician a written report and a printed copy of the aberrant cells from the electronic display. It is recommended that a positive outcome prompts a subsequent traditional incisional biopsy. The utility of this technique has sparked considerable debate, primarily centered around concerns regarding the potential for false negative outcomes.

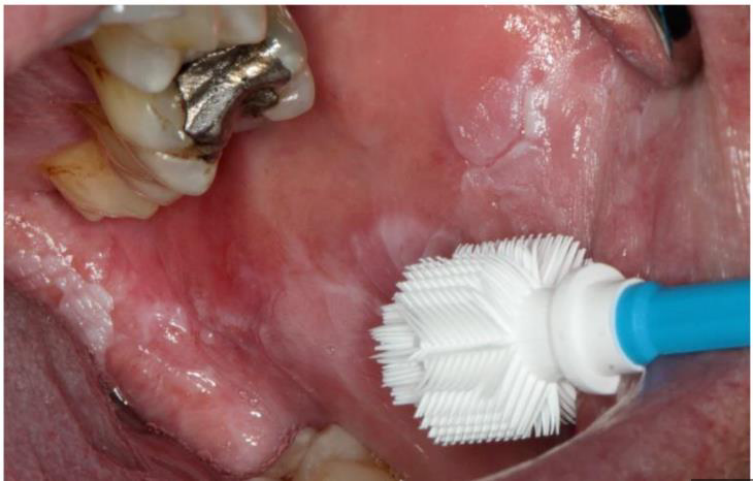

Fig 1: The Orcellex brush, a cell collector, is positioned in front of a leukoplakia on the buccal mucosa. [18]

1.7 Optical systems

When light interacts with the tissue, it might be able to observe changes in tissue structure and metabolic activity. Optical spectroscopy devices take advantage of the reality that the light spectrum emanating through cell carries crucial details about its histology and biochemical properties in order to detect these changes. Utilizing such optical improvements may aid in the early diagnosis of lesions of the mouth, including oral squamous cell carcinoma and possibly malignant lesions (PML). these adjuncts may help in recognizing cellular and molecular anomalies that escape the naked eye during regular examination, as well as in highlighting the surface texture and lesion boundaries and pinpointing the locations of biopsies. The complexity of histopathology can be rivaled by a number of optical technologies that are accessible and produce information that is similar to that Theoretically, these systems offer a method that is more objective and quantitative, providing real-time, non-invasive, and in-situ information [19].

Table 3. Vizilite Studies Overview

Author(s)	Study Population	Number of Cases	Findings
Haber (2004)	Consecutive patients	150	In contrast to a conventional oral examination (COE), one lesion was discovered by fluorescence.
			Fluorescence made spotting lesions on COE a little bit simpler.
Ram (2005)	Patients with prior oral cancer or premalignant lesions	40	Fluorescence is not very useful for small samples.
Epstein et al. (2006)	Oral Medicine patients with white lesions	134	By fluorescence but not by COE, two lesions were found.
			Fluorescence failed to detect the lesions apparent on COE.
Farah et al. (2007)	40-year-old women with tobacco use	55	A single lesion was found using fluorescence rather than COE.
Dental screening patients		301	By fluorescence rather than COE, six lesions were found.
			A repeat COE reveals lesions
			Nothing particularly better than COE

Note: Information presented in the table provides an overview of studies involving Vizilite, their respective study populations, number of cases, and key findings.

Early detection of mucosal lesions may be improved by using a diluted solution of acetic acid rinsing and subsequent inspection with a chemiluminescent light. An overview of several pertinent studies is presented in Table 3 for reference.

1.8 Oral cancer detection through saliva analysis

Exfoliate samples of cells were used to find gene mutations in the epithelium of the mouth of people who are more likely to develop oral cancer [20]. Moreover, these samples have proven effective in detecting microsatellite [21] alterations in cases of OSCC. Salivary mRNAs, including β-actin, SAT, and interleukin-8, exhibit noteworthy stability despite the presence of salivary ribonucleases [22]. In the context of OSCC, more than 300 samples of healthy and sick people's saliva have been evaluated, consistently revealing elevated levels of the designated signature in OSCC patients' saliva compared to healthy counterparts. This signature's presence demonstrated an overall accuracy rate of approximately 85% [23]. Additional prospective diagnostic systems are outlined in Table 4.

Table 4. Emerging Prospects In Diagnostic Technologies
Future technologies that could be used for diagnosis
Fluorescence Caused by Lasers Spectroscopy
The Fluorescence Induced by Light Spectroscopy

Infrared Elastic Scattering Spectroscopy
RMAn Spectroscopy
Imaging with light and sound
Particle Fluorescence
The OPS (Orthogonal Polarization Spectral) Quantum Dots in Image
Coherence in Light (OCT) Tomography
Combining Sensitivity with Several Spectroscopic Methods
Magnetic Resonance Imaging with Doppler
Broad Range Chromoendoscopy Scanning
Strategies for Immunophotodiagnosis
Comparative Path Length Spectroscopy
Dual Photon Fluorescence
Imaging using Terahertz of the Second Harmonic Generation
Future technologies that could be used for diagnosis
Fluorescence Caused by Lasers Spectroscopy
The Fluorescence Induced by Light Spectroscopy
Infrared Elastic Scattering Spectroscopy

Note: The Table Outlines Various Potential Diagnostic Technologies That May Emerge In The Future, Encompassing A Range Of Innovative Approaches For Oral Health Assessment.

1.9 Saliva as a Perfect Diagnostic Medium

Complete saliva arises from the combined secretions of the three principal salivary glands (parotid, submandibular, sublingual), alongside contributions from various minor salivary glands. It intermingles with crevicular fluid, nasal and bronchial secretions, as well as blood components from gum bleeding or wounds, and also incorporates microbes such as bacteria, viruses, and fungi [24]. Given its convenient accessibility and the non-intrusive nature of its collection process, saliva has long been proposed and harnessed as a diagnostic medium [25]. This approach boasts advantages such as minimal invasiveness, swift collection, cost-effectiveness, simplicity, and suitability for large-scale population screenings [26]. Saliva has established a substantial role in monitoring drug abuse involving addictive substances like cocaine, heroin, amphetamine, and barbiturates [27]. Additionally, salivary analysis has been prominently applied for diagnosing HIV infection [28]. Similar changes in specific proteins have been seen in the saliva of people who have different types of cancer [29].

1.10 Salivary Genotypic and Phenotypic Markers

Saliva can contain serum proteins and nucleic acids via a variety of methods. They may either enter saliva naturally through the acinar cells' [30] secretions or via intracellular processes like active transport or passive diffusion [31] across cell membranes. Extracellular paths are also a possibility, such as inclusion in crevicular fluid outflow or ultrafiltration through tight junctions [32]. Cellular necrosis may have an impact on the release of cell-free nucleic acids and proteins in saliva, a theory that is supported by the amount of DNA found in the plasma of cancer patients. The release of exosomes or microvesicles by living cells is another notable method. These membrane vesicles, which normally have a diamete [33]of 40 to 100 nanometers, leave the endoplasmic reticulum and are released after merging with the cell membrane. The presence of mRNA, miRNA, and proteins in the payload of these exosomes suggests that they may play a role in promoting cell-free intercellular communication [34].

1.11 Altered mRNA Transcripts

For a considerable period, the assumption prevailed that RNA undergoes swift degradation in saliva [35], a notion attributed to the presence of various RNAses within saliva. Contrary to this, emerging reports indicate the presence of both intact and fragmented cell-free RNA molecules within saliva. Notably, microRNAs, compact RNA molecules spanning 18 to 24 units in length, which exert influence over transcriptional processes, have been identified in saliva [36.] Furthermore, the detection of mRNA in saliva has garnered substantial attention, proving instrumental in the realm of Forensic Medicine[37] for identifying body fluids. Significantly, mRNA markers within saliva have been proposed not only for diagnosing primary Sjögren's syndrome[38] but also for delineating sleep drive, a phenomenon observed in both flies and humans [39].

1.12 Early Diagnosis

Implementing effective strategies to mitigate the elevated mortality rates associated with this condition is imperative. Early detection holds the potential to alleviate the disease's morbidity and the often taxing treatments it entails, which can lead to substantial functional impairment, psychological distress, disfigurement, and compromised quality of life. Regrettably, despite data gleaned. According to the National Cancer Institute's SEER program, which regularly compiles data on mouth cancer, there has been little to no progress in the last twenty years for the early diagnosis of oral malignancies [40]. At the moment, a sizable percentage of individuals are diagnosed after the illness has already evolved to a severe level Following the appearance of dysplastic lesions, which are obvious precancerous lesions, oral squamous cell carcinoma (OSCC)

almost always develops. In order to reduce the incidence and mortality of cancer, the American Dental Association emphasizes the need of identifying and treating these white and red patches that are demonstrating dysplastic alterations [41]. The development of malignancy from dysplasia, which has a course that is fundamentally unpredictable, occurs gradually over the course of several years. This prolonged period offers a window for intervention, enabling the potential treatment of the lesion and averting the progression to full-fledged oral cancer.

1.13 Light-based oral cancer screening aids

Diverse light-based techniques have developed as a result of increased interest in improving the effectiveness of early detection for precancerous and cancerous oral lesions. These cutting-edge options have been painstakingly designed to increase the efficiency of standard oral cavity examinations while making it easier to spot abnormalities. The VELscope, a portable device that uses visible light at a 430 nm wavelength to cause fluorescence excitation in specific tissue components, is one amazing example. This ground-breaking tool highlights the dedication to better diagnoses and patient outcomes and is a testament to the ongoing advancements in oral healthcare technology.

1.14 Diagnostic Tests

To enhance the quick identification of malignant and cancerous oral tumors, a number of light-based technologies have been created. These tools are especially made to support standard oral cavity examinations and make lesions easier to see. Notably, the VELscope, a portable device, stimulates the fluorescence excitation of particular tissue components using visible light at a wavelength of 430 nm. In contrast, patients using the Vizilite Plus with TBlue system (Zila Pharmaceuticals, Phoenix, Arizona, U.S.) rinse with acetic acid before having their eyes examined with a lit chemiluminescent light stick. Microlux takes a similar tack, requiring an acetic acid rinse and using a battery-operated fiberoptic visible light source to examine the oral cavity.

1.15 Oral Carcinoma Detection

A simple, affordable, and highly sensitive additional method for the identification of early oral squamous cell carcinoma and high-grade dysplasia's is toluidine blue (TB) staining. Metachromatic acidophilic nuclear staining is produced after 30 seconds of the application of a 1% aqueous TB solution to a suspicious lesion, which helps distinguish invasive or in situ cancer from healthy tissue. Although TB has a high sensitivity and a low specificity for malignant lesions, it is less successful in detecting premalignant lesions, with reported false negative rates of up to 58% for detecting mild-to-moderate dysplasia. Additionally, it has been shown that toluidine blue can help with assessing the margin status surrounding oral cancer after resection.

1.16 Laser Capture Microdissection

Laser capture microdissection has greatly advanced efforts to elucidate the molecular basis of malignancy [42] and improved the accuracy of cancer biology research. In order to isolate cells from samples while maintaining the exact morphology of both the captured cells and the surrounding tissue, LCM offers the best method. The accuracy of microscopically dividing particular cellular subsets is further improved by combining fast immune histochemical staining techniques with LCM [43].

1.17 Dna-Analysis

DNA image cytometry analyzes the ploidy status of cells to determine their propensity for malignancy. The cytological specimens are compared to a control cell group after being stained with Feulgen dye. To identify differences in cellular DNA content, a brand-new computer-assisted technique has been devised. Atypical DNA levels may be able to distinguish precancerous dysplastic lesions that have the potential to develop into cancer from those that do not since genomic instability plays a role in the beginning of cancer. Through the integration of morphological and cytogenetic evaluations, analysis of oral brush sample samples was done to identify non-diploid cells (NDC). It has been suggested that morphological and cytogenetic analyses of cells collected with a non-invasive brush could be used in conjunction to potentially detect cancer cells [44] at an early stage.

1.18 Epidemiology

In the majority of ethnic groups, the occurrence of oral cancer is found to be two to three times higher in men compared to women, according to data from the Surveillance, Epidemiology, and End Results (SEER) program by the National Cancer Institute. Globally, when considering all instances of oral cavity and pharynx cancers, these collectively rank as the sixth most prevalent type of cancer. Recent data originating from the International Center for Cancer Research depicts an annual worldwide [45] count surpassing 300,000 confirmed cases of oral cancer, resulting in approximately 145,000 fatalities. This classification, identified as ICD-10 C00-08, impacts various regions such as the lips, tongue, gingiva, mouth floor, parotid, and salivary glands. The geographical breakdown by the World Health Organization provides a comprehensive outline of the occurrence and mortality rates linked to oral cancer.

Table 5 : GLOBOCAN Cancer Incidence and Mortality, All Ages, Both Sexes by Population

Frequency of occurrence				
Population	**Numbers**	**Crude rate**	**ASR (W)**	**Accumulative risk**
AFRO Region	13,484	1.5	2.7	0.30
PAHO Region	49,200	5.2	4.1	0.48
EMRO Region	20,681	3.3	4.6	0.52
EURO Region	65,933	7.3	4.6	0.53
SEARO Region	103,464	5.6	6.4	0.73

WPRO Region	47,524	2.6	2.0	0.22
Very High HDI	92,338	8.0	4.8	0.54
Low HDI	40,954	3.1	5.2	0.59
More Developed*	100,823	8.1	4.7	0.54
Less Developed*	199,550	3.4	3.7	0.42
Mortality				
AFRO Region	8,530	1.0	1.8	0.20
PAHO Region	12,803	1.3	1.0	0.12
EMRO Region	10,997	1.8	2.5	0.30
EURO Region	25,202	2.8	1.7	0.19
SEARO Region	65,734	3.5	4.1	0.48
WPRO Region	22,068	1.2	0.9	0.09
Very High HDI	26,970	2.3	1.2	0.14
Low HDI	25,238	1.9	3.3	0.39
More Developed*	33,313	2.7	1.4	0.16
Less Developed*	11,2040	1.9	2.1	0.24

Note: Age-specific rates for the world are denoted by the abbreviation "ASR (W)". The international community Development Program and the WHO are two of the organizations that contributed to the Global Burden of Cancer Study 2012, which is where the data is obtained from. The terms "More Developed" and "Less Developed" are used for statistical ease and make no assessment of the level of development of any particular nations or areas.

1.19 Risk factors

1.1.1 Tobacco

In 2007, the IARC reached the conclusion that there exists substantial evidence supporting the carcinogenic nature of snuff smoke. For instance, it has been linked to the development of oral and pancreatic cancer [46]. Smokers face a threefold increase in the likelihood of developing oral cancer compared to non-smokers [47]. Furthermore, individuals who ceased smoking four years ago demonstrate a 35% reduction in the risk of oral cancer compared to those who continue to smoke. Surprisingly, the risk of oral cancer in individuals with a smoking-free history spanning over two decades is not higher than that of individuals who have never smoked [48]. This smoke comprises a variety of components that have the potential to foster cancer development, and they can be categorized into three main groups: nitrosamines, benzopyrenes, and aromatic amines. These substances are referred to as pre-carcinogens, which necessitate orchestrated modifications by oxidative enzymes. This transformation leads to a final product with reduced electron content, enabling it to form a covalent bond with DNA, resulting in the formation of a mutated adduct. The consumption of tobacco exposes the oral epithelium to oxygen and nitrogen free radicals, which may impact the body's antioxidant defense mechanisms

1.20 Alcohol

Alcohol (ethanol) can pose risks at both localized and systemic levels. It can enhance the permeability of the oral mucosa, dissolve lipid components within the epithelium, leading to epithelial atrophy, and disrupt DNA synthesis and repair processes. Moreover, alcohol exhibits genotoxic and mutagenic properties, resulting in reduced salivary flow and impairing the liver's capacity to process toxic or potentially cancer-causing substances.

1.21 Carcinogenesis

Evidently, oral squamous cell carcinoma (OSCC) progresses gradually over an extended span, during which numerous neoplastic changes occur within the oral cavity [49]. The development of oral cancer involves a intricate and multifaceted process influenced by a range of genetic modifications impacting epithelial cells, with certain genes being associated with translocations [50]. The process of oral carcinogenesis initiates with the conversion of a small group of regular keratinocytes. This conversion involves alterations in cytogenetics and epigenetic mechanisms that impact the advancement of cell cycle, DNA repair procedures, cell differentiation, and programmed cell death. These modifications might arise from random mutations, exposure to diverse biological elements, carcinogenic substances, or flaws in DNA repair mechanisms. Consequently, an initially unstable keratinocyte transforms into a field of pre-cancerization, ultimately progressing towards malignant and cancerous transformations [51]. Tumorigenesis necessitates several crucial components, including unlimited replicative capacity, self-reliance on growth stimuli, insensitivity to anti-growth cues, evasion of apoptosis, heightened angiogenesis, and enhanced capabilities for invasion and metastasis [52].

1.22 Tumor Microenvironment (TME)

The components of the tumor microenvironment (TME) in oral squamous cell carcinoma (OSCC) consist of cancer-associated fibroblasts (CAFs), immune cells, and various other supportive cell types [53]. The oncogenic modifications in gene expression patterns play a significant role in bringing about changes within the microenvironment. These alterations include the buildup of reactive oxygen species, excessive cytokine production, and the beginning of epithelial-mesenchymal transition (EMT). Among these elements, cancer-associated fibroblasts (CAFs) emerge as particularly pivotal entities within the TME, contributing significantly to processes like proliferation, invasion, and metastasis.

1.23 Nanotechnology-Based Detection And Diagnostic

The ability of magnetic resonance imaging (MRI) to detect bone penetration, determine the presence of a primary tumor, and help surgeons define the limits of tumors during surgical procedures is well established. Two typical MRI contrast agents that can shorten tissue longitudinal relaxation times are gadolinium combined with diethyltriamine-pentaacetic acid and the gadolinium added with diethylenetriaminepentaacetic acid. The use of several kinds of nanoparticles as particular cancer MRI contrast agent's diagnosis is the result of substantial developments made in the field of nanotechnology [54]. Nano-contrast agents are capable of identifying specific cell surface features, which increases their potential for cancer screening. Improved MRI contrast qualities are shown by markers with longer blood circulation half-lives. For this reason, Iron oxides that are extremely small and highly responsive to magnetic fields received the greatest research attention. These nanoparticles have been employed as neutral contrast agents in order to detect disorders of the liver and spleen because they can shorten both the T2 and T2* relaxation durations. Researchers have also looked on nano-contrast compounds in relation to oral malignancies [55]. To create an MRI contrast-enhancing agent, Asifkhan et al., for example, mixed magnetic poly nanoparticles with folate preconjugated chitosan.

1.24 Optical Coherence Tomography

OCT produces fine cross-sectional pictures of underlying tissues like epithelial layers and basement membranes, acting as a direct equivalent to ultrasound. This method, which makes use of infrared light that can penetrate up to around 2 mm, is appropriate for monitoring oral dysplasia and early identification of oral cancer [56]. OCT boasts a resolution of about 10 μm, greater than equivalent simple tests like magnetic resonance imaging, CT, and ultrasound [57]. While OCT stands as a real-time, non-invasive means for imaging cell and stromal morphology in clinical settings, it still grapples with insufficient contrast, particularly when distinguishing between neoplastic and normal tissues [58]. Meanwhile, the combination of microneedles and ultrasound was employed to surmount the challenge of delivering Au NPs. This multimodal approach demonstrated its efficacy in enhancing OCT penetration depth and led to an approximate 150% increase in contrast levels for detecting oral carcinogenesis. Photoacoustic imaging, a nascent optical diagnostic technique, utilizes a brief laser pulse to induce ultrasound signals from tissues [59]. This results in temporary thermoelastic expansions due to optical absorption. An ultrasound transducer detects the arrival of these photoacoustic waves, which are then translated into pictures based on their timing. In addition to being useful in assessing lymph nodes following major surgery, ultrasound offers excellent spatial resolution for describing tissue architecture [60]. Luke and colleagues pioneered the utilization of ultrasound-guided spectroscopic photoacoustic imaging technique to identify micro-metastases in lymph nodes within a murine model of metastatic OSCC. The research employed molecularly activated plasmonic nanosensors that were linked to anti-EGFR antibodies. Notably, the study revealed a significant absorption spectrum shift of the MAPS towards the near-infrared range.

1.25 Surface-Plasmon Resonance Scattering

The coordinated mobility of electrons that conduct in noble metals produces surface-plasmon waves [61]. nanoparticles of gold are now widely used to take advantage of surface Plasmon resonance scattering. They may effectively spread light that is visible or near-infrared through Plasmon surface oscillations [62], which explains this. Furthermore, they are ideally suited for jobs like precision targeting [63] and bio molecular labeling because to their straightforward preparation procedure, adaptability for bio conjugation, and low toxicity. According to research, compared to their unlinked counterparts, these linked nanoparticles have a propensity to agglomerate, leading to a much higher amount of surface Plasmon resonance scattering.

1.26 Raman Spectroscopy With Surface Enhancement

Raman spectroscopy functions through inelastic interactions between light and substance, serving as a vibrational spectroscopic approach [64]. The differentiation between regular, pre-malignant, and malignant anomalies relies on the alteration in the trajectory of scattered light. The use of lasers with known, near-infrared, or near-ultraviolet frequencies can cause this scattering. Healthy organs exhibit homogeneous warnings, while cancerous cells exhibit heterogeneous signals [65]. These variations reflect modifications in the chemical composition and molecular structure of the anomalies.

1.27 Imaging Through Diffusion And Reflection

In diffusion reflection imaging, only a small portion of incident white light that penetrates the tissue is either taken in or passes through, while the majority is subjected to several occurrences of elastic scattering and becomes diffusely reflected [66]. As a result of epithelial tissue cancerization, changes in cellular and structural properties have a substantial impact on the reflected light that results [67]. These alterations include things like the size of the nucleus, the makeup of the collagen, how the extracellular matrix is arranged, the thickness of the epithelial layer, and variations in blood flow. It has been documented that the capture of diffuse reflectance images holds potential in delineating surgical boundaries and serves as a valuable tool in distinguishing between normal mucosa, potentially malignant disorders (OPMD), and oral cancer.

1.28 Quantum Dots Imaging

The crystals known as quantum dots glow as a result of quantum confinement effects [68]. There are a number of benefits that quantum dots have over traditional fluorescent dyes. These advantages include the ability to size-adapt their emission, a range of activation spectrum, powerful glow, and very good photo bleaching resistance [69]. Furthermore, by adjusting the size and composition of quantum dots, an extensive range of wavelengths can be created, ranging from visible to near-infrared [70]. Currently, both in vitro and in vivo genetic and cell visualization of oral squamous cell carcinoma uses quantum dots. According to studies, when used to visualize specific mouth cancer cells, such as Tca8113, SCC-25, and BcaCD885 [71], in the laboratory, quantum dots show excellent photo bleaching resistance, low nonspecific binding, and high fluorescence intensity. Many quantum dots are paired with targeting molecules for in vivo imaging, allowing for cancer cell selectivity,

tissue penetration, and biocompatibility [72]. Notably, tissue auto fluorescence interference is efficiently avoided by quantum dots emitting in the 400–600 nm range, which makes them ideal for bio imaging applications.

1.29 Nano-Based Ultrasensitive Biomarker Detection

Tumor necrosis factor-alpha , vascular endothelial growth factor , epidermal growth factor receptor , and interleukin 6 are just a few examples of the molecular biomarkers that have been studied within tumors and have shown tremendous promise in sometime cancer identification and detection [73]. However, frequently used measurement methods such as ELISA, immunohistochemistry, Western blot, and polymerase chain reaction, have inherent limitations in their detection sensitivity, functioning between pM and fM (10-12 to 10-15 M) levels [74]. Leveraging nanotechnology offers the opportunity to amplify the sensitivity of detection for biomarkers present in minute quantities within tissue samples or bodily fluids [75]. An effective method in the realm of salivary proteomics analysis is the saliva peptide fingerprint technique [76], which aids in the identification of potential cancer diagnostic biomarkers.

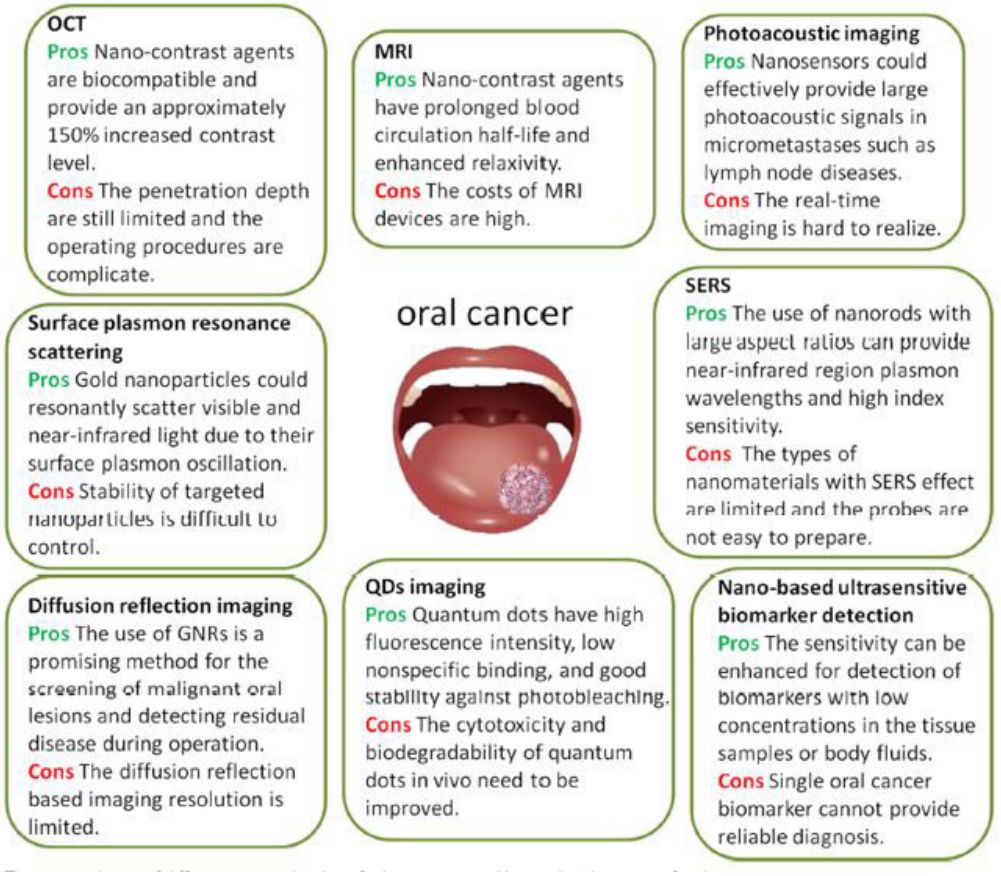

Fig 2. The pros and cons of different nanotechnology for bio imaging and biomarker detection of oral cancer

1.30 Reliable Clinical Studies

For early detection, a number of diagnostic techniques have been established. For instance, scanning, clinical evaluation, lymph node cellular biology, and conclusive histology following excisional biopsy can all be used to diagnose OSCC. The gold standard for identifying oral cancer is still histological investigation. However, occurrences of moderate dysplasia frequently go unnoticed and develop into severe dysplasia and then malignant tumors [77]. Specific gene mutations and protein alterations brought on by particular genetic traits and environmental factors are typically implicated in the progression of oral malignancies. Similar to how the prognosis and diagnosis of oral malignancies are determined by individual genes. Because of this, improved proteomic and genomic approaches have been widely employed to track changed protein and gene expression in a variety of oral malignancies. Biomarkers associated with OSCC have been detected in bodily fluids, offering a convenient option for early diagnosis. Saliva, recognized for its abundance of biomarkers, emerges as a non-invasive, simpler, and more cost-effective alternative to blood for biomarker identification.

1.31 Saliva

Specific gene mutations and protein alterations brought on by particular genetic traits and environmental factors are typically

implicated in the progression of oral malignancies [78]. Similar to how the prognosis and diagnosis of oral malignancies are determined by individual genes. Because of this, improved proteomic and genomic approaches have been widely employed to track changed protein and gene expression in a variety of oral malignancies. Epitope Inc., situated in Beaverton, Oregon, and Saliva Diagnostic Systems, based in Vancouver, Washington State, both pioneering manufacturing firms, vied to develop cutting-edge instruments and gadgets to cater to the evolving profession.The OraSure device's development by Epitope Inc, now known as OraSure Technologies (www.orasure.com), was crucial. This tool was used to collect oral fluid in a broad context in the early 1990s, which helped establish the first oral test kit for the HIV virus. This kit was made feasible thanks to collaboration with the the HIV-1 virus Western Blotting Test offered by OraSure, which received approval from the regulatory body responsible for supervising medicines and medical devices in the United States. Saliva Diagnostic Systems (SDS) developed a comparable product named the Saliva-Sampler within the United States. In Europe, it acquired the trademark Omni-SAL, and following the OraSure device, this tool received FDA approval for broad saliva collection applications. The Saliva Collection System by Greiner Bio-One in Austria, the OraSure Oral Fluid Drug Collection device from OraSure Technologies in Bethlehem, PA, USA, and the Salivette from Sarstedt, Germany are notable products in the field. Quantisal was developed in the USA and is an adaptation of the Saliva-Sampler device for drug monitoring.

Table 6: lists the main benefits of commercially available saliva collection devices.

Saliva Collection Device	Applications	Features	Additional Information
OraSure	HIV-1 testing	Convenient and secure for public health	Suitable for assessing life insurance risks and implementing outreach community initiatives
Quantisal	Saliva drug testing, including marijuana (THC)	Rapid saliva absorption with paper-based absorbent material	FDA cleared for forensics, criminal justice, and other applications.
Salivette	HIV antibody detection, oxidative stress assessment, steroid hormone testing	-	Wide application range for general wellness purposes.
UltraSal-2	Large sample of saliva (24 mL)	"Split" between two containers, the specimen	-
Greiner Bio-One SCS	measuring saliva with an internal dye (tartrazine)	utilizes colorimetry	-
RNAPro•SAL	simultaneous gathering of proteins and RNA	removing large DNA and obstructing elements	Sample Volume Adequacy Indicator (SVAI) built-in.
Pure•SAL	Collection of cell-free DNA, RNA, or proteins	Built-in filtration system removes significant pollutants	SVAI which is already present.
Super•SAL	Whole saliva collection system	Absorbent pad material removes interfering mucinous material	SVAI that is already there.
Versi•SAL	whole system for collecting saliva	Interfering mucinous material is removed by absorbent pad material.	SVAI that is already present.
Pedia•SAL	passively salivary sampling from newborns and babies	based on the shape of a pacifier	Saliva is collected intact.
DNA•SAL	Kit for collecting salivary DNA with buccal cell scraping	-	after which an oral rinse.
SimplOFy	genomic DNA collection kit for saliva	a consumer-friendly saliva collection tool	by spitting (expectoration).
Micro•SAL	collection of babies' and newborns' saliva	-	For the collection of small animals, there is a separate setup..

1.32 Salivary Biomarkers

Saliva, a multifaceted biological fluid, is increasingly recognized for its potential in exploring circulating cancer biomarkers. It offers enhanced specificity and sensitivity in various aspects of disease investigation, including diagnosis, prognosis, monitoring, and treatment. Distinguishing itself from other bodily fluids, saliva boasts a minimal presence of inhibitory substances and ordinary materials, resulting in lower complexity than blood [79]. Proteins, DNA, mRNA, and different metabolites all present in saliva have the potential to serve as biomarkers in clinical and translational research [80]. The bodily fluids has an exact

composition that is predetermined as a response to numerous events in the mouth. According to researchers like Schapher et al., salivary gland cancers and high leptin levels may be related. Furthermore, during the initial phases of gastric cancer [81], alterations in salivary proteomes could potentially serve as indicators of the presence of cancer. As a result, the analysis of saliva presents a viable avenue for activities such as prevention, monitoring, diagnosis, and prognostication. Saliva possesses a specific makeup that is predetermined as a reaction to various occurrences within the mouth. Researchers, like Schapher et al., have proposed an association between elevated levels of leptin in saliva and salivary gland tumors. Furthermore, during the initial phases of gastric cancer, alterations in salivary proteomes could potentially serve as indicators of the presence of cancer. As a result, the analysis of saliva presents a viable avenue for activities such as prevention, monitoring, diagnosis, and prognostication [82].

1.33 Diagnostic Toolboxes

The fluid has an innate ability to respond to specific situations, which causes it to express proteins, genes, RNAs, and a variety of inflammatory cytokines differently as cancer progresses and spreads [83]. These distinctive expressions hold potential benefits for the surveillance of individuals susceptible to cancer.

2. AUTHOR CONTRIBUTION STATEMENT

Dr. Pooja Kabra, Dr.R. Roghini, Dr. Somenath Ghosh conceived of the presented idea. Dr. Sucheta Karande, Dr. M. Chandran , Dr. Abikesh Prasada Kumar developed the theory. All authors discussed the results and contributed to the final manuscript.

3. CONCLUSION

In conclusion, the utilization of microfluidics for oral cancer screening offers a promising and innovative approach. By harnessing the inherent capabilities of saliva to reveal specific biomolecular changes associated with cancer progression, this method holds the potential to revolutionize early detection and monitoring. As technology continues to advance, integrating microfluidic techniques into routine screening protocols could significantly enhance our ability to identify and address oral cancer at its earliest stages, ultimately leading to improved patient outcomes and a higher quality of life.

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