The Application of Salivary Exosomes in the Diagnosis of Oral Disease

Ming Yang YU[1#], Xing Chi LIU[1#], Zi Li YU[1,2], Jun JIA[1,2]

Oral diseases not only greatly impact patients' daily lives, but also pose a severe threat to their overall health. Due to the constant exposure of saliva to oral diseases, the former plays a vital role in their diagnosis and monitoring. Exosomes, nanosized bilayer lipid encapsulated nanovesicles, are widely present in saliva and can be released by any type of cell. Exosomes inherit features from their mother cells in both physiological and pathological conditions. The molecular characteristics and expression levels of exosomes depend on their cellular origin, and they can directly reflect the physiological state of the body and cells. This makes salivary exosomes a promising source for early detection and monitoring of oral diseases. As a result, researchers have been exploring the potential use of exosomes as biomarkers for diagnosing and predicting various oral diseases. This review provides an overview of the composition, separation and function of salivary exosomes. It also discusses their potential as diagnostic and prognostic markers for several oral diseases, including periodontitis, primary Sjögren's syndrome, oral mucosal diseases, hand-foot-mouth disease and oral squamous cell carcinoma. By studying salivary exosomes, researchers hope to improve the early detection and monitoring of oral diseases, leading to better outcomes for patients.

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According to the latest Global Burden of Disease (GBD) study, 74.6% of the global population suffer from oral diseases,[1] which means they rank in the top 10 leading causes of years lived with disability globally.[2] Early detection of oral disease greatly increases the likelihood of successful treatment. Conversely, when treatment is delayed or inaccessible, there is a lower chance of survival, the problems associated with treatment become more severe, and the cost of care rises. Therefore, it is crucial to detect and identify pathogens early and adopt effective management to reduce the negative impacts of the disease. Currently, tissue biopsy remains the gold standard for diagnosing various diseases, including oral lesions. However, oral mucosal diseases, especially those with a high incidence rate, are not always homogeneous. This lack of homogeneity poses a serious challenge to the diagnostic accuracy of biopsy specimens due to the limited sample size. For instance, the process of carcinogenesis among different sites of oral leucoplakia (OLC) often varies greatly. During the lengthy carcinogenesis from OLC to oral cancer, it is difficult to decide when the biopsy specimen should be taken, since it is unavoidable and associated with invasion, pain and wounds.[3] In addition, accurately harvesting lesions for biopsy becomes even more frustrating when they are located in deep tissues, such as salivary gland tumours. This difficulty further hampers the early diagnosis of oral diseases. To address this issue, it is crucial to develop detection methods that enable early diagnosis of oral diseases in a convenient, minimally invasive, highly sensitive and selective manner. These methods

1 State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University, Wuhan, P.R. China.
2 Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University, Wuhan, P.R. China.
# These authors contributed equally to this work as co-first authors.

Corresponding authors: Dr Zi Li YU and Dr Jun JIA, Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University, LuoYu Road No. 237, Wuhan 430079, P.R. China. Tel: 86-27-87686215. Email: zili09@whu.edu.cn; junjia@whu.edu.cn.

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should aim to overcome the limitations of traditional biopsy techniques and provide health care professionals with reliable tools to detect and diagnose oral diseases at an early stage. This, in turn, would improve patient outcomes, reduce the invasiveness of diagnostic procedures and enhance overall convenience for patients.

Taking a liquid biopsy specimen holds great potential in overcoming the limitations of tissue-based biopsy specimens for early lesion identification, disease detection and the prediction of progression in oral mucosal diseases. It offers an alternative method for tumour monitoring without the need for repeated surgical biopsy specimens, minimising the associated risks and invasiveness. Among the various options for liquid biopsy specimens, blood is the most tested in clinical settings, as it contains extensive health-related information. However, in the case of oral diseases, saliva was believed to be superior to blood as a liquid biopsy tool.[4] Saliva is a complex mixture of fluids primarily secreted by the major salivary glands (parotid, submandibular and sublingual) as well as numerous minor salivary glands.[5] Lesions in the oral cavity are constantly immersed in saliva and engage in uninterrupted exchange of substances with it. The saliva contains various bioinformation molecules, including RNA, DNA, proteins and metabolites, which can reflect the relevant stage of oral disease. This characteristic of saliva offers the potential for early diagnosis and monitoring of various oral diseases. The exchange of bioinformation molecules between lesions and saliva allows for the detection of specific molecules or biomarkers that are indicative of the presence and progression of oral diseases. By analysing these molecules in saliva, researchers can gain valuable insights into the molecular changes associated with different stages of oral diseases. The availability of these biomarkers in saliva opens possibilities for the early diagnosis, monitoring and management of oral diseases. It offers a non-invasive and convenient approach, eliminating the need for invasive biopsy specimens in many cases. This can lead to earlier detection and intervention and personalised treatment strategies for individuals with oral diseases.[6]

Exosomes are nanosized bilayer lipid-coated nanovesicles that are released by all types of cells and widely present in saliva.[7] It is widely accepted that exosomes carry various bioactive molecules that are detected in their parental cell.[8] Most importantly, the molecular profiles of exosomes vary with cellular and tissue origins. Thus, the molecular profiles of exosomes can well recapitulate their parental cells. This is also the theoretical basis of exosome-based liquid biopsy specimens. In recent years, interest has increased among research scholars regarding exploration of the potential of exosomes as biomarkers for the diagnosis and prediction of oral diseases. Previous studies have shown promising results regarding the use of salivary exosomes as biomarkers in oral cancer patients. Zhong et al[9] observed that the levels of salivary exosomes are significantly increased in individuals with oral cancer compared to healthy individuals. Furthermore, the elevated levels of salivary exosomes have been found to correlate with the prognosis, staging and clinical outcomes of oral cancer patients.[9] The present authors have also designed a wedge-shaped and high magnetic field gradient-mediated chip to realise the one-step detection of multiple salivary exosome–based biomarkers to differentiate oral cancer from oral ulcers.[10] The aforementioned studies have provided compelling evidence for the significant potential of salivary exosomes as diagnostic and prognostic biomarkers in various oral diseases.

In this comprehensive review, the authors summarise recent studies focusing on salivary exosomes, including their composition, separation techniques and functional roles. They also explore and discuss the application potential of salivary exosomes as diagnostic and prognostic markers in a range of oral diseases, including periodontitis, primary Sjögren's syndrome, oral mucosal disease, foot, and mouth disease, and oral squamous cell carcinoma (Fig 1). In summary, the comprehensive review of these studies confirms the substantial application potential of salivary exosomes as diagnostic and prognostic markers in various oral diseases. The characterisation of exosomal cargo and their correlation with disease status may lead to improved diagnostic accuracy, personalised treatment approaches and better patient outcomes. Further research in this field is warranted to fully exploit the clinical potential of salivary exosomes in oral disease management.

Overview of salivary exosomes

Characteristics of salivary exosomes

Salivary exosomes are usually defined as nanovesicles that are secreted by oral epithelial cells. Many studies have identified their size range of 30 to 150 nm and cup-shape morphology though advanced microscopy techniques such as field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM).[11-13] As in other exosomes from body fluids, salivary exosomes are also enriched in components, including proteins, RNA, microRNAs (miRNAs), enzymes and

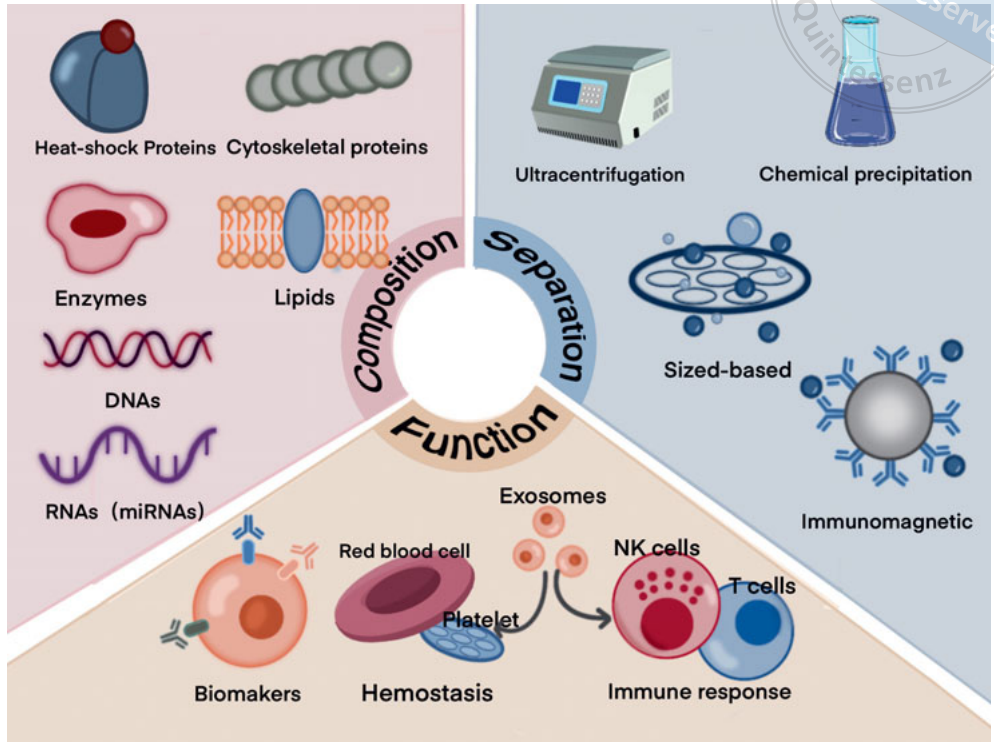

Fig 1 Exosomes as a new target for liquid biopsy. Exosomes are rich in body fluids and are closely related to disease occurrence, progression and metastasis. They are also rich in components, various proteins, enzymes, lipids and genetic factors. Traditional and advanced techniques have been used to isolate exosomes from a variety of body fluids and detect exosomes. Exosomes play an active role in coagulation and immune response, and can be used as biomarkers to provide novel strategies for disease inference, prediction of progression and prognostic monitoring.

lipids.[14] The differential expression of these proteins can help distinguish salivary exosomes from other types of exosomes. Salivary exosomes can be categorised into two types according to their size and protein features.[15] Salivary exosome I is slightly larger than exosome II. Until now, 101 proteins for exosomes have been detected in salivary exosome I, whereas 154 have been found in exosome II. The most common proteins are Alix, TSG101, HSP70, CD81, CD63, IgA and pIgR. Approximately 40% of them belong to a family of secretory proteins. Notably, unlike exosomes derived from other sources such as plasma or tissues, CD81 is accepted as more representative marker for salivary exosomes than CD63. In addition, aquaporin-5, associated with salivary secretion, has been identified to exist in salivary exosomes. The differential expression of the above proteins can distinguish salivary exosomes from other exosomes.[15,16]

Many different proteins, thought to be involved in intracellular communication, have been identified in exosomes. Tetraspanins are a family of membrane proteins that play a crucial role in various cellular processes. They are involved in cellular migration, adhesion, proliferation and immune response.[14] Tetraspanins, such as CD9 CD63 and CD81, are particularly rich in exosomes and have been found to be important for the formation and function of some exosomes.[17] These proteins help in the organisation and biogenesis of exosomes, as well as in sorting specific cargo molecules into exosomes. In terms of cellular migration, tetraspanins have been involved in processes such as cell adhesion, migration and invasion. In addition, tetraspanins are involved in cellular proliferation. They can regulate cell cycle progression, cell growth and cell survival through various signalling pathways.[18] The abundance of tetraspanins in exosomes indicates their active role in mediating intercellular communication through exosomes.[19] Some scholars have studied human saliva and identified exosomes according to their size and protein characteristics. So far, the most common proteins identified are Alix, TSG101 and HSP.[15] The presence of these proteins suggests that salivary exosomes originate from circulating lymphocytes and intravascular fluids.[14] Additionally, exosomes carry different types of RNA, which can be used to identify mutations associated with malignancy in related cells. A study successfully isolated miRNAs (e.g., miR-125a, miR-200a, miR-31 and miR-17-92) from salivary exosomes, confirming that the composition of the exosome is a reflection of the physiological state.[20] As mentioned earlier, exosomes can be regarded as biomarkers for diagnosing and predicting different diseases, thereby greatly facilitating the advancement of disease diagnosis and therapeutic strategies through improved accuracy and efficiency.

Separation of salivary exosomes

Salivary exosomes can be isolated in various ways, according to their physical and chemical properties. Both glandular and whole saliva can be used as excellent sources for separating salivary exosomes. The possible influence on classification by contamination of normal cells must be considered in whole saliva; glandular saliva is obviously more suitable for exosome isolation.[21] At present, there are two commonly used methods of isolating the salivary exosomes: ultracentrifugation and chemical precipitation.[22] Different forms of separation have their own unique limitations. Ultracentrifugation is time-consuming and yields low recovery, whereas chemical precipitation operation is simple but yields a low purity; these methods are limited by specific conditions.[23] It is important to consider these limitations when choosing a separation method and to explore alternative techniques based on the specific requirements of the experiment or application in question. Therefore, separation technologies that require higher accuracy, lower loss, lower cost and less time spent must be explored. In a particular study, salivary exosomes were separated using differential centrifugation.[24] The researchers then measured the mid-infrared absorbance spectra of these exosomes and machine learning technology was utilised to establish a discriminant model based on the absorbance data.[25] By training the machine learning model on known patterns and characteristics of protein, lipid and nucleic acid changes in exosomes, it was able to accurately classify and detect these changes in the salivary exosomes being analysed. This highlights the potential for using such methods in studying disease biomarkers, diagnostics, and monitoring changes in biological samples. Beyond that, the researchers also used density gradient centrifugation,[26] immunoaffinity capture,[27] size exclusion chromatography[28] and polymer-based precipitation[29] to try to isolate salivary exosomes precisely. Researchers are continuing their efforts to find efficient and precise methods for isolating salivary exosomes.

Function of salivary exosomes

Given the role of other exosomes in diagnosing and treating diseases, salivary exosomes may have similar diagnostic and therapeutic potential.[4] Exosomes can cross epithelial barriers,[29] and blood and saliva are exchanged for DNA, RNA, proteins, metabolites and microbiota. These can be used as diagnostic evidence of disease. Particularly in the diagnosis and treatment of malignant tumours, salivary exosomes may serve as biomarkers.[30] They also have various functions. Salivary exosomes have been shown to shorten coagulation time and achieve haemostasis through the participation of tissue factors in the initial stage of blood coagulation.[31] They could also modulate and participate in the body's humoral immune response.[22] Minor variations in salivary exosomes can also be used to diagnose various oral diseases, including periodontitis,[32] oral lichen planus,[33] oral squamous cell carcinoma and some oral precancerous lesions.[34]

Exosomes in oral disease

Potential role of exosomes in periodontitis

Periodontitis is a chronic inflammatory disease characterised by an imbalance of the periodontal biofilm caused by plaque buildup.[35] Patients usually present local or total oral irreversible periodontal membrane damage, periodontal pocket deepening, alveolar bone absorption and other symptoms, which eventually lead to tooth loosening and loss, masticatory dysfunction and even arch defects.[36] Early detection and appropriate management are crucial to prevent the progression of periodontitis and mitigate its consequences. Thus, given the irreversible nature of periodontitis, the development of reliable tests for early detection of both periodontitis and periimplantitis is subject to significant focus in current research.[37] Salivary exosomes have been shown to be biomarkers for early detection and timely prevention of periodontitis. The disease was shown to lead to a significant increase in the secretion of certain components of exosomes by comparing the protein profiles of exosomes from healthy donors and patients with periodontitis and gingivitis.[38] In addition, the high specificity and sensitivity of global 5mC hypermethylation in salivary exosomes can differentiate periodontitis patients from healthy control subjects.[38] Researchers successfully isolated exosomal mRNA from the saliva samples of 61 patients and 30 controls for comparison. The results revealed that compared to the control group, the expression of PD-L1 was significantly elevated in patients with periodontitis, and there were significant differences in salivary exosomal PD-L1 mRNA levels among different stages of periodontitis.[39] In one study, researchers discovered that out of the ten mature miRNAs found in saliva, only three (hsa-miR-140-5p, hsa-miR-146a-5p and hsa-miR-628-5p) exhibited a significant increase within the exosomes of individuals with periodontitis when compared to the healthy control group.[33] These miRNAs have demonstrated con-

siderable potential in accurately identifying periodontitis and can be employed for diagnostic purposes.[40] Salivary exosomes miR-25-3p were significantly enriched in periodontitis patients with type 2 diabetes.[41] Interestingly, in patients with periodontitis, a notable reduction in the levels of CD9 and CD18 exosome-related tetraspanins was observed. These decreases exhibited a negative correlation with clinical measurements.[42,43]

Potential role of exosomes in primary Sjögren's syndrome

Primary Sjögren's syndrome (pSS) is an autoimmune disease that primarily affects female patients. It is considered one of the three most prevalent autoimmune disorders. Patients with this condition often experience symptoms such as dry mouth and dry eyes, which result from the focal infiltration of lymphocytes into the exocrine glands.[44,45] While the exact principles and mechanisms of action are yet to be fully understood, experimental studies have shown that exosomes play a role in both regulating and dysregulating the immune system.[46] Autoimmune responses targeting Ro/SSA and La/SSB antigens are significant in pSS. Salivary gland epithelial cells (SGECs) are crucial in initiating and facilitating the local immune response.[47,48] SGECs can also mediate the exposure of Ro/SSA and La/SSB autoantigens to the immune system, increase the apoptosis of apoptotic bodies, regulate the release of autoantigens and promote the secretion of exosomes containing autoantigens (Fig 2). Studies have reported the isolation and detection of APMAP, GNA13 and WDR1 in saliva-derived exosomes, as well as APEX1, PRDX3 and CPNE1 in tear-derived exosomes from patients with pSS.[49-51] These proteins are the components that exhibit the greatest deviation in biological replicates when compared to the control group. These exosomal components hold promise as potential biomarkers for early diagnosis and subsequent treatment of pSS, thereby enhancing diagnostic accuracy.[49]

The Epstein-Barr virus (EBV) has been found to be another critical factor in the pathogenesis of pSS, with a strong salivary gland homogeneity and the ability to infect B cells preferentially.[52] EBV-miR-BART13-3p, a specific microRNA derived from the EBV, has been found to be present in both EBV-infected B cells and epithelial cells in saliva. The levels of this microRNA were significantly higher in individuals infected with EBV compared to those who were not.[53] This exosome directly targets the mechanism that interacts with molecule 1 (STM1) to regulate and influence Ca^{2+} entry into SOCE channels, leading to SOCE loss and Ca^{2+}-dependent NFAT activation, ultimately affecting the salivary function of pSS.[44]

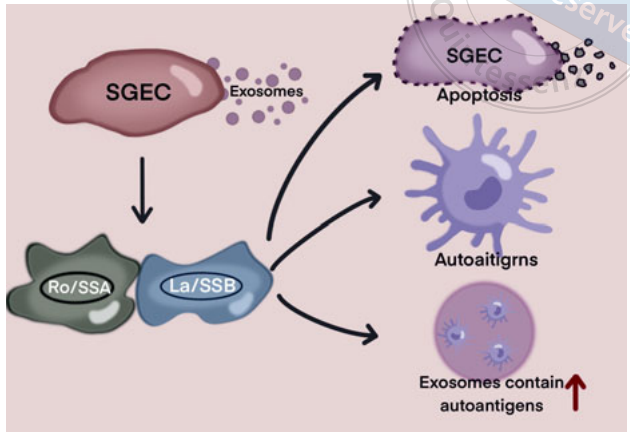

Fig 2 Schematic representation of the development and regulation of local autoimmune responses in pSS. In inflamed salivary tissues, exposure to danger signals or apoptosis-inducing factors prompts salivary gland epithelial cells (SGECs) to present Ro/SSA and La/SSB to the immune system, and these antigens are released into the microenvironment via exosomes or apoptotic bodies. This progress increases the apoptosis of apoptotic bodies, regulates the release of autoantigens and promotes the secretion of exosomes containing autoantigens.

Potential role of exosomes in oral mucosal disease

The oral mucosa is in prolonged contact with saliva in the mouth. It is reasonable to assume that the development of oral mucosal diseases can impact the secretion and composition of salivary exosomes. Salivary exosomes hold potential as biomarkers for various types of oral mucosal diseases and can contribute to their diagnosis and subsequent treatment.

Exosomes in leucoplakia

According to the World Health Organisation's definition, "white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" is known as leucoplakia.[54] Leucoplakia is a typical precancerous lesion of the mouth. Risk factors include smoking, heavy alcohol consumption and areca chewing. There is no significant sex difference with regard to its incidence.[55-57] Experimental studies in oral medicine and research are continuously conducted to enhance the convenience and accuracy of diagnostic methods used in clinical practice.[58-61] These studies aim to develop and refine techniques that can detect the early stages of malignant transformation in oral leucoplakia, as well as identify potential indica-

tors of early oral malignancies.[58-61] One study referred to the OncAlert oral cancer rapid test, where saliva was collected and tested to compare pan-CD44 and total protein expression in patients with oral leucoplakia and an average healthy population.[62] Elevated levels of SolCD44 and complete protein have been observed in the saliva of patients diagnosed with oral leucoplakia, and these levels tend to increase with the severity of the clinical pattern of leucoplakia. These findings indicate that SolCD44 and complete protein could potentially serve as biomarkers for leucoplakia, as they show statistical differences in their expression between individuals with the disease and healthy individuals.[49] It is important to note that further research and validation studies are required to confirm the specificity and sensitivity of SolCD44 and complete protein as biomarkers for leucoplakia. Some findings suggest that the delivery of microRNAs through exosomes can modulate the inflammatory response, inhibit cell proliferation, suppress angiogenesis and induce apoptosis.[63] This approach holds promise as a potential novel treatment for precancerous lesions such as oral leucoplakia. By targeting specific pathways and mechanisms, exosomal-delivered microRNAs have the potential to offer new therapeutic options for managing precancerous conditions.

Exosomes in oral lichen planus

Oral lichen planus (OLP) is an idiopathic inflammatory mucosal form of autoimmune disease.[64] Its exact pathogenesis is not known. OLP lesions often involve the oral mucosa, tongue and gums, and the intraoral lesions always show a bilateral symmetrical distribution. Clinically, six types of OLP, namely reticular, papular, plaque-like, atrophic/erosive, ulcerative and bullous, can be identified.[65] Recent studies have indicated that microRNAs present in salivary exosomes may play a role in the development and progression of OLP.[33,66] One study found that salivary exosomes obtained from OLP patients exhibited significantly higher levels of miR-4484 compared to healthy individuals.[33] Another study found that salivary exosomes from OLP patients showed notably higher levels of miR-21 and substantially lower levels of miR-125a.[67] Additionally, an increase in miR-31 levels was observed in saliva from OLP patients with developmental abnormalities, but this pattern was not observed in patients with non-developmental abnormalities in OLP. These findings indicate that salivary exosomes have the potential to serve as biomarkers for diagnosing OLP, predicting prognostic outcomes and more.[67] Further research is needed to fully understand the diagnostic and prognostic implications of these specific microRNAs in OLP. MicroRNAs have been shown to be closely associated with cytokines in a variety of inflammation-related diseases, suggesting that in OLP, these specific microRNAs have the potential to contribute to the pathogenesis of OLP and may also participate in the regulation of inflammatory processes within the body.

Exosomes in hand, foot and mouth disease

Hand, foot and mouth disease (HFMD) is an acute viral infection, and the two most common pathogens are human enterovirus 71 (EV71) and coxsackievirus A16 (CVA16), which account for more than 70% of outbreaks.[68,69] It has also been reported that coxsackievirus A10 can lead to the occurrence of HFMD infection, which needs to be taken seriously.[70] Jia et al[71] verified that miRNAs (miR-671-5p, miR-16-5p and miR-150-3p) were significantly abnormally expressed in the serum exosomes of patients by testing the blood of children with HFMD compared to healthy children, suggesting that exosomes could be used as potential biomarkers for HFMD. Similar exosomal changes in saliva need to be confirmed by further experiments; miR-16-5p expression in exosomes was found to be especially higher, and miR-671-5p and miR-150-3p levels in exosomes were particularly lower than those in healthy children.[71] In their study, Jia et al[71] demonstrated significant dysregulation of specific microRNAs (miR-671-5p, miR-16-5p and miR-150-3p) in serum exosomes of patients with HFMD when compared to healthy children. These observations highlight the potential diagnostic value of these microRNAs in HFMD; however, further experiments are required to validate whether similar exosomal changes occur in saliva.

Potential role of exosomes in oral squamous cell carcinoma

Head and neck tumours are the sixth most common malignancy globally, with more than 500,000 patients diagnosed each year. Approximately 30% of head and neck cancers are oral cancers.[72] Lip and oral cancers accounted for around 354,000 new cases and over 177,000 deaths in 2018 alone.[73] Saliva plays a crucial role as the first line of defence against oral cancer due to its composition of various enzymes, proteins and immunoglobulins. Studies have confirmed that saliva contains high levels of immunoglobulins that are involved in the immune regulation of the body and exhibit promising anti-inflammatory effects.[15,50] Immunoglobulins, also known as antibodies, are produced by immune cells in response to foreign substances and play a significant role in the immune response. In the case of oral cancer,

the presence of high levels of immunoglobulins in saliva suggests that they may contribute to the immune surveillance and defence mechanisms against cancer cells in the oral cavity. The anti-inflammatory effects of saliva have been observed and acknowledged. Inflammation is closely linked to the development and progression of cancer. By having a good anti-inflammatory effect, saliva may help prevent or reduce the inflammatory processes that can contribute to the initiation or growth of oral cancer cells.[15] By comparing the morphological and molecular characteristics of salivary exosomes in oral cancer patients and healthy subjects, a group of researchers found significant differences between the two, confirming that salivary exosomes also possess the potential to become biomarkers in oral cancer patients involved in diagnosis and later treatment.[74]

By processing the corresponding saliva samples using ultracentrifugation, it was observed that exosomes isolated from saliva samples of head and neck cancer patients carried more PD-L1, FasL and TGF-β compared to those isolated from healthy patients.[75] Furthermore, the levels of these components were associated with tumour staging.[75] miRNA-365 has been noticed and studied as a potential biomarker for oral squamous cell carcinoma (OSCC). miRNA-365 can be detected as significantly elevated in different oral cancer cell lines in culture, and the expression level varies among cell lines. This implies that miRNA-365 has the potential for differential diagnosis of oral cancer and its phenotype.[34,76] OSCC accounts for over 95% of oral cancer cases. A study conducted on head and neck squamous cell carcinoma (HNSCC) discovered that using circulating tumour DNA (ctDNA) extracted from oral saliva as a biomarker resulted in a 100% positive rate for early tumour diagnosis, and this diagnostic rate was significantly higher compared to blood tests.[77] A group of researchers compared salivary exosomes from oral cancer patients and healthy controls by quantitative real-time polymerase chain reaction (qRT-PCR) and found that increased miR-31 expression could promote exosome-mediated miR-29a-3p expression by regulating the macrophage SOCS1/STA T6 signalling pathway. miR-125a and miR-200a expression decreased, and miRNA expression was reduced after tumour resection.[78] This experimental result demonstrated that salivary exosomes may be used not only for early diagnosis of oral cancer, but also for prognostic monitoring of tumours.

In a study comparing oral saliva samples from patients with OSCC and healthy individuals, researchers found that the size and concentration of OSCC-derived exosomes were significantly higher than those

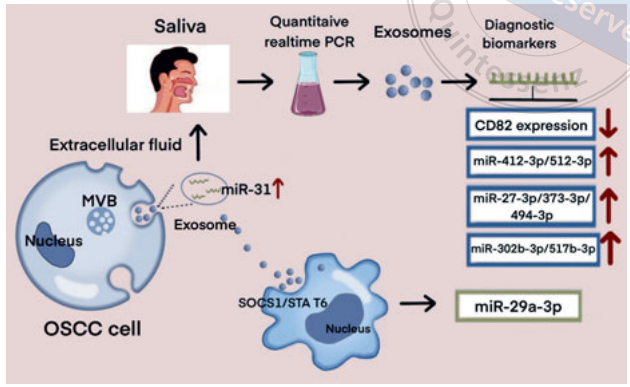

Fig 3 Schematic diagram of the proposed mechanism. Through the separation and detection of salivary exosomes, a significant decrease in CD82 expression[29] and upregulation of miR-412-3p, miR512-3p, miR-27a-3p, miR-373-3p and miR-494-3p occurred in OSCC patients. Salivary exosomal miR-31 is a potential novel diagnostic biomarker for OSCC. Exosomal miR-31 promotes exosome-mediated miR-29a-3p expression by regulating the macrophage SOCS1/STA T6 signalling pathway.

in the healthy group.[79] Additionally, there was a notable decrease in the expression of a molecule called CD82 in OSCC patients, and several miRNAs including miR-412-3p, miR-512-3p, miR-27a-3p, miR-373-3p and miR-494-3p were upregulated in OSCC patients compared to the healthy group.[79] Interestingly, OSCC patients exhibited the specific expression of miR-302b-3p and miR-517b-3p, which was not observed in the healthy group (Fig 3). The role of biomarkers in salivary exosomes in oral disease diagnosis is summarised in Table 1.

Recent advancements in saliva biopsy techniques have led to the development of innovative methods such as Fourier-transform infrared-based salivary exosome spectroscopy.[80] This technique has shown promising potential in distinguishing OSCC from healthy individuals. With a sensitivity of 100% and a specificity of 89%, it effectively detects the specific mid-infrared spectral features of OSCC salivary exosomes. Changes in salivary exosomal proteins, lipids and nucleic acids in OSCC patients contribute to these discernible differences. Furthermore, researchers have explored combining exosome technology with nanoplatforms, such as microfluidics, flow cytometry and electrochemical analysis, to add a new dimension to cancer diagnosis.[81]

Conclusion

In recent years, research has focused on studying the role of salivary exosomes in oral diseases like periodontitis, oral lichen planus and oral precancerous lesions. Salivary exosomes have shown potential as biomarkers

Table 1 The role of RNA, DNA, and protein in salivary exosomes in the diagnosis of oral disease.

Class	Exosome biomarker	Type of disease	Description	References
RNA	PD-L1 mRNA	Periodontitis	Significantly elevated in patients with periodontitis and expression levels vary when different stages of periodontitis are classified	39
	PD-L1 mRNA	Head and neck cancer	PD-L1 is highly expressed in saliva samples from patients, and the levels of these components were associated with tumour staging	75
	hsa-miR-140-5p, hsa-miR-146a-5p, hsa-miR-628-5p	Periodontitis	Exhibited a significant increase within the exosomes of individuals with periodontitis	40
	miR-25-3p	Periodontitis	Significantly enriched in periodontitis patients with type 2 diabetes	41
	EBV-miR-BART13-3p	Sjögren's syndrome	Higher in individuals infected with EBV compared to those who were not	53
	mRNA	Leucoplakia	The delivery of microRNAs through exosomes can modulate the inflammatory response, inhibit cell proliferation, suppress angiogenesis, and induce apoptosis	63
	miR-4484	OLP	Salivary exosomes obtained from OLP patients exhibited significantly higher levels of miR-4484 compared to healthy individuals	33
	miR-21	OLP	Notably higher levels of miR-21 from OLP patients	67
	miR-125a	OLP	Substantially lower levels of miR-125a from OLP patients	67
	miRNA-365	OSCC	Can be detected as significantly elevated in different oral cancer cell lines in culture, and the expression level varies among cell lines	34,75
	miR-125a, miR-200a	OSCC	miR-125a, miR-200a expression decreased, and miRNA expression was reduced after tumour resection	34,82
	miR-412-3p, miR-512-3p, miR-27a-3p, miR-373-3p, miR-494-3p	OSCC	Upregulated in OSCC patients compared to the healthy group	79
	miR-302b-3p, miR-517b-3p	OSCC	Specific expression in patients' salivary exosomes	79
DNA	ctDNA	OSCC	Using circulating tumour DNA (ctDNA) extracted from oral saliva as a biomarker resulted in a 100% positive rate for early tumour diagnosis which was significantly higher compared to blood tests	77
Protein	CD82	OSCC	Notable decrease in the expression of a molecule called CD82 in OSCC patients	79
	CD9\CD18	Periodontitis	A notable reduction in the levels of CD9 and CD18 exosome-related tetraspanins was observed	42,43

for noninvasive disease diagnosis and predicting therapeutic response. Salivary exosomes can also be used to monitor disease progression and treatment response. However, more research is needed to understand their regulation and specific roles in recipient cells. Despite the enormous diagnostic and therapeutic potential of salivary exosomes in oral diseases, there are still numerous limitations in their actual clinical application.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Ming Yang YU and Xing Chen LIU drafted the manuscript and made the figures; Drs Ming Yang YU and Zi Li YU discussed and revised the manuscript; Drs Zi Li YU and Jun JIA designed the study. All authors read and approved the final manuscript.

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