UNIVERSITÀ DEGLI STUDI DI PALERMO

Dottorato di ricerca in Oncologia e Chirurgia Sperimentali
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Salivary biomarker study for early diagnosis of oral cancer

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Years 2021/2024– Cycle XXXVII

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Title of Doctor Europaeus

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Abstract

Oral squamous cell carcinoma (OSCC) is the most prevalent form of head and neck cancer (HNC), often arising from oral potentially malignant disorders (OPMDs). Despite advancements in cancer diagnostics, OSCC remains associated with a high mortality rate due to late-stage diagnosis and limited diagnostic tools. Salivary biomarkers have emerged as a promising avenue for non-invasive and early detection of OSCC. Saliva, being easily accessible and reflective of systemic and oral health, provides an ideal medium for biomarker discovery. Recent studies have identified various salivary biomarkers, including enzymes, cytokines, microRNAs, proteins, and metabolites, along with changes in oral microbiota composition. Dysbiosis, characterized by an imbalance in the oral microbiota, has been linked to OSCC pathogenesis. The aim of this research was to identify and validate salivary biomarkers for the early and non-invasive diagnosis of oral cancer, investigating specific molecular and biochemical changes in saliva associated with the onset of oral neoplasms. By advancing early diagnosis through non-invasive tools, a further objective was to improve patients' quality of life, facilitate the development of personalized prevention strategies, and optimize the follow-up care for individuals with a history of OSCC or OPMDs.

Summary

OSCC represents a major global health burden, ranking as the 16th most common cancer. It predominantly affects individuals aged 50 and older, although an increasing incidence in younger populations has been noted. OSCC's prognosis remains poor, with a five-year survival rate below 50%, largely due to late-stage diagnosis and diagnostic delays caused by non-specific clinical features. OPMDs, such as leukoplakia and oral lichen planus, often precede OSCC and carry varying risks of malignant transformation.

This doctoral research focused on salivary biomarkers for the early diagnosis of OSCC, emphasizing the role of oral microbiota. Saliva's non-invasive collection and reflection of oral and systemic conditions make it an ideal biofluid for biomarker identification. A systematic review of 11 studies revealed significant microbial alterations in OSCC patients, particularly an overrepresentation of periodontal pathogens such as Fusobacterium and Prevotella. These findings were corroborated by observational studies utilizing Oxford Nanopore Technology (ONT), which demonstrated microbial shifts linked to OSCC stages and surgical treatment. Dysbiosis, marked by a loss of beneficial microbes and an increase in pathogenic taxa, emerged as a key feature of OSCC-associated microbiota. Mechanistically, dysbiosis may contribute to OSCC through chronic inflammation, direct anti-apoptotic actions, and the production of carcinogenic metabolites. Periodontal pathogens such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* have been implicated in tumor progression via inflammatory cytokine release, E-cadherin degradation, and carcinogenic metabolite production.

While the diagnostic potential of salivary microbiota is evident, challenges such as methodological heterogeneity and small sample sizes must be addressed.

The findings of this research provide a foundation for future studies exploring microbiome-based diagnostic and therapeutic approaches. Standardized methodologies and longitudinal studies integrating multi-omics techniques are crucial for validating salivary microbiota's role in OSCC. Ultimately, incorporating oral health management into cancer care protocols may improve patient outcomes and advance personalized medicine in oncology.

Original papers

This dissertation is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Panzarella V, Di Fede O, Tripodo C, Campisi G. Salivary Microbiota Composition in Patients with Oral Squamous Cell Carcinoma: A Systematic Review. Cancers (Basel). 2022 Nov 4;14(21):5441. doi: 10.3390/cancers14215441. PMID: 36358859; PMCID: PMC9656014.

II. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Cancila V, Tripodo C, Campisi G. No Clear Clustering Dysbiosis from Salivary Microbiota Analysis by Long Sequencing Reads in Patients Affected by Oral Squamous Cell Carcinoma: A Single Center Study. Cancers (Basel). 2023 Aug 22;15(17):4211. doi: 10.3390/cancers15174211. PMID: 37686487; PMCID: PMC10486367.

III. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Belmonte B., Campisi G. Oral Microbiota composition in patients affected by Oral Cancer Before and After surgical treatment: A Single Center Pilot Study. *Under submission.*

CHAPTER 1 Background Rationale and Objectives

1.1 Salivary biomarkers of oral cancer

OSCC is the most common form of head and neck cancer. It can arise de novo or from oral precursor lesions, a group known as OPMDs (1).

Various efforts have been undertaken to identify biomarkers that could facilitate the diagnosis of OSCC or predict the malignant transformation of OPMD (2).

Biomarkers have been obtained from tissues, urine, serum, blood, and saliva. Saliva is the most attractive biofluid for biomarker research since its collection is easy, non-invasive, and fast. Currently, enzymes, cytokines, microRNAs, proteins, metabolites, and microorganisms have been studied as possible biomarkers in patients with oral cancer (2).

Saliva and oral microbiota composition can be influenced by several factors (e.g., diet and lifestyle) and their alteration can represent an important indicator of certain diseases, including OSCC (3).

Demonstrating a correlation between the alteration of the oral microbiota composition and carcinogenesis would lead to new tools for malignant transformation prevention of OPMD and early diagnosis of OSCC, evaluate the prognosis of patients and the risk of recurrence during follow-up.

1.2 Oral cancer

Oral cancer is one of the most common cancers globally, representing the 16th most common cancer diagnosed in 2020, according to GLOBOCAN (4).

In 2020, there were an estimated 377,713 new cases of oral cancer worldwide, with global age-standardized incidence rates of 6.0 per 100,000 men and 2.3 per 100,000 women. In 2020, the incidence rates of oral cancer (including lip cancer) were highest in Melanesia and South Asia(4).

The incidence increases with age: most cases occur in patients aged 50 and over (range 50-74 years); although, in recent years, an increase in OSCC onset in individuals under 45 years has been observed (5,6).

Globally, there were an estimated 177,757 deaths from oral cancer, with age-standardized mortality rates of 2.8 per 100,000 men and 1 per 100,000 women. In 2020, the mortality rates of oral cancer (including lip cancer) were highest in Papua New Guinea, followed by Pakistan, Bangladesh, India, and Sri Lanka (7).

Both incidence and mortality rates were consistently higher in men than in women across the world, although changes have been observed in recent years (8–10).

In the past, the incidence rates for men were significantly higher than in women (ratio of 7:1), but on average, the age-standardized rates for men are currently just twice as high as the rates for women (in most countries in a ratio of 2:1) (11).

In 2020, the incidence rates of oropharyngeal cancer were highest in Europe; in detail, the incidence rates were highest in Denmark, France

and Romania and the mortality rates were highest in Slovakia, Republic of Moldova and Romania, respectively (11).

The mortality rate of patients with oral cancer is unacceptably high in most countries and, unlike the recent increase in cancer treatment success rates observed for many malignancies (e.g. colorectal cancer, breast cancer and melanoma), the mortality rate for oral cancer has remained stable for over 20 years (8).

Currently, the 5-year survival rate for patients affected by OSCC is less than 50%, and the treatments these patients must undergo are highly invasive and debilitating (12). Among survivors, the quality of life is often so severely compromised that it significantly impacts their physical and psychological well-being.

Survival data is strongly influenced by the timing of diagnosis: more than 50% of patients with oral cancer are diagnosed at an advanced stage, and the 5-year survival rate of these patients is less than 50%, a fact which is mainly due to diagnostic delay (13).

The diagnostic delay may be attributable both to patient-related factors (e.g., a lack of knowledge regarding OSCC, fear) and to physician-related factors (e.g., a lack of clinical competence, the prescribing of inappropriate diagnostic investigations) (13).

The absence of the pathognomonic signs or symptoms of OSCC often can lead the clinician to a misdiagnosis (14). The clinical features of oral cancer vary depending on the site and the stage of clinical presentation. The two most affected sites by oral cancer are the tongue and the buccal mucosa (11). The clinical manifestations of OSCC are heterogeneous, including non-ulcerated lesions, exophytic or ulcerated lesions, bleeding

and characterized by irregular margins with high margins and loss of elasticity(15). The lesions are usually painless in the early stages and may cause only some nonspecific discomfort. Some months can elapse between the initial discomfort and the appearance of ulceration. The pain gradually increases as the ulcerated surface increases. The presence of rolled margins and induration are the most significant features of malignancy(16).

1.3 Oral potentially malignant disorders

The term OPMD was introduced in 2005, replacing the terms “oral precancerous/premalignant lesions and conditions”(18). An OPMD is defined as any oral mucosal abnormality associated with a statistically increased risk of developing oral cancer(18). OPMDs comprise a wide range of disorders with varying rates of malignant transformation ranging from 1.4% to 49.5% (19). In 2020, the World Health Organization (WHO) Collaborating Centre for Oral Cancer recommended a new list of OPDMs, which include leukoplakia, proliferative verrucous leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus, actinic cheilitis, palatal lesions in reverse smokers, oral lupus erythematosus, dyskeratosis congenita, oral lichenoid lesions and graft-versus-host disease (1). In some cases, oral cancer is preceded by OPMD (17).

1.4 Oral carcinogenesis

Oral carcinogenesis is a multistep process, arising from the accumulation of genetic and epigenetic alterations over the years (20). Oral carcinogenesis is

based on the alteration of the cell cycle through two key mechanisms: the activation of oncogenes and the inactivation of tumor suppressor genes (21). Oncogenes, which drive uncontrolled cell proliferation, become activated through mutations or overexpression, while tumor suppressor genes, which typically regulate cell division and repair DNA damage, are often silenced by mutations, deletions, or epigenetic changes like DNA methylation(22). These changes result in a loss of cellular growth control and resistance to apoptosis, leading to malignant transformation of oral epithelial cells(22). These genetic changes disturb cellular proliferation and differentiation, resulting in a spectrum of architectural and cytological aberrations of the mucosal epithelium recognized as oral epithelial dysplasia (OED)(23). According to a stepwise theory, OED represents an intermediate stage of carcinogenesis (24). OPMDs are mucosal diseases with different aetiologies in which OED may be present. The risk of malignant transformation varies among the OPMDs and has been correlated with the OED pattern (25). The evolutional tumor theories do not dictate OED malignant transformation per se. As with any other malignant neoplasm, OSCC initiation and progression depend on interactions with the microenvironment, immunomodulatory mechanisms, and epigenetic influences, besides genetic changes (26). The oral cavity represents a unique environment for carcinogenesis. Its epithelium is a self-renewing tissue where somatic mutations accumulate during each renewal cycle (27). Additionally, continuous exposure to potent environmental mutagens, such as tobacco and microbial products, exacerbates genetic instability. Crucially, mutations in TP53, a gene essential for DNA repair and frequently altered in OED and OSCC, often result in tumors harboring thousands of mutations (28).

1.5 Risk factors

OPMDs share common risk factors with OSCC (29). Some risk factors for oral and oropharyngeal cancers are well known, including tobacco smoking, alcohol consumption, betel quid chewing, and Human Papilloma Virus infection. However, a portion of oral cancers cannot be attributed to known risk factors, in fact in recent years there has been a change in the trend of OSCC with an increase in new cases in women who have never smoked and in young people(30).

For this reason, there are more and more cases reported in the literature associated with several additional potential risk factors, such as:

- Environmental factors (e.g. second-hand smoke, occupational exposures);
- Lifestyle factors (e.g., diet, mate drinking, cannabis smoking, mouthwash use);
- Familiar or genetic predisposition;
- Systemic factors (e.g., immunosuppression, haematinic and micronutrient deficiency);
- Local factors (e.g., chronic mechanical irritation, oral microbiota composition) (31).

Carcinogenicity is a dose-dependent process and is magnified by multiple exposures to different risk factors. Conversely, low and single exposures do not exclude oral cancer risk (32).

1.6 Oral microbiota

Oral microbiota is a complex dynamic ecological community conditioned by continuous changes in the availability of oxygen, nutrients, and the pH of saliva, since it contains very distinct niches adhering to various surfaces (e.g., supragingival and subgingival plaque, tongue dorsum, attached gum, buccal mucosa, hard palate, saliva) (33).

According to the Human Oral Microbiome Database, only approximately 57% of the oral bacterial species were identified, 13% were cultivated but they remain nameless, and 30% are neither isolable nor replicable in cultures microbiological (34).

The terms microbiota and microbiome are often mistakenly used as synonyms, although this is not the case.

The term “microbiome” defines the collective genome of microorganisms that reside in a particular environment, especially the human body (e.g., skin, gastrointestinal tract).

The term “microbiota” indicates all the microorganisms found in an environment and it includes bacteria, viruses, and fungi (35).

The oral cavity is a dynamic and complex ecosystem, in which more than 700 species of bacteria, fungi, viruses, and protozoa reside. The balance of the components of oral microbiota is maintained by a continuous interplay with the host (36). Variations in this state of equilibrium are termed “dysbiosis,” which may allow pathogens to cause both systemic and local diseases. Among systemic pathologies, those most frequently associated with alterations in the oral microbiota composition include Alzheimer's disease, diabetes, cardiovascular diseases, and certain types

of cancer (pancreatic, esophageal, and colorectal) (37,38). Locally, changes in the oral microbiota have been observed in patients with caries, periodontitis, and peri-implantitis (39).

The roles of microorganisms have been demonstrated in modulating and maintaining the biological functions of the human host. In cases of dysbiosis, caused by external factors (e.g., smoking habits, alcohol consumption) or infections with pathogenic microorganisms, the risk of developing diseases, including OSCC, may increase (35).

1.7 Aim

General aim

The overall aim of this research project was to identify and validate salivary biomarkers for the early and non-invasive diagnosis of oral cancer. Our study aims to enhance diagnostic accuracy by investigating specific molecular and biochemical changes in saliva associated with the onset of oral neoplasms. By advancing early detection through non-invasive tools, a further objective was to improve patients' quality of life, facilitate the development of personalized prevention strategies, and optimize the follow-up care for individuals with a history of oral neoplasia.

Specific aims

The aim of this research is to investigate the relationship between the salivary microbiota and OSCC. Specifically, this study aimed to identify a possible association between oral dysbiosis and OSCC by systematically reviewing the existing literature, with particular emphasis on studies analyzing the salivary microbiota composition.

Furthermore, it aimed to investigate the salivary microbiota composition in

OSCC patients using ONT, a novel sequencing approach employed for the first time in this context, to explore its practicability and potential for microbiome profiling.

Additionally, this research aimed to evaluate changes in the salivary microbiota composition in OSCC patients before and after surgical treatment and to compare these profiles with those of healthy controls, thereby providing insights into the impact of surgical intervention on the oral microbiome.

Taken together, these objectives aim to elucidate the role of the salivary microbiota in OSCC pathogenesis and progression, offering a foundation for future microbiome-based diagnostic and therapeutic strategies.

CHAPTER 2 Materials/ Patients and Methods

2.1 Systematic Review

Paper I

2.1.1. Protocol Research Strategy

A systematic literature search was independently conducted by two authors (Martina Coppini and Rodolfo Mauceri). The study protocol was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (40).

The research question was designed based on PICo items in which:

P: patients affected by OSCC;

I: alterations of salivary microbiota composition;

Co: worldwide.

The systematic review was based on the following research question: Do OSCC patients have alterations in the composition of their salivary microbiota?

A selection of studies analyzing the salivary microbiota of oral cancer patients was performed. Relevant records were identified using various search engines (e.g., Medline/PubMed) and by reviewing the reference lists of retrieved articles. The search strategy employed a combination of MeSH terms and free-text keywords, connected using Boolean operators, as follows: *Microbiota* AND (*oral cancer* OR *Squamous Cell Carcinoma of Head and Neck* OR *oral carcinoma*). The literature search was completed in January 2022.

2.1.2. Eligibility criteria

The inclusion criteria required studies to be human based, published in English, and involve at least 30 patients. Only studies with a histologically confirmed diagnosis of OSCC, with a well-defined site classification, were considered. The study focused on salivary microbiota composition analysis in patients affected by OSCC before therapy.

Exclusion criteria were: studies focused on tumors different from OSCC; narrative and systematic reviews and meta-analyses; case reports and studies with less than 30 patients; in vitro studies; in silico studies; animal studies; analysis of oral microbiome in patients affected by OSCC, during or after cancer therapy; studies with HNC or Oropharyngeal Squamous Cell Carcinoma (OP-SCC), no defined information for subgroup of OSCC; supragingival plaque analysis and tongue swab analysis; oral scraping.

2.1.3. Statistical Analysis

The selected studies were reviewed to identify relevant outcomes, and data were extracted using a pre-designed Excel sheet. Extracted information included study characteristics (e.g., first author, publication year, country, and study design), details of case-control groups (e.g., case population, control population, age of participants, and risk factors), and methodological aspects (e.g., sample type, collection methods, DNA extraction and amplification techniques, sequencing platforms, and reference databases). Outcome data encompassed raw reads, microbial abundance, detected genera, species, phyla, and operational taxonomic units (OUT). Not all studies provided all the specified outcomes. Continuous variables were summarized as mean values with standard deviations, while

categorical variables were reported as counts and percentages.

2.2 Observational Studies

Paper II and III

2.2.1. Etichs

The study protocol conformed to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All the procedures reported were approved by the Internal Ethical Committee of the University Hospital A.U.O.P "Paolo Giaccone" of Palermo (Italy) (approval #11/2020). Patients signed an informed consent form before recruitment for the study. The study reported was assessed following the STROBE guidelines for observational studies (41).

2.2.1. Population Recruitment

Paper II

Subjects were consecutively recruited between December 2020 and May 2022 from the Unit of Oral Medicine at the "Paolo Giaccone" Policlinico University Hospital in Palermo, Italy. Participants were eligible if they were over 18 years old, had a diagnosis of OSCC confirmed by pathological examination before surgical treatment, and could provide informed consent. Exclusion criteria included pregnancy or nursing, recent use of antibiotics or periodontal therapy within the last three months, severe periodontitis, immunocompromising conditions, or prior surgical, radiotherapy, or chemotherapy treatments.

Paper III

Subjects were consecutively recruited between December 2022 and May 2024 from the Unit of Oral Medicine at the “Paolo Giaccone” Policlinico University Hospital in Palermo, Italy. Inclusion criteria required participants to be over 18 years of age, have a diagnosis of OSCC confirmed by pathological findings both before and after surgical treatment, and be able to provide informed consent. Exclusion criteria included pregnancy or nursing, recent antibiotic or periodontal therapy within the last three months, severe periodontitis, or the presence of immunocompromising conditions.

Paper II and III

2.2.2. Oral squamous cell carcinoma diagnosis

To confirm the diagnosis of OSCC, a histopathological examination was conducted at the Oral Pathology Unit of the “Paolo Giaccone” Policlinico University Hospital in Palermo, Italy.

Following local anesthesia, an incisional biopsy was performed, and a section from the sample was fixed in formalin solution before being sent to the pathology laboratory for analysis. If the histopathological examination confirmed the diagnosis of OSCC, an unstimulated salivary sample was then collected from the patient. Subsequently, the patients were referred to the Oncological and Plastic Surgery Unit for further treatment.

2.2.3. Outcome Measures

For each patient, several data points were recorded, including demographic

information, the localization of the OSCC lesion based on the International Classification of Diseases (ICD, 10th revision), and the TNM stage (version 8.0) following the guidelines of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC). Additional factors such as smoking habits, alcohol consumption, and potential traumatic risk factors (e.g., ill-fitting prostheses or sharp dental cusps) were also documented. Patients were then classified into two groups based on their TNM stage: early stage (stage I and II) or advanced stage (stage III and IV).

2.2.4. Sample Collection

Once the diagnosis of OSCC was confirmed through histopathological examination, an unstimulated salivary sample was collected. As controls, patients with oral diseases other than OSCC and healthy individuals were enrolled. Non-stimulated saliva was collected in the morning from each participant following a standardized protocol. Participants were instructed to refrain from eating, drinking, or smoking for at least two hours before sample collection and to rinse their mouths with saline solution for 60 seconds. Saliva was allowed to accumulate in the floor of the mouth before being collected in sterile Falcon tubes, which were immediately stored frozen at -80°C until analysis. The analysis of the salivary microbiota composition was performed at the Microbiology Unit of the “Paolo Giaccone” Policlinico University Hospital in Palermo, Italy. A second unstimulated salivary sample was collected during the follow-up visit at least three months after surgical excision.

2.2.5. DNA extraction

From each sample has been extracted DNA using the QIAamp DNA Blood Kit

(Qiagen, Cat No 51183, Hilden, Germany), following the QIAamp® DNA Mini and Blood Mini protocol—Blood or Body fluid spin section. Briefly, each falcon tube was centrifuged for 30–40 min at 300 x g to pellet the lactescent, like-mucus phase and to remove the supernatant. Next, the pellet was resuspended in 400 µL of 1 x PBS pH 7.4, homogenized by pipetting and transferred in new 2 µL Eppendorf tubes. Next, any solution was mixed with 40 µL of Qiagen proteinase k and 400 µL di AL buffer, then incubated at 56°C for 16 to 24 h, until the pellet was dissolved completely. Then, 8 µL of Qiagen Rnase A was added to each sample at a concentration of 100 ng/ µLL and then it was mixed by inversion at least 3 times and was incubated for 2 min at room temperature. Next, 400 µL of pure ethanol was added to each sample, and after it has been well mixed by pipetting, all mixture was filtered through the QIAampMini spin column. Subsequently, in the spin columns were applied 500 µL of both AW1 and AW2 buffer was for a total of 2 washes. In the elution step, each QIAamp Mini spin column was placed in a new clean 1.5 mL microcentrifuge collection tube and 70 µL di Tris-EDTA pH 8 was added directly onto the membrane. Each sample was incubated for 5 min at room temperature and successively centrifuged for 1 min at 20,000 x g to recover the DNA. The elution step was repeated another time, recovering the DNA, and centrifuging in the same tube.

2.2.6. Samples Sequencing

From DNA samples, we prepared metagenomic sequencing libraries with a Rapid PCR Barcoding Kit (SQK-RPB004—RPB_9059_v1_revL_14Aug2019) and then ran on the MinION device (ONT, Oxford, UK). We base called the output (i.e., converted the sequencing device

output into nucleic acid base sequences) with the MinKNOW software v20.06.4 (Oxford Nanopore Technologies Ltd., Oxford, UK) and used the ONT platform, EPI2ME, for quality control, species identification [What's In My Pot (WIMP) pipeline]. DNA initial concentration was 5 ng per sample, in 3 µL of nuclease-free water. For the DNA tagmentation, 1 µL fragmentation mix (FRM) was added for each sample, which was incubated at 30 °C and then at 80 °C for 1 min per step, in a thermocycler. Next, 4 ng of the tegmented DNA was added to a mixture that included 1 µL of Rapid Barcode Primer at 10 µM, 25 µL of LongAmp® Hot Start Taq 2× Master Mix (NEB cat No. M0287), and Nuclease-free water, up to a final volume of 50 µL. PCR profile included an initial denaturation of 3 min at 95 °C; 14 cycles of 15 s at 95 °C, 15 s at 56 °C, 6 min at 65 °C; and a final extension step of 6 min at 65 °C with a Hold at 4 °C. The amplified DNA was cleaned up with 30 µL of Agencourt AMPure XP beads (Beckman coulter, Munich, Germany) at 1× concentration, incubated in a rotator mixer for 10 min, and washed twice on a magnetic rack, with 200 µL of freshly made 70% ethanol. Next, after having to remove the ethanol and air drying the pellet, it was eluted in 10 µL of freshly T50 Buffer, made with 1% of Tris-HCl 1 M pH 8, 5% of NaCl 1 M, and 94% of Nuclease-free water; and then 1 µL per sample was quantified by Qubit™ fluorometer (Thermo Scientific: Waltham, MA, USA). To determine the average length of the library in each sample, 1 µL was analyzed in 2100 Bioanalyzer Instrument, model G2939B using Agilent DNA 12000 protocol (Part. Number 5067-1508), according to manufacturers' instructions. The NEBioCalculator v1.15.0 web was used to convert ng of the sample into fmol, and barcoded DNA was then pooled into a 1.5-mL microcentrifuge tube, at the final concentration of 100 fmol in 10 µL of T50 buffer. The rapid Adapter ligation step was performed by

mixing the pooled barcoded sample with 1 µL di Rapid Adapter buffer (Oxford Nanopore Technologies Ltd., Oxford, UK.; SQK-PBK004) and incubating for 5 min at room temperature. The priming and loading step was conducted following the protocol instructions. Sequencing was performed on ONT MinION flow cell (FLO-MIN106 R9 Version) connected to an Mk1B device (ONT Ltd., Oxford, UK.; MIN-101B). The sequencing was run up to 48 h, using nanopore software MinKNOW v20.06.4, in live basecall mode with default parameters, so that it uses neural networks to directly translate and barcode the raw signals Fast5 file in barcoded fastq format. The data set was analyzed by cloud-based analysis WIMP application from the EPI2ME platform (Oxford Nanopore Technologies Ltd., Oxford, UK), which is based on Centrifuge which assigns taxonomy by comparing read sequences against the NCBI reference database.

2.2.7. Bioinformatics and Statistical Analysis

We used the tax_name function of the taximize R package to retrieve the class and the family names from the NCBI database. The absolute frequencies of the organism correspond to the WIMP reads, and the absolute frequencies of classes have been calculated summing the reads of the organisms that belong to the same class. To consider the environmental contaminants that could occur during the sequencing, we have associated each human sample with a control experiment (white run), and we have analyzed the control experiments using WIMP. In this way we have identified the possible environmental contaminants related to each sample run. The absolute organism reads of each control experiment have been subtracted from the organism reads of the respective human sample. For hierarchical clustering analysis of the patients, the Bray Curtis was used to calculate the distance among patients and the Ward-D2 aggregation method was used for building the dendrogram through the R package hclust. We used the bootstrap *t*-test to compare the average absolute

frequencies among patient groups. All statistical analyses have been performed using R statistical software v4.2.3.

CHAPTER 3 Results

3.1.Systematic Review

Paper I

3.1.1. Study Screening and Selection

The initial search strategy identified 1 226 records. After applying the inclusion criteria, 668 studies were excluded. The remaining 558 studies were screened based on their titles and abstracts, resulting in the exclusion of 510 records. A full-text evaluation of the remaining 92 studies was then conducted, leading to the exclusion of 81 records. Ultimately, 11 papers were included in the current review. A detailed flowchart of the selection process is presented in Figure 1.

3.1.2. Study characteristics

Eleven articles were included in this review, with their main characteristics outlined in Tables 1 and 2. Specifically, Table 1 presents the characteristics of the included studies along with the methodologies used to analyze the salivary microbiota, while Table 2 summarizes the statistically significant abundance of phyla, genera, and species found in the study groups.

All the included articles were observational studies published between 2017 and 2021 (42–52). However, a significant amount of heterogeneity was observed across the studies, making advanced statistical analysis unfeasible. Of the eleven studies, eight were case–control studies (42,43,46–48,50–52), two were cohort studies (45,49), and one was a cross-sectional cohort study

(44). Geographically, five studies were conducted in Taiwan (44,45,49–51), three in China (42,46,52), one in the USA (43), one in Sudan (47), and one in Japan (48).

The average age of the patients across the studies ranged from 25 to 87 years, with a mean age of approximately 56.8 ± 7.1 years. Only four studies included both male and female participants (179 males and 113 females), while four studies focused exclusively on male patients (n = 534). In three studies, the gender of the participants was not specified. In total, the oral microbiomes of 1,335 patients were analyzed, including 687 OSCC patients and 648 controls. Among the control groups, 480 were healthy individuals, 153 had OPMDs, and 15 had periodontitis.

Several different comparisons were made across the studies. Four studies compared salivary samples from OSCC patients with healthy controls (47,48,50,51). One study analyzed salivary samples from OSCC patients without a control group (49). Three studies compared the oral microbiomes of OSCC patients, healthy controls, and individuals with OPMD (43,45,51). One study focused on comparing the microbiomes of OSCC patients, healthy controls, and individuals with gingivitis or periodontitis (46). Finally, two studies examined the microbiomes of cancer lesions and anatomically matched normal sites within the same patients(44,52).

3.1.3. Sample collection

The main details regarding the salivary collection and analysis techniques are outlined in Table 1. In general, participants were instructed to refrain from eating, drinking, smoking, or performing any oral hygiene procedures for 30 minutes to 2 hours before saliva collection. In one study, participants were

asked to avoid food intake and not brush or floss their teeth for at least 12 hours before sample collection (42). Saliva samples were typically collected following a mouth rinse with 10 mL of sterile saline for 30 seconds. Different techniques were used for sample collection, including sputum collection, oral rinse, and mucosal swabbing. In addition to saliva, some studies also analyzed subgingival plaque, as well as healthy and tumor tissues (42,46). All studies focused on unstimulated saliva, except for one study in which participants were asked to chew gum for 5 minutes before saliva collection (48). After collection, all samples were stored frozen at -80°C, except for one study where swab samples were stored at -20°C (44).

3.1.4. DNA extraction and amplification

The different types of commercial DNA kits used were: QIAamp® DNA Blood Mini Kit, QIAamp® MinElute Virus Spin Kit, Modified QIAGEN® DNA extraction method, FastDNA™ Kit, Gene Prep Star PI-80X device, E.Z.N.A.® soil DNA Kit, and QIAampFast DNA Stool Mini Kit. DNA amplifications were carried out by targeting different hypervariable REGIONS of bacterial 16S rRNA genes. Few studies focused only on a single variable region such as V4 (44,49,51), while others focused on multiple regions, for instance V3–V4 (43,45,46,48), V3–V5 (50), and V4–V5 (42,52).

3.1.5. DNA Sequencing and Analysis of Data

The main data regarding microbial abundance are described in detail in Table 2. At the phylum level, only two studies provided specific counts of the phyla detected, reporting 11 and 12 phyla, respectively(43,52). In the OSCC group, the most abundant phyla identified compared to healthy controls were

Firmicutes, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria*. Zhao et al. observed a significant increase in *Spirochaetes*, *Fusobacteria*, and *Bacteroidetes*, alongside a decrease in *Firmicutes* and *Actinobacteria* in the saliva of OSCC patients (52). In contrast, other studies reported a reduction in *Actinobacteria* and *Fusobacteria* in the OSCC group compared to controls.

At the genus level, four studies defined the number of genera detected, with an average ranging from 36 to 130, and a mean of approximately 92 genera(43,47,48,52). *Fusobacterium* was identified as the most abundant genus in OSCC patients across multiple studies (42–44,46,48–52). In addition, high levels of *Prevotella* and *Alloprevotella* were commonly observed in OSCC patients, while *Streptococcus* levels were generally lower in these patients compared to healthy controls. Zhao et al. identified 17 taxa that were significantly more abundant in OSCC samples compared to controls, including *Megasphaera*, *Stomatobaculum*, *Granulicatella*, *Lautropia*, *Veillonella*, *Streptococcus*, *Scardovia*, *Rothia*, and *Actinomyces*, which were notably more prevalent in the control group (52). Su et al. reported an increase in *Fusobacterium*, *Peptostreptococcus*, *Campylobacter*, *Prevotella*, and *Capnocytophaga* in OSCC patients (44), while Lee et al. highlighted the prevalence of *Prevotella*, *Veillonella*, *Streptococcus*, *Neisseria*, *Rothia*, *Fusobacterium*, *Haemophilus*, *Actinomyces*, and *Porphyromonas* across all groups (51). They also noted the exclusive presence of *Megasphaera* in healthy controls, *Escherichia* in the PMD group, and *Atopobium* in OSCC patients.

Interestingly, the prevalence of *Capnocytophaga* was linked to advanced-stage OSCC, as reported by Yang et al. (49), and was found to be more abundant in patients with OSCC recurrence by Ganly et al (43). The same study also noted a high prevalence of *Veillonella* and *Fusobacterium* in patients with OPMD

lesions. Takahashi et al. observed a significant abundance of *Streptococcus* and a lower level of *Haemophilus* in female OSCC patients compared to male patients (48). Furthermore, *Rothia*, *Haemophilus*, and *Porphyromonas* were found to be less abundant in OSCC patients.

In healthy individuals, Zhou et al. reported the exclusive presence of *Rothia* and higher levels of *Streptococcus*, *Neisseria*, *Prevotella*, *Porphyromonas*, and *Haemophilus* compared to OSCC patients (42). The fungal composition of the oral microbiome was examined by Mohamed et al., who found that *Candida* and *Saccharomyces* were more abundant in the saliva of OSCC patients, while *Cyberlindnera* was more prevalent in healthy controls (47). Notably, *Candida* levels were higher in female OSCC patients than in males.

Regarding species, only three studies provided specific data, with an average range of 172 to 389 species, and a mean of 253.7 species (43,50,52). Zhao et al. identified 14 species as part of the oral mucosal core bacteriome of OSCC patients, reporting increases in *Neisseria flavescens* and *Fusobacterium periodonticum* in OSCC patients compared to healthy controls (52). Su et al. observed higher levels of *Campylobacter* spp. and a decrease in *Streptococcus pneumoniae* in OSCC patients (44). Two studies also reported an increased presence of periodontal pathogens, such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, *Prevotella tanneriae*, *Fusobacterium nucleatum*, and *Prevotella intermedia* in OSCC samples (46,50). Additionally, Hsiao et al. found a decrease in *Streptococcus tigurinus* in the OSCC cohort compared to healthy controls (50).

Chen et al. reported a high abundance of *Capnocytophaga sputigena*, *Catonella morbi*, *Prevotella oris*, *Peptostreptococcus stomatis*, and *Parvimonas micra* in OSCC patients, with *Veillonella parvula*, *Rothia dentocariosa*,

Porphyromonas gingivalis, and *Tannerella forsythia* more abundant in the oral verrucous hyperplasia cohort (OVH) (45). Mohamed et al. identified several species of *Candida*, *Malassezia*, and *Saccharomyces cerevisiae* in all salivary samples, with *C. orthopsilosis* and *C. sake* found exclusively in the saliva of OSCC patients (47).

Three studies also explored the association between salivary microbial composition and clinical factors (45,48,50). Hsiao et al. found that higher levels of *P. intermedia* were associated with alcohol consumption and betel quid chewing, while *F. nucleatum* levels correlated with cigarette smoking (50). Moreover, infrequent tooth brushing and lack of regular dental visits were linked to higher levels of *P. tanneriae*, and no use of dental floss was associated with higher levels of *F. nucleatum*. Takahashi et al. observed a higher prevalence of *Haemophilus* in patients who consumed alcohol (48). Chen et al. reported an association between HPV infection and *Haemophilus* and *Gemella (45)*. Mohamed et al. detected *Lodderomyces* exclusively in smokers, while *Phlebiopsis* and *Filobasidium* were found only in non-smokers. Additionally, OSCC patients with locoregional lymph node involvement had a higher abundance of *Candida* and *Aspergillus*, and a lower abundance of *Malassezia*, compared to those without lymph node involvement (47).

In summary, the oral microbiota in OSCC patients is marked by an abundance of *Fusobacterium* and *Prevotella* species, which are commonly associated with periodontal disease. While it was not possible to identify a single, distinct microbiota associated with OSCC, the findings suggest that specific bacterial genera, particularly periodontal pathogens, may play a role in the pathogenesis of the disease.

3.2.Observational Studies

Paper II

3.2.1 Characteristics of Participants Study

Sputum samples were collected from 31 individuals, including 24 patients diagnosed with OSCC and 7 free from disease. Among the latter group, 3 individuals had OPMD (control disease group), while 4 were healthy (control health group). The main characteristics of the study participants are summarized in Table 3. In the OSCC group, 13 patients were male (54.2%) and 11 were female (45.8%), with a mean age of 65.5 ± 13.9 years (range: 32–88 years). The most affected site was the tongue border (41.7%), followed by the buccal mucosa (25%), the floor of the mouth (12.5%), the lower gingiva (12.5%), the upper gingiva (4.1%), and the hard palate (4.1%). Based on the TNM staging system, 7 patients were classified as stage I (29.2%), 6 as stage II (25%), 5 as stage III (20.8%), and 6 as stage IV (25%). Overall, 13 patients were categorized as having early-stage OSCC (54.2%), while 11 were classified as advanced-stage (45.8%). Regarding lifestyle factors, 7 patients (29.2%) were current smokers, including 3 who were also alcohol consumers (12.5%), and 3 were former smokers (12.5%). Clinical examinations identified incongruous dental prostheses in 4 patients (16.7%), which may have contributed to mechanical trauma. Information on missing and decayed teeth was not available. Additionally, 6 patients used dental prostheses, of whom 4 were in the OSCC group and 2 were healthy individuals.

3.2.2 Microbiota Composition

In this study, the taxonomy of salivary samples was analyzed using the cloud-based WIMP application on the EPI2ME platform. WIMP software version 20.06.4 was utilized to perform an in-silico estimation of organism read counts

across samples (relative frequencies are presented in Figure 2). The average read counts were then compared between OSCC and control groups to identify microorganisms with significant differences in abundance. As previously reported in the literature, ONT confirmed the predominance of periodontal bacteria, such as *Prevotella*, in OSCC patients. Specifically, this study identified *Prevotella melaninogenica*, *Prevotella intermedia*, and *Prevotella jejuni* as the most abundant in the OSCC group. Average counts of microbial classes were compared between OSCC and control groups using a non-parametric bootstrap t-test, after excluding classes found in fewer than five samples and with a total count below five. Sixteen microorganism classes demonstrated statistically significant differences ($p < 0.05$), including an increase in *Chlamydiia, Tissierellia, Calothrix, Leotiomycetes, Firmicutes,* and *Zetaproteobacteria,* and a decrease in *Saccharibacteria* within the OSCC group. However, due to low statistical power, the Benjamini-Hochberg adjusted p-values exceeded the 5% threshold. Hierarchical cluster analysis of read counts did not reveal distinct clustering patterns among OSCC patients (Figure 3). A Venn diagram illustrated shared and unique microorganisms across groups, identifying 714 microorganisms common to all groups, 3,333 unique to the OSCC group, 59 specifics to the disease control group, and 238 exclusives to the healthy control group (Figure 4a). Similarly, for microbial classes, 59 were common to all groups, 72 were exclusive to the OSCC group, 2 to the disease control group, and 4 to the healthy control group (Figure 4b). The Shannon and inverse-Simpson biodiversity indices were calculated to assess intra-sample variability. These indices, widely used to quantify microbiome diversity, indicated similar intra-sample variability across patient groups. To further explore variations within the OSCC group, patients were

stratified into early and advanced stages. Comparisons of average read counts between early and advanced OSCC stages did not reveal significant differences in organism abundance. A Venn diagram showed that 3,186 microorganisms were shared between early and advanced stages. The early-stage group uniquely featured 873 microorganisms, with 53 shared between early and disease control groups, 175 shared between early and healthy control groups, and 14 shared across all three groups. Conversely, the advanced stage group had 1,132 unique microorganisms, with 65 shared between advanced and disease control groups, 241 shared between advanced and healthy control groups, and 23 common across all groups. For microbial classes, 137 were shared between early and advanced stages. The early-stage group had 15 unique classes, with one shared between early and healthy controls, and none shared with disease controls. In the advanced stage group, 17 classes were unique, 6 were shared with healthy controls, and 1 was shared with disease controls.

Paper III

3.3.1 Characteristics of Participants Study

Sputum samples were collected from 16 patients affected by OSCC before surgical treatment, 16 patients affected by OSCC after surgical treatment, 5 patients with OPMD and 5 healthy patients. The main characteristics of the study participants are summarized in Table 4.

Among patients affected by OSCC, 8 were males and 8 females with a mean age of 63.2 ± 12.9 years. Among control patients, 5 were males and 5 females with a mean age of 63 ± 18.6 years. The border of the tongue was the most affected site by OSCC, followed by buccal and masticatory mucosa. Regarding

the TNM staging, 3 patients were diagnosed with stage I, 6 with stage II, 4 patients with stage III, and 3 patients with stage IV.

3.3.2 Microbiota Composition

Despite no statistically significant data was observed, *Bacteroidia* and *Fusobacteriia* were the most common classes identified in OSCC patients before surgical therapy and *Bacilli* was the most common class identified in OSCC patients after surgical therapy (Figure 5).

CHAPTER 4 Discussion

This dissertation provides an integrated perspective on the composition and role of the salivary microbiota in the development and progression of OSCC. The analysis is informed by findings from both a systematic review of existing literature and novel observational data employing ONT (35,53). Evidence consistently highlights alterations in the salivary microbiota of OSCC patients, with an increased prevalence of periodontal pathogens such as *Fusobacterium* and *Prevotella*. The systematic review revealed that these genera, along with other anaerobic bacteria like *Porphyromonas*, dominate the microbiota profiles of OSCC patients. These findings align with observational data that identified *Prevotella melaninogenica*, *Prevotella intermedia*, and *Prevotella jejuni* as the most abundant species in OSCC samples. Such microbial shifts suggest a state of dysbiosis, characterized by a loss of microbial diversity and an overrepresentation of pathogenic taxa.

The use of ONT in the observational study provided unique insights, with its capacity for long-read sequencing enabling the identification of less abundant taxa that may have been overlooked in previous research. Interestingly, taxa such as *Chlamydiia*, *Tissierellia*, and *Zetaproteobacteria* were identified as significantly enriched in OSCC patients, suggesting novel microbial signatures associated with oral carcinogenesis. Conversely, a reduction in beneficial

microbes, such as *Saccharibacteria*, further underscores the imbalance in the oral microbial ecosystem.

The association between alterations of the oral microbiota and carcinogenesis has been studied for years, not only locally in the head and neck region but also in distant organs (e.g., pancreatic and gastrointestinal carcinomas) (37–39). Three paradigms have been proposed to describe the pathogenic processes that involve the microbiota in the development of oral cancer:

a) Chronic pro-inflammatory processes (54);
b) Direct anti-apoptotic action (55);
c) Production of carcinogenic metabolites (56).

Chronic pro-inflammatory processes

Oral bacteria, particularly anaerobic species, such as *Porphyromonas, Prevotella* and *Fusobacterium*, are key contributors to periodontitis (57). These pathogenic bacteria stimulate the production of inflammatory mediators, including cytokines such as interleukin-1ß (IL-1ß), IL-6, IL-17, IL-23, tumor necrosis factor-α (TNF-α), and matrix metalloproteinases (MMPs), with MMP-8 and MMP-9 being notably involved. These inflammatory mediators have harmful effects on fibroblasts, epithelial and endothelial cells, and components of the extracellular matrix(58,59).

Within periodontal tissues, IL-1ß is released by immune cells under the influence of lipopolysaccharides (LPS), a major component of the outer membrane of Gram-negative bacteria. IL-1ß not only promotes bone resorption but also enhances the release of other inflammatory factors, such as prostaglandins, IL-6, TNF, various MMPs, and pro-angiogenic mediators like vascular endothelial growth factor (VEGF) (60,61). These factors collectively

contribute to creating an inflammatory microenvironment that facilitates angiogenesis and tumor progression. Elevated levels of IL-1ß have also been linked to increased tumor cell migration and invasion, reduced E-cadherin expression, and the development of more aggressive tumor phenotypes (62,63). IL-6, another cytokine released by periodontal cells in response to bacterial LPS and inflammatory stimuli like IL-1ß and TNF, similarly plays a dual role. It promotes bone resorption and influences cell cycle genes to suppress apoptosis (64). Additionally, IL-6 exacerbates oxidative stress at the mitochondrial level, potentially leading to mitochondrial damage. It also increases the expression of MMPs, which facilitate tissue invasion and metastasis and enhances the production of inflammatory cytokines and endothelial adhesion molecules, thereby promoting the attachment of tumor cells to endothelial cells (65,66). TNF-α, predominantly released during chronic inflammatory responses to LPS, is another critical cytokine in this context (67). It stimulates the production of prostaglandins and reactive oxygen species (ROS), which can cause DNA damage and contribute to tumorigenesis. TNF-α also upregulates MMPs, aiding in tissue invasion by tumor cells, and promotes the release of additional pro-inflammatory cytokines such as IL-8 and VEGF. Together, these factors create a pro-inflammatory and pro-tumorigenic environment that fosters tumor development and progression (68–71).

Direct anti-apoptotic action

Porphyromonas gingivalis influences the cellular replication cycle through various mechanisms (72). It promotes the activation of signaling pathways that regulate mitochondrial apoptosis, accelerates progression through the S phase of the cell cycle, and reduces the expression of the tumor suppressor protein

p53 (73,74). Additionally, *P. gingivalis* inhibits apoptosis in gingival epithelial cells by increasing the release of anti-apoptotic molecules, such as Bcl-2, and secreting anti-apoptotic enzymes like nucleoside diphosphate kinase. These processes enhance mitochondrial reactive oxygen species (ROS) production, further contributing to cellular dysfunction (75,76).

Moreover, *P. gingivalis* produces a cysteine protease called gingipain, which damages the pro-enzyme form of MMP-9, activating it. This activation leads to the degradation of the cellular basement membrane, facilitating tumor cell migration and invasion into surrounding tissues (73). Similarly, the lipopolysaccharides (LPS) produced by *Fusobacterium nucleatum* stimulate the release of inflammatory cytokines, including IL-1ß, IL-6, and TNF-α. Chronic tissue inflammation triggered by these cytokines not only leads to the development of periodontitis and bone resorption but also activates various anti-apoptotic mechanisms (77)

F. nucleatum produces an adhesin called FadA, which binds to E-cadherins on tumor cells, enhancing Wnt signaling, inflammatory cytokine activation, oncogene expression, and neoplastic cell proliferation (78). Additionally, *F. nucleatum* activates p38 signaling, resulting in the secretion of MMP-9 and MMP-13, both of which play critical roles in tissue invasion and metastasis (58,59,79,80).

Production of carcinogenic metabolites

The carcinogenic effects of bacterial metabolites are less well understood, but several key compounds have been identified, including reactive oxygen species (ROS), reactive nitrogen species (RNS), volatile sulfur compounds (VSCs), and organic acids. The microbial metabolism of alcohol into acetaldehyde also

plays a significant role in carcinogenesis.

During inflammatory responses, cytokines such as IL-6, TNF-α, and TGF-ß stimulate epithelial cells and the immune system to release ROS and RNS. These reactive molecules can activate enzymes like NADPH oxidase and nitric oxide synthase (NOS), leading to the production of hydrogen peroxide (H_2O_2) and nitrogen dioxide (NO_2) (81–83). Chronic inflammation and the associated production of free radicals have been shown to promote the development and progression of neoplasms. Specific bacterial species, including *Streptococcus oralis*, *S. mitis*, and *S. sanguinis*, are known to produce H_2O_2 and NO_2, linking them to these processes. Other bacteria, such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, produce volatile sulfur compounds (VSCs), such as hydrogen sulfide. These compounds are not only toxic and critical in the progression of periodontitis but are also genotoxic agents that facilitate the development and accumulation of genetic mutations (72,84,85). Additionally, bacteria from genera such as *Lactobacillus*, *Lactococcus*, and *Streptococcus* produce acids like lactic acid, which lower tissue pH. This acidification contributes to the creation of a hypoxic tumor microenvironment, enhancing metastatic potential (86–88). Furthermore, certain microorganisms possess alcohol dehydrogenase (ADH) enzymes, enabling them to metabolize alcohol into acetaldehyde, a known carcinogen. The primary species involved in this process include *S. mitis*, *S. oralis*, *S. salivarius*, *S. sanguini*s, and *Candida spp* (89,90).

While the findings from both the systematic review and the observational study underscore the potential diagnostic value of salivary microbiota analysis, some

limitations must be acknowledged. The systematic review highlighted significant methodological heterogeneity across studies, including variations in sample collection, DNA extraction, and sequencing approaches, which complicates direct comparisons and meta-analyses. A limitation observed is the high cost of salivary microbiota composition analysis.

For this reason, observational studies, despite employing state-of-the-art sequencing technology, faced challenges related to small sample size and limited statistical power. Future research should prioritize longitudinal studies with standardized methodologies to establish causal relationships between microbial dysbiosis and OSCC. Additionally, integrating multi-omics approaches, such as metabolomics and proteomics, with microbiome analysis could provide a more comprehensive understanding of the functional roles of the salivary microbiota in OSCC. The complex relationship between oral microbiota and OSCC represents a promising frontier in cancer research, offering new perspectives on pathogenesis, prevention, and patient management. While the current evidence highlights significant alterations in the salivary microbiota composition in OSCC patients, identifying a definitive microbial signature remains a challenge.

In conclusion, the oral microbiota serves as both a mirror and a mediator of oral and systemic health. If future studies confirm a direct association between periodontal pathogens (e.g. *Fusobacterium* and *Prevotella*) and OSCC, the role of periodontal health management could become pivotal. Regular monitoring and control of periodontal status during follow-up visits may not only reduce localized inflammatory insults but also slow the progression of OPMD and OSCC. The prospect of integrating periodontal care into cancer follow-up protocols underscores the significance of oral health in systemic disease

management.

CHAPTER 5 Figures and Tables

Figure 1. PRISMA 2020 flow chart.

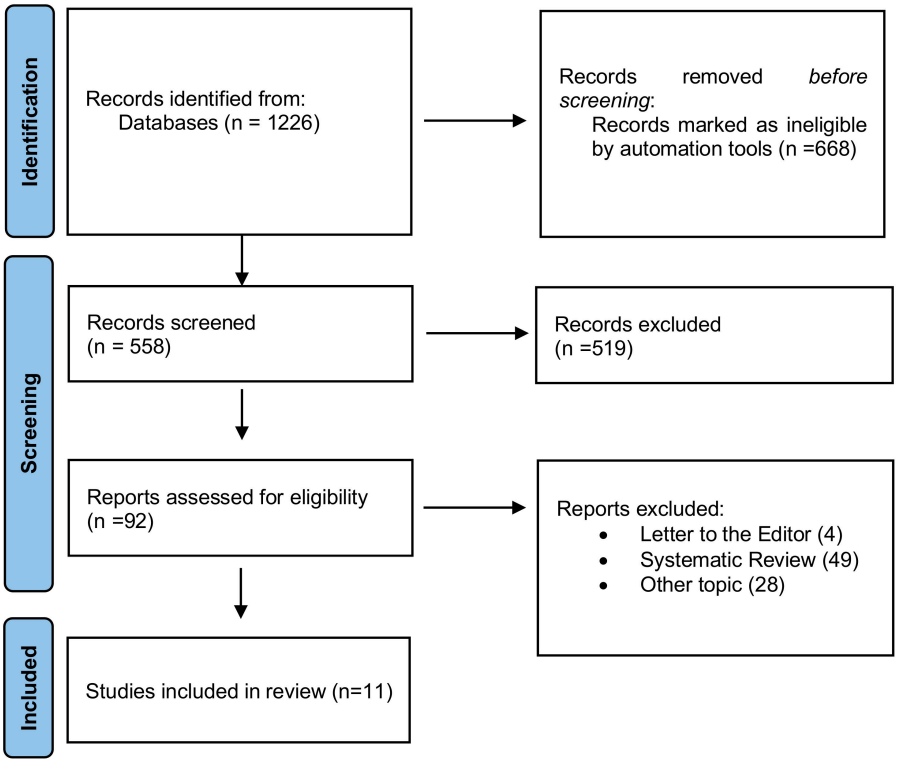

Figure 2. Relative frequencies of organism classes over patients.

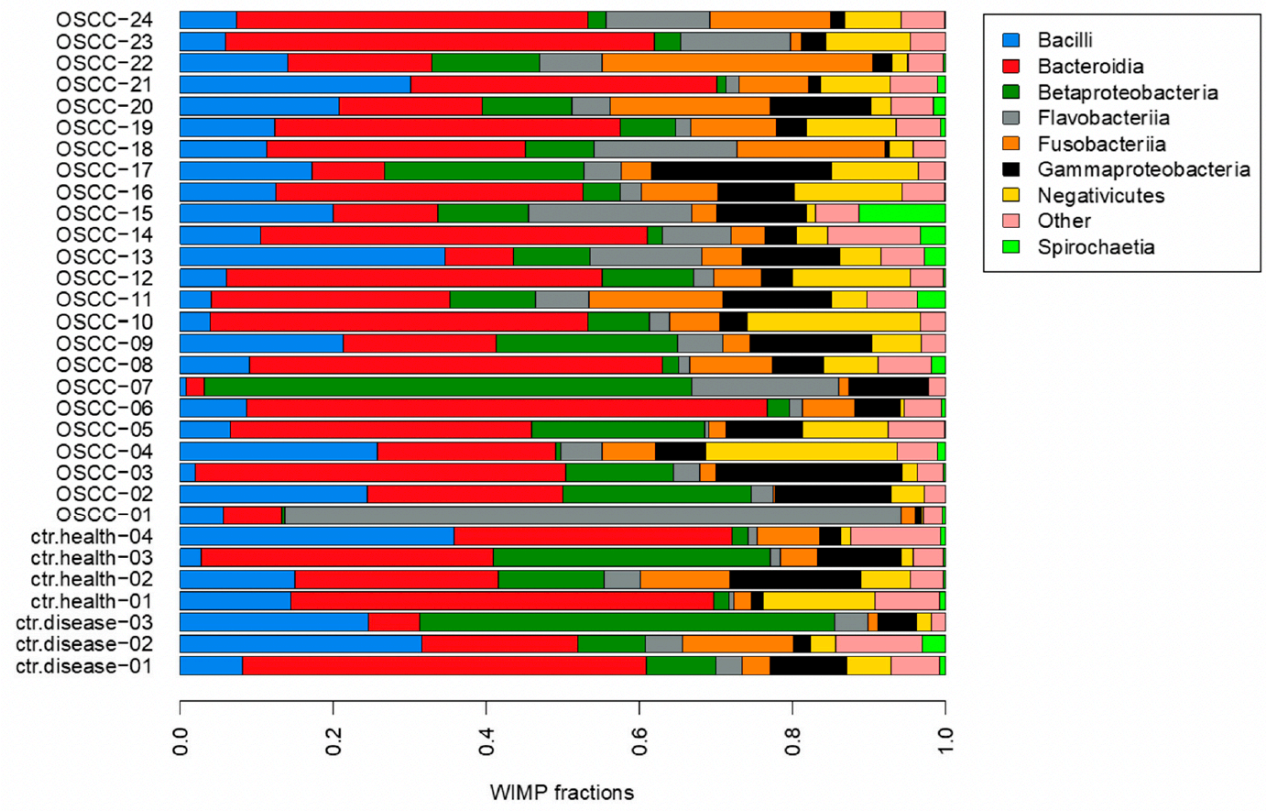

Figure 3. Hierarchical clustering over patients.

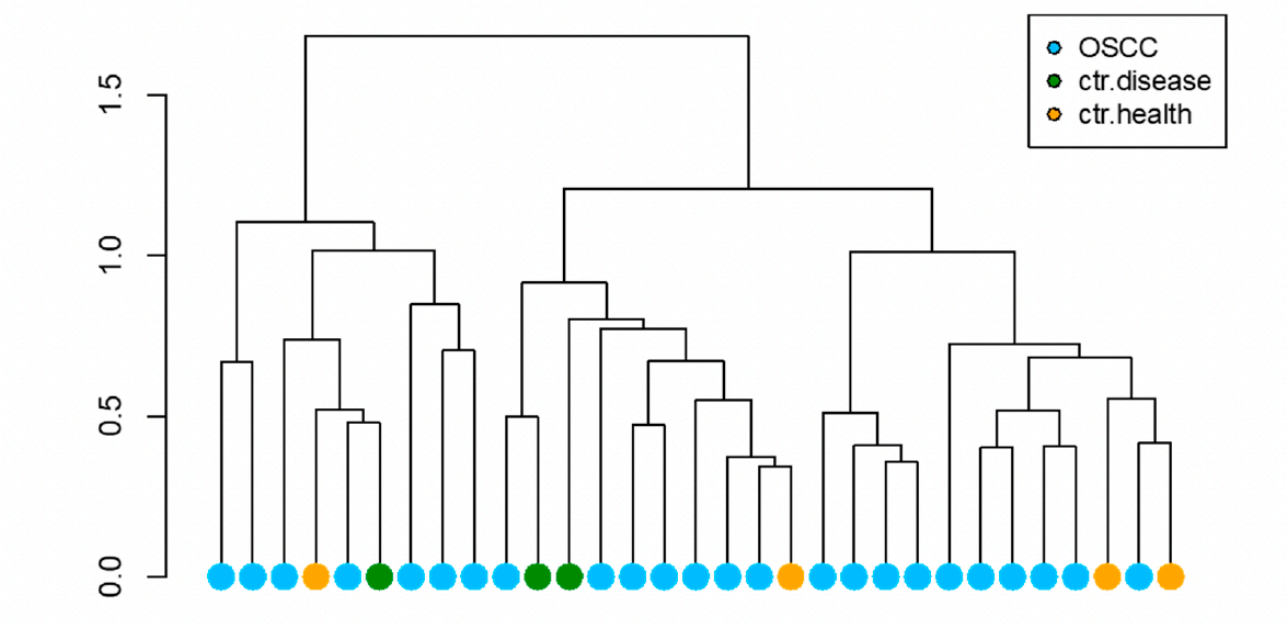

Figure 4. Organisms and class overlap among groups. (a) Organisms overlaps among groups. (b) Class overlaps among groups.

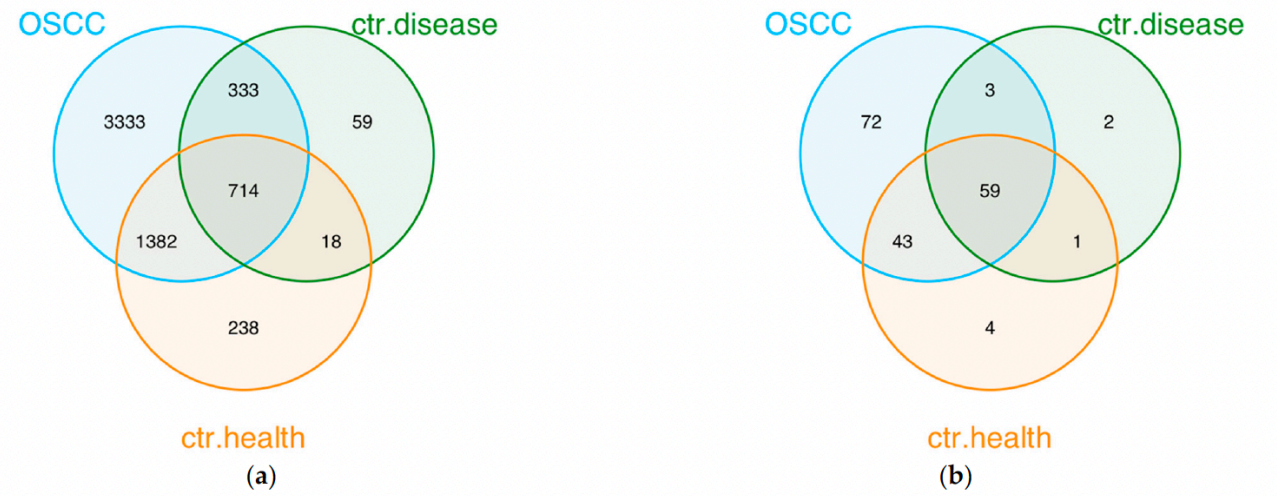

Figure 5. Relative frequencies of organism classes over patients.

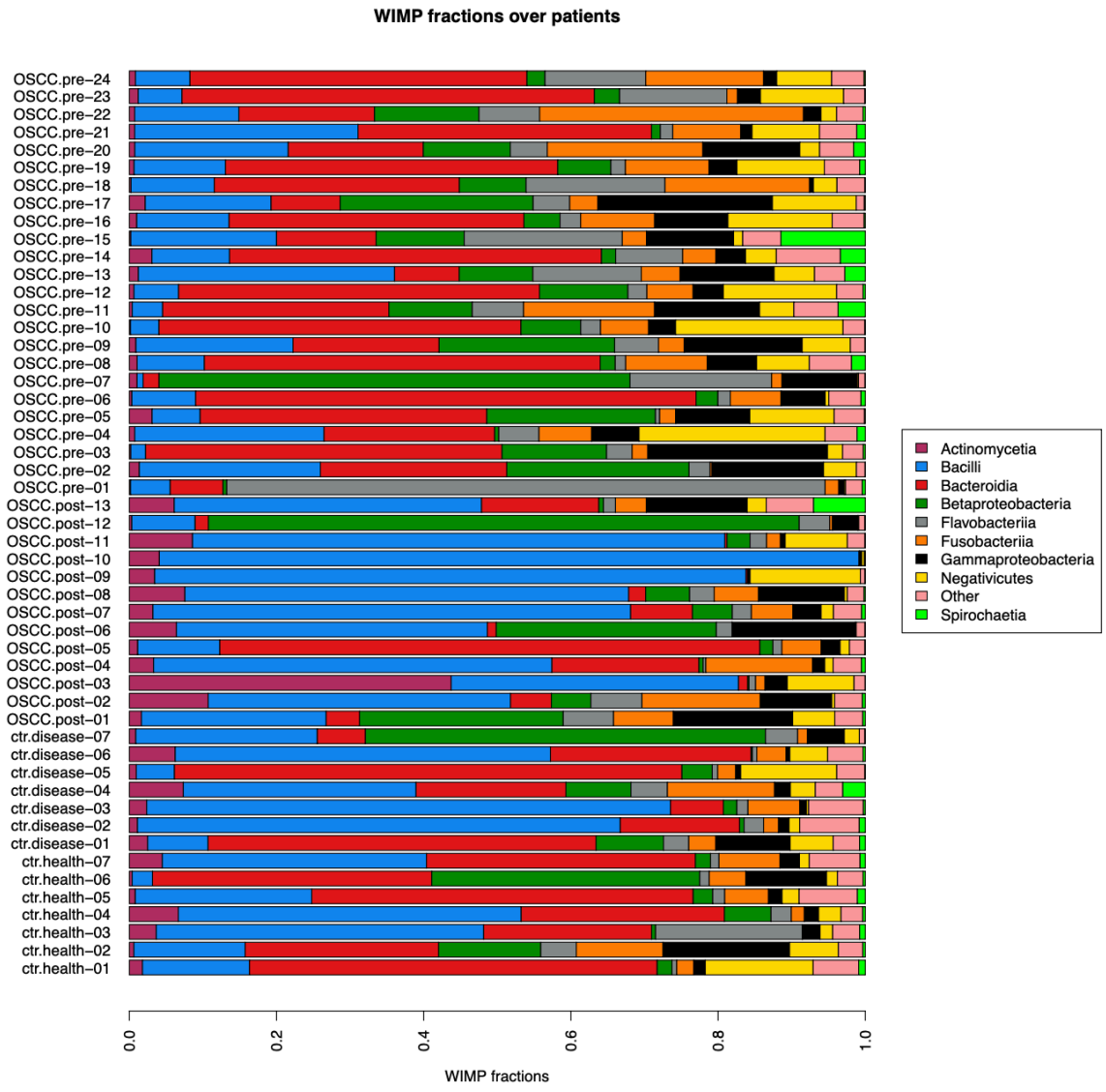

Table 1. Characteristics of the included studies and the methodology employed for the analysis of the salivary microbiota.

N.	Author, Year	Country	Study Design	N. of Case	N. of Control	Sample	Methods of DNA Extraction	DNA Amplification	Sequencing	Reference Database
1	Lee WH, 2017 [25]	Taiwan	Case-control	125	251	Sputum	QIAamp® DNA Blood Mini Kit	V4	Illumina MiSeq System	SILVA
2	Zhao H, 2017 [26]	China	Case-control	40	40 *	Oral swabs	QIAamp® DNA Blood Mini Kit	V4–V5	Illumina MiSeq System	RDP
3	Hsiao JR, 2018 [24]	Taiwan	Case-control	138	151	Sputum	QIAamp® MinElute Virus Spin Kit	V3–V5	Illumina MiSeq System	RDP
4	Yang SF, 2018 [27]	Taiwan	Cohort study	39	No control group	Sputum	QIAamp® DNA Blood Mini Kit	V4	Illumina MiSeq System	Greengenes
5	Mohamed N, 2019 [28]	Sudan	Case-control	59	13	Sputum	FastDNA™ Kit	fungal ITS2 region	Illumina MiSeq System	UNITE
6	Takahashi Y, 2019 [29]	Japan	Case-control	60	80	Sputum	Gene Prep Star PI-80X device	V3–V4	Illumina MiSeq System	SILVA 128
7	Li Y, 2020 [30]	China	Case-control	10	30	Saliva, subgingival plaque, tumour and healthy surface	QIAampFast DNA Stool Mini Kit	V3–V4	Illumina MiSeq System	SILVA
8	Chen JW, 2021 [31]	Taiwan	Cohort study	27	48	Sputum	QIAamp® DNA Blood Mini Kit	V3–V4	Illumina MiSeq System	HOMD
9	Ganly I, 2021 [32]	USA	Case-control	18	20	Ora rinse	Modified QIAGEN® DNA extraction method	V3–V4	454 FLX platform	Greengenes
10	Su SC, 2021 [33]	Taiwan	Cross-sectional	116	116 *	Oral swabs	QIAamp® DNA Blood Mini Kit	V4	Illumina MiSeq System	SILVA
11	Zhou X, 2021 [34]	China	Case-control	47	48	Saliva, subgingival plaque, tumour and healthy surface	E.Z.N.A.® soil DNA Kit	V4–V5	Illumina MiSeq System	SILVA

* Studies that analysed contralateral normal regions of the same patients as control group.

Table 2. Description of the statistically significant results about phyla, genera, and species of the studies included.

Author, Year	N. of Phyla Detected	Phyla		N. of Genera Detected	Genera		N. of Species Detected	Species	
		↑ in OSCC Group	↓ in OSCC Group		↑ in OSCC Group	↓ in OSCC Group		↑ in OSCC Group	↓ in OSCC Group
Zhao H, 2017 [26]	11	Spirochaetes, Fusobacteria, Bacteroidetes	Firmicutes, Actinobacteria	130	Mycoplasma, Treponema, Campylobacter, Eikenella, Centipeda, Lachnospiraceae, Alloprevotella, Fusobacterium, Selenomonas, Dialister, Peptostreptococcus, Filifactor, Peptococcus, Catonella, Parvimonas, Capnocytophaga, and Peptostreptococcaceae	Megasphaera, Stomatobaculum, Granulicatella, Lautropia, Veillonella, Streptococcus, Scardovia, Rothia, and Actinomyces	389	n.d.	n.d.
Hsiao JR, 2018 [24]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	120	*P. tannaerae* and *F. nucleatum*	n.d.
Yang SF, 2018 [27]	n.d.	n.d.	n.d.	n.d.	Capnocytophaga *	n.d.	n.d.	n.d.	n.d.
Takahashi Y, 2019 [29]	n.d.	n.d.	n.d.	85	Peptrostreptococcus, Fusobacterium, Alloprevotella, Capnocytophaga	Rothia, Haemophilus	n.d.	n.d.	n.d.
Ganly I, 2021 [32]	12	n.d.	n.d.	116	Fusobacterium, Prevotella, Alloprevotella	Streptococcus	172	n.d.	n.d.
Su SC, 2021 [33]	n.d.	n.d.	n.d.	n.d.	Fusobacterium, Peptostreptococcus, Campylobacter, Prevotella, Capnocytophaga	Streptococcus	n.d.	*Campylobacter* spp.	*Streptococcus pneumoniae*

* Statistically significant differences only in OSCC diagnosed at the late stage. ↑ phyla, genera, and species increased in the OSCC group. ↓ phyla, genera, and species reduced in the OSCC group

Table 3. Characteristics of patients enrolled in the study (I observational study)

Characteristics	**Patients Affected by OSCC**	**Patients OSCC free**
	24	7
Gender		
Male	13 (54.2)	5 (71.4)
Female	11 (45.8)	2 (28.6)
Demographic data		
Median age	64.5	55
Q1-Q3	53.5 – 79.7	30 - 76
Mean age ± SD	65.5 ± 13.9	51.4 ± 19.2
Risk factors		
Tobacco smoking	4 (16.6)	0
Tobacco smoking and alcohol consumption	3 (12.5)	0
Former smoking	3 (12.5)	0
Characteristics of Patients Affected by OSCC (n.24)		
OSCC anatomical site		
Border of the tongue	10 (41.7)	
Buccal mucosa	6 (25)	
Floor of the mouth	3 (12.5)	
Lower gingiva	3 (12.5)	
Upper gingiva	1 (4.1)	
Hard palate	1 (4.1)	
TNM staging		
Stage I	7 (29.2)	
Stage II	6 (25)	
Stage III	5 (20.8)	
Stage IV	6 (25)	

Table 4. Characteristics of patients enrolled in the study (II observational study)

Characteristics	**Patients Affected by OSCC**	**Patients OSCC free**
	16	10
Gender		
Male	8 (50)	5 (50)
Female	8 (50)	5 (50)
Demographic data		
Median age	61	71
Q1-Q3	55 – 74.7	42.5 – 77.7
Mean age ± SD	63.2 ± 12.9	63 ± 18.6
Risk factors		
Tobacco smoking	4 (25)	1 (6.2)
Tobacco smoking and alcohol consumption	2 (12.5)	0
Former smoking	3 (18.7)	0
Characteristics of Patients Affected by OSCC (n.16)		
OSCC anatomical site		
Border of the tongue	9 (56.2)	
Buccal mucosa	5 (31.2)	
Upper gingiva	1 (6.2)	
Hard palate	1 (6.2)	
TNM staging		
Stage I	3 (18.7)	
Stage II	6 (37.5)	
Stage III	4 (25)	
Stage IV	3 (18.7)	

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Scientific Products (bound)

Publications discussed in this thesis:

I. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Panzarella V, Di Fede O, Tripodo C, Campisi G. Salivary Microbiota Composition in Patients with Oral Squamous Cell Carcinoma: A Systematic Review. Cancers (Basel). 2022 Nov 4;14(21):5441. doi: 10.3390/cancers14215441. PMID: 36358859; PMCID: PMC9656014.

II. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Cancila V, Tripodo C, Campisi G. No Clear Clustering Dysbiosis from Salivary Microbiota Analysis by Long Sequencing Reads in Patients Affected by Oral Squamous Cell Carcinoma: A Single Center Study. Cancers (Basel). 2023 Aug 22;15(17):4211. doi: 10.3390/cancers15174211. PMID: 37686487; PMCID: PMC10486367. ***Best paper award 2024 of Department of Precision Medicine in Medical, Surgical and Critical Care, University of Palermo, Italy***

III. Mauceri R, Coppini M, Vacca D, Bertolazzi G, Belmonte B., Campisi G. Oral Microbiota composition in patients affected by Oral Cancer Before and After surgical treatment: A Single Center Pilot Study. *Under submission.*

Publications not discussed in this thesis:

1. Diagnostic delay of oral squamous cell carcinoma and the fear of diagnosis: A scoping review
   Mauceri R, Bazzano M, **Coppini M**, Tozzo P, Panzarella V, Campisi G.
   Front Psychol. 2022 Nov 3;13:1009080. doi: 10.3389/fpsyg.2022.1009080. PMID: 36405204; PMCID: PMC9669962.

2. MRONJ in breast cancer patients under bone modifying agents for cancer treatment-induced bone loss (CTIBL): a multi-hospital-based case series.
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3. Oral Secondary Syphilis in an HIV-Positive Transgender Patient: A Case Report and Review of the Literature.
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4. Polymorphous Adenocarcinoma: A Systematic Review of the Literature and Presentation of Two Cases in a Less-Considered Anatomical Site.
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5. Challenges in the diagnosis of oral squamous cell carcinoma mimicking medication-related osteonecrosis of the jaws: a multi-hospital-based case series.
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6. Efficacy of topical agents in oral mucositis prevention: Systematic review and network meta-analysis.
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7. Drug assumption and awareness about adverse drug reactions. The right to know. The case of the Bone-Modyfing Agents: a systematic review
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8. Letter to Editor regarding "Why are osteoporosis patients treated with antiresorptive therapies considered like oncology patients regarding their oral health care?"
   Campisi G, Mauceri R, **Coppini M**, Bedogni A, Bertoldo F, Fusco V
   Osteoporos Int. 2024 Aug 16. doi: 10.1007/s00198-024-07192-4. Epub ahead of print. PMID: 39150534.

9. Aesthetic lip filler augmentation is not free of adverse reactions: lack of evidence-based practice from a systematic review
   Coppini M, Caponio VCA, Mauceri R, Pizzo G, Mauceri N, Lo Muzio L and Campisi G (2024).

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10. Oral epithelial dysplasia with lichenoid features shares proteomic overlap with oral epithelial dysplasia without lymphocytic immune response.
    Alejandro I. Lorenzo-Pouso, Elina Pérez-García, Susana B. Bravo, **Martina Coppini**, Fábio França-Vieira-E-Silva, Cintia M. Chamorro-Petronacci, Vito Carlo Alberto Caponio, María Elena Padín-Iruegas, Irene Lafuente-Ibañez-de-Mendoza, Pilar Gándara-Vila, Mario Pérez-Sayáns, Andrés Blanco-Carrión,
    Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 2024, ISSN 2212-4403, https://doi.org/10.1016/j.oooo.2024.10.085.

Abstracts presented at national conferences:

2022:

I. Malignant transformation of oral leukoplakia in oral squamous cell carcinoma: a case report. **M. Coppini**, F. Buttacavoli, V. Panzarella, G. Seminara, G. Campisi, R. Mauceri. Abstract presentation - Oral Potentially Malignant Disorders (OPMD), Brescia, February 25, 2022

II. Optical Coherence Tomography (OCT) and Oral Potentially Malignant Disorders: critical review on potential diagnostic patterns.
F. Buttacavoli, **M. Coppini**, G. Seminara, R. Mauceri, O. Di Fede, G. Campisi, V. Panzarella. Abstract presentation - Oral Potentially Malignant Disorders (OPMD). Brescia, February 25, 2022

III. A case report of 2 metachronous oral squamous cell carcinomas and 1 recurrence in 7 years. **M. Coppini**, F. Buttacavoli, M. Bazzano, G. La Mantia, P. Tozzo, F. Spirito, L. Lo Muzio, O. Di Fede, G. Campisi, R. Mauceri. DentalCadmos – Pathology and oral medicine, page 32; doi: 10.19256/abstract.cduo.15.2022. Proceeding del XXIX Congresso Nazionale CDUO. Bologna, April 7-9 2022

IV. Oral squamous cell carcinoma and patients delay: a systematic review.
M. Bazzano, R. Mauceri, **M. Coppini,** G. La Mantia, P. Tozzo, V.C.A. Caponio, E. Lo Muzio, F. Buttacavoli, V. Panzarella, G. Campisi. DentalCadmos – Pathology and oral medicine, page 314. doi: 10.19256/abstract.cduo.15.2022. Proceeding del XXIX Congresso Nazionale CDUO. Bologna, April 7-9 2022

V. Optical coherence tomography (OCT) patterns for oral leukoplakia and lichen planus.
F. Buttacavoli, **M. Coppini**, G. La Mantia, R. Mauceri, P. Tozzo, F. Spirito, L. Lo Muzio, O. Di Fede, G. Campisi, V. Panzarella. DentalCadmos – Pathology and oral medicine, page 317 doi: 10.19256/abstract.cduo.15.2022. Proceeding del XXIX Congresso Nazionale CDUO. Bologna, April 7-9 2022

VI. "IO POSSO" (pazienti oncologici e supporto salute orale): a focus group for cancer patients. G. La Mantia, M. Bazzano, F. Buttacavoli, **M. Coppini**, G. Troiano, M. Dioguardi, K. Zhurakivska, V. Panzarella, O. Di Fede. DentalCadmos – Pathology and oral medicine, page

328. doi: 10.19256/abstract.cduo.15.2022. Proceeding del XXIX Congresso Nazionale CDUO. Bologna, April 7-9 2022

VII. Transformation of a leukoplakia located on the lateral margin of the tongue into oral squamous cell carcinoma: a case report.
M. Coppini, R. Mauceri R, M. Bazzano, F. Buttacavoli, G. La Mantia, O. Di Fede, V. Panzarella, G. Campisi. Congresso regionale ANDI Sicilia. Agrigento, July 2 2022.

VIII. Salivary microbiota alterations in patients with oral squamous cell carcinoma: a systematic review
M. Coppini, F. Buttacavoli, G. La Mantia, P. Tozzo, L. Togni, F. Spirito, M. Mascitti, V. Panzarella, R. Mauceri
Simposio internazionale SIPMO in associazione con European Association Of Oral Medicine: La saliva e le patologie salivari in odontoiatria
Parma, November 25-26 2022
Proceeding abstract in atti di convegno pubblicato su rivista: special issue of Medicina Oral, Patologia Oral y Cirugia Bucal

IX. In vivo Optical Coherence Tomography for the evaluation of ultrastructural site-specific characteristics of healthy oral mucosa
Buttacavoli F., **Coppini M.,** La Mantia G., Bazzano M., Lo Muzio L., Spirito F., Di Fede O., Mauceri R., Panzarella V.
Simposio internazionale SIPMO in associazione con European Association Of Oral Medicine: La saliva e le patologie salivari in odontoiatria
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Proceeding abstract in atti di convegno pubblicato su rivista: special issue of Medicina Oral, Patologia Oral y Cirugia Bucal

X. Automated Detection and Classification of Oral Lesions Using Deep Learning: results of a Narrative Review
La Mantia G., **Coppini M**., Buttacavoli F., Tozzo P., Mascitti M., Spirito F., Panzarella V., Campisi G., Di Fede O.
Simposio internazionale SIPMO in associazione con European Association Of Oral Medicine: La saliva e le patologie salivari in odontoiatria
Parma, November 25-26 2022
Proceeding abstract in atti di convegno pubblicato su rivista: special issue of Medicina Oral, Patologia Oral y Cirugia Bucal

XI. Oral Manifestation of Langerhans cell histiocytosis recurrence: a case report
OS Pometti, G. Seminara**, M. Coppini**, G. La Mantia, A. Santarelli, E. Lo Muzio, R. Mauceri, G. Oteri, G. Campisi
Simposio internazionale SIPMO in associazione con European Association Of Oral Medicine: La saliva e le patologie salivari in odontoiatria
Parma, November 25-26 2022
Proceeding abstract in atti di convegno pubblicato su rivista: special issue of Medicina Oral, Patologia Oral y Cirugia Bucal

2023:

XII. The most common genera detected in salivary microbiota of patients affected by oral cancer.
M. Coppini, M. Bazzano, F. Buttacavoli, G. La Mantia, M. Mascitti, G. Troiano, L. Lo Muzio, V. Panzarella, G. Campisi, R. Mauceri
XXX Congresso Nazionale CDUO - La moderna odontoiatria tra tecnologie avanzate e umanizzazione.
Catania, April 20-22 2023
Proceeding abstract in atti di convegno pubblicato su rivista: Supplemento Dental Cadmos

XIII. Oct-trained learning algorithms for oral Carcinogenesis: project phase I
Buttacavoli F., Seminara G., **Coppini M**., Bazzano M., Dioguardi M., Zhurakivska K., Tozzo P., Campisi G., Panzarella V.
XXX Congresso Nazionale CDUO - La moderna odontoiatria tra tecnologie avanzate e umanizzazione.
Catania, April 20-22 2023
Proceeding abstract in atti di convegno pubblicato su rivista: Supplemento Dental Cadmos
WINNER OF THE BEST POSTER AWARD IN THE ORAL PATHOLOGY AND MEDICINE CATEGORY

XIV. Location and gender differences in MRONJ: a meta- analysis and trial sequential analysis.
F. Spirito, M. Dioguardi, V.C.A. Caponi, L. Memè, M. Mascitti, **M. Coppini**, G. La Mantia, K. Zhurakivska, L. Lo Muzio
XXX Congresso Nazionale CDUO - La moderna odontoiatria tra tecnologie avanzate e umanizzazione.
Catania, April 20-22 2023
Proceeding abstract in atti di convegno pubblicato su rivista: Supplemento Dental Cadmos
WINNER OF THE BEST POSTER AWARD IN THE ORAL PATHOLOGY AND MEDICINE CATEGORY

XV. Levels of anxiety and depression in patients with potentially malignant oral disorders awaiting biopsy.
Bazzano M., Buttacavoli F., Seminara G., **Coppini M**., Santarelli A., Spirito F.,
Caponio V.C.A., Panzarella V., Di Fede O., Campisi G.
XXX Congresso Nazionale CDUO - La moderna odontoiatria tra tecnologie avanzate e umanizzazione.
Catania, April 20-22, 2023
Proceeding abstract in atti di convegno pubblicato su rivista: Supplemento Dental Cadmos

XVI. Combining Deep Learning and Case-Based Reasoning for early detection of OSCC: a pilot study
La Mantia G., **Coppini M**., Bazzano M., Seminara G., Togni L., Lo Muzio L., Caponio V.C.A., Mauceri R., Campisi G., Di Fede O.

XXX Congresso Nazionale CDUO - La moderna odontoiatria tra tecnologie avanzate e umanizzazione.
Catania, April 20-22 2023
Proceeding abstract in atti di convegno pubblicato su rivista: Supplemento Dental Cadmos

XVII. Analysis of salivary microbiota composition in OSCC patients using ONT technology.
R. Mauceri, G. Campisi, V. Panzarella, **M. Coppini**, F. Buttacavoli, G. La Mantia, G. Seminara, O. Di Fede
Project proposal - Closed Meeting contest 2023 Clinical research and innovation
TAOBUK - DA VINCI AWARD
Taormina, June 15-19, 2023
WINNER OF THE SPECIAL MENTION AWARD CATEGORY: Oncological predictive models and new technologies in oral medicine.

XVIII. SYMILIS OCT: SYsteMatical trained learning aLgorithms for oral carcinogenesiS interpretation by Optical Coherence Tomography
Panzarella V., Campisi G., Buttacavoli F., Mauceri R., Di Fede O., La Mantia G., **Coppini M.,** Seminara G.
Project proposal - Closed Meeting contest 2023 Clinical research and innovation
TAOBUK - DA VINCI AWARD
Taormina, June 15-19, 2023
WINNER OF THE SPECIAL MENTION AWARD IN THE CATEGORY OF ADVANCED DIAGNOSTIC SYSTEMS IN ORAL CAVITY NEOPLASMS

XIX. DoctOral: a smartphone-based decision support tool for the early diagnosis of Potentially Malignant Oral Disorders
Gaetano La Mantia, Giuseppina Campisi, **Martina Coppini**, Fortunato Buttacavoli, Giuseppe Seminara, Rodolfo Mauceri, Vera Panzarella, Olga Di Fede.
Project proposal - Closed Meeting contest 2023 Clinical research and innovation
TAOBUK - DA VINCI AWARD
Taormina, June 15-19, 2023
WINNER OF THE SPECIAL MENTION AWARD CATEGORY: New technologies for the diagnosis of precancerous lesions of the oral cavity

XX. Comparative evaluation of the effect of Denosumab and Bisphosphonates on Healing in cancer patients undergoing oral surgery
La Mantia G., **Coppini M.**, Campisi G., Tozzo P., Di Fede O., Mauceri R.
SIDCO - Young Surgeons Contest 2023
Napoli, October 26-28, 2023

2024:

XXI. MRONJ in patients under low-dose bone modifying agents for cancer treatment-induced bone loss: a case series.
M. Coppini, R. Mauceri, M.E. Mauceri , M.R. Valerio, G. Campisi
ONJ UPDATE 2024
Torino, February 24, 2024
HONORABLE MENTION

XXII. Patients treated with LD-BMAs for the prevention of CTIBL: a multicenter study on a new category of patients at risk of MRONJ
Coppini M, Mauceri R, Caponio VCA, Bizzoca ME, Togni L, Santarelli A, Tenore G, Valerio MR, Romeo U, Campisi G
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024
HONORABLE MENTION

XXIII. Osteonecrosis of the jaws in a patient undergoing therapy with Lenvatinib: a case report
Tozzo P, **Coppini M**, Dioguardi M, Ballini A, Santarelli A, Mascitti M, Mauceri R, Campisi G
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024

XXIV. Transformation of oral lichen planus into squamous cell carcinoma: a case report
Mauceri M.E., Mauceri R., Troiano G., Fanelli F., Togni L., Mascitti M., **Coppini M., Campisi** G
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024

XXV. Detection of metabolic pathway alterations in recurrent OSCC by unsupervised co-clustering
G. Musella, M. E. Bizzoca, M. E. Mauceri, **M. Coppini**, M. Mascitti, L. Togni, F. Fanelli, V. C. A. Caponio
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024

XXVI. Eco-friendly dentistry: cold extraction system olive wastewater applications in oral health
M. E. Bizzoca, M. Dioguardi, M. E. Mauceri, M. Coppini, L. Togni, A. Santarelli, L. Lo Russo, A. Ballini
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024
BEST POSTER AWARD

XXVII. Eco-friendly pediatric dentistry: tooth buds as a reliable sustainable source of stem cells
M. E. Bizzoca, K. Zhurakivska, **M. Coppini**, F. Buttacavoli, M. Mascitti, L. Togni, S. Cantore, A. Ballini
31° Congresso Nazionale del Collegio dei Docenti Universitari di Discipline Odontostomatologiche
Trieste, June 20-22, 2024
BEST POSTER AWARD

XXVIII. Oral field cancerization in non-smokers and non-alcohol users: a case series regarding a gender issue
M. Coppini, V.C.A. Caponio, M.E. Mauceri, G. La Mantia, L. Togni, G. Seminara, R. Mauceri, G. Campisi
16° Congresso Nazionale SIPMO
Napoli, October 11-12, 2024

XXIX. Malignant Transformation of Oral Lichen Planus in patients treated with topical corticosteroids: a case series.
Mauceri M.E., **Coppini M**., Tozzo P., Troiano G., Togni L., Buttacavoli F., Mauceri R., Campisi G
16° Congresso Nazionale SIPMO
Napoli, October 11-12, 2024

XXX. Pilot study on the efficacy of SIPMO-SIOMMMS protocols for the prevention of MRONJ in cancer patients undergoing low-dose anti-resorptive drug therapy requiring dental extractions
M. Coppini, R. Mauceri, S.M. Marchese, A. Bedogni, V. Fusco, G. Campisi
Oral Presentation XXIV Congresso Nazionale SIOMMMS
Padova, December 7, 2024

Abstracts presented at international conferences:

1. MRONJ in patients under low-dose bone modifying agents for cancer treatment-induced bone loss (CTIBL): a descriptive study
   Mauceri R., **Coppini M.,** Caponio V.C.A., Lo Muzio L., Di Fede O., Campisi G.
   Proceeding abstract in atti di convegno pubblicato su rivista: Medicina Oral, Patología Oral y Cirugía Bucal
   Oral comunication. Congreso Medicina Oral 2023.
   Santiago de Compostela, Spain. 25-27 May 2023.

2. Analysis of salivary microbiota composition in patients affected by oral squamous cell carcinoma: a single center observational study
   Coppini M, Mauceri R, Panzarella V, Buttacavoli F, Vacca D, Bertolazzi G, Tripodo C, Campisi G
   EAOM 2023 - European Association of Oral Medicine 16th Biennial conference
   29-30 September 2023

3. Oral Secondary Syphilis in an HIV-Positive Transgender Patient
   Presentacion de un caso clinico en SEMO JOVEN
   Barcelona, January 26-27, 2024

4. Malignant Transformation of Oral Lichen Planus in non-smokers and non-alcohol users: a case series
   M. Coppini, G. Campisi
   3rd OPMD Day Cost Interceptor – Interception of oral cancer development
   Porto, June 6-7, 2024

5. Patients under low-dose bone modifying agents for cancer treatment-induced bone loss (CTIBL): a new emerging category of patients at risk of MRONJ
   M. Coppini, R. Mauceri, G. La Mantia, F. Buttacavoli, V. Panzarella, V. Fusco, M. Bazzano, G. Campisi
   MASCC/AFSOS/ISOO 2024 Annual Meeting
   Lille, 27-29 June 2024

6. Evaluation of topical agents in the prevention of oral mucositis onset in adult patients: systematic review and network meta-analysis
   Martina Coppini, Carlo Caponio, Giuseppe Troiano, Diana Russo, Lorenzo Lo Muzio, Rodolfo **Mauceri, Giuseppina** Campisi
   MASCC/AFSOS/ISOO 2024 Annual Meeting
   Lille, 27-29 June 2024

7. Optical Coherence Tomography (OCT) in assessing oral mucositis: a systematic literature review
   Fortunato Buttacacavoli, Vera Panzarella, **Martina Coppini**, Rodolfo Mauceri, Giuseppina Campisi
   MASCC/AFSOS/ISOO 2024 Annual Meeting

Lille, 27-29 June 2024

8. Smoking habits and awareness about the health risks associated with smoking in the Sicilian adolescent population
   M. Coppini, M. Bazzano, G. Marcon, R. Mauceri, G. Campisi
   CED-IADR 2024 Ora Health Researc Congress
   Geneva, Switzerland 12-14 September 2024

9. Analysis of salivary microbiota composition in patients with oral squamous cell carcinoma: a single center observational pilot study
   Coppini M., Belmonte B., Vacca D., Bertolazzi G., Mauceri R., Campisi G.
   1° International Conference Head and Neck Oncological Peronsalized Outcome by Artificial Intelligence Genomic Analysis
   Napoli, 10 October 2024
   BEST POSTER AWARD

10. Evaluation of topical agents in the prevention of oral mucositis onset in adult patients: systematic review and network meta-analysis
    Coppini M., Caponio V.C.A., Lo Muzio L., Troiano G., Bizzoca M.E., Mauceri R., Campisi G.
    International Conference Translational Insights in Oral Precision Medicine
    Foggia, 25th of October 2024
    BEST POSTER CLINICAL RESEARCH

11. Analysis of salivary microbiota composition in patients affected by oral squamous cell carcinoma before and after surgical treatment: a single center pilot study
    Coppini M, Mauceri R, Vacca D, Bertolazzi G, Tripodo C, Campisi G.
    Joint Meeting of The European Association of Oral Medicine: Region V
    Istanbul, 27th of October 2024

Invited presentations

1. "Adverse Oral Drug Reactions". University of Santiago de Compostela, USC (Spain). December 2, 2024

2. "Adverse Oral Drug Reactions". Fernando Pessoa University, Canary Islands (Spain). November 27, 2024

3. "Oral Microbiota: From Caries to Oral Cancer. The Invisible Ecosystem Affecting Our Oral Health". Official College of Dentists of Las Palmas (Spain). November 23, 2024

4. "Adverse Drug Reactions Involving the Oral Mucosa, Hard Tissues of the Oral Cavity, and Salivary Glands". XXXIV National AIDI Congress "Medical Humanities: The Systemic Vision of Oral Care". Bologna, November 15-16, 2024

5. "Smoking Habits and Awareness of Associated Health Risks Among 7,213 Sicilian Students". 2nd Italian CED-IADR Award. 31st National Congress of the Association of University Professors of Dental Disciplines. Trieste, June 20-22, 2024

6. "Clinical Cases in Oral Medicine: ONJ". 31st National Congress of the Association of University Professors of Dental Disciplines. Trieste, June 20-22, 2024

7. "Study of Salivary Biomarkers for the Early Diagnosis of Oral Cancer". VI PhD Day. 31st National Congress of the Association of University Professors of Dental Disciplines. Trieste, June 20-22, 2024

8. "Medication-Related Osteonecrosis of the Jaw: Italian Position Paper and Clinical Experience at the University Hospital of Palermo". Master in Oral Medicine, Surgery, and Implantology. University of Santiago de Compostela (Spain). June 2-5, 2024

9. AIO Agrigento Day "Update on Special Care for the Modern Dentist" – "Dental Management of Oncology Patients". November 18, 2023