

# Extracellular Vesicles in Oral and Craniofacial Diseases: from Basic Knowledge to Clinical Perspectives

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*Extracellular vesicles (EVs), produced by exocytosis or membrane budding of cells, are membranous vesicles that carry specific proteins, nucleic acids and other bioactive molecules. EVs are indispensable carriers of biological information and play a critical role in cell-to-cell communication. Due to their involvement in physiological and pathological processes, EVs have shown great potential in the diagnosis and treatment of various diseases in recent years. The present review focuses on the regulatory role of EVs in oral and craniofacial diseases to provide valuable insights into possible applications in translational medicine.*

**Key words:** diagnosis, extracellular vesicles, oral diseases, therapy  
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Extracellular vesicles (EVs) are membranous vesicles that mediate intercellular communication by delivery of functional cargos, such as proteins and nucleic acids<sup>1</sup>. According to their biogenesis, EVs can be classically divided into exosomes and microvesicles (Fig 1). Exosomes are released after multivesicular bodies (MVBs) fuse with the plasma membrane of donor cells,

whereas microvesicles are formed directly via membrane budding and shedding. The International Society for Extracellular Vesicles (ISEV) proposed a new classification in 2018 regarding EV size, with “small EVs” (< 200 nm) and “large EVs” (> 200 nm) introduced as the defined consensus to distinguish EVs<sup>2</sup>. A growing body of evidence has shown the critical functions and applications of EVs in systemic diseases, especially malignant tumours, cardiovascular diseases and neurological disorders, but the understanding of the roles of EVs in oral and craniofacial diseases is still at a relatively early stage. The present review systematically outlines the latest studies on the regulatory roles of EVs in oral and craniofacial diseases to potentially provide new perspectives in future applications.

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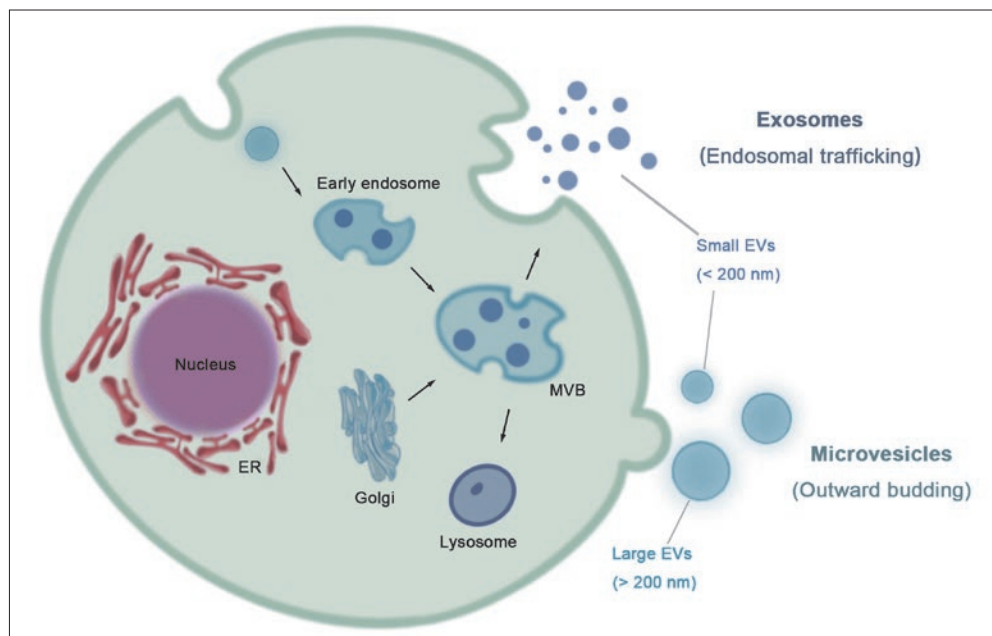
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## EVs in oral and craniofacial diseases

### *Infectious and inflammatory diseases*

Studies have suggested that EVs in saliva or gingival crevicular fluid (GCF) are closely related to the occurrence and development of oral and craniofacial diseases. Periodontitis, mainly caused by localised pathogenic bacteria, is one of the main infectious oral and craniofacial diseases<sup>3</sup>. It was found that EVs in saliva significantly induced the proliferation activity of periodontal ligament fibroblasts and may be involved in periodontal regeneration<sup>4</sup>. By comparing the messenger ribonucleic



**Fig 1** Classification of EVs. EVs are generally classified into exosomes and microvesicles. Exosomes (50 to 150 nm) are assembled through endosomal trafficking and microvesicles (100 to 1000 nm) are formed by direct outward budding. EVs < 200 nm are considered as “small”, and those > 200 nm are considered as “large”.

acid (mRNA) expression profile of salivary EVs from periodontitis patients with that from healthy individuals, it was found that PD-L1, which played a vital role in immunosuppression, was greatly upregulated in salivary EVs from periodontitis patients<sup>5</sup>. Furthermore, their expression was positively correlated with the severity of periodontitis. It was suggested that salivary EVs were closely related to the occurrence and development of periodontitis, which may provide new insights for the examination and treatment of periodontal disease<sup>5</sup>. Oral lichen planus (OLP) and oral leukoplakia are common chronic inflammatory diseases<sup>6,7</sup>. Byun et al<sup>8</sup> compared the microRNA (miRNA) expression profile of salivary EVs from OLP patients and healthy individuals and found that miR-4484 was significantly upregulated in the salivary EVs of OLP patients. Peng et al<sup>9</sup> identified the expression profiles of circulating exosomal miRNAs and found that the exosomal miR-34a-5p was positively correlated with the severity of OLP. Wang et al<sup>10</sup> showed that as a novel biomarker, the decreased expression of miR-185 in salivary EVs is significantly correlated with the malignant transformation process of oral leukoplakia to oral cancer, and this progression can be blocked by the local application of miR-185-rich EVs. They suggested that salivary EVs, especially their carried miRNAs, may be potential biomarkers and therapeutic targets for OLP and oral leukoplakia<sup>10</sup>.

#### *Trauma and malformations*

Fractures are common traumatic injuries in the oral clinic<sup>11</sup>. Wei et al<sup>12</sup> demonstrated that EVs released by osteoblasts at the mid-to-late stage of differentiation promoted osteogenesis. Chen et al<sup>13</sup> indicated that EVs derived from miR-375-overexpressing human adipose mesenchymal stem cells induced bone regeneration. Studies also demonstrated that secreted miRNAs are closely associated with EVs in the GCF. During orthodontic tooth movement (OTM), the expression profile of EV-miR-29 was significantly upregulated in the GCF of orthodontic patients<sup>14,15</sup>. It was then suggested that EV-miR-29 in GCF may serve as a potential biomarker for remodelling<sup>15</sup>. EVs in GCF were also reported to be crucial in bone remodelling and may contain biomarkers for OTM and orthodontic-associated root resorption<sup>15</sup>.

EVs may be also involved in malformation in the craniofacial region. Venous malformation (VM) is the most common type of congenital vascular malformation, the symptoms of which present at birth or later in the development<sup>16</sup>. Zhu et al<sup>17</sup> found that VM patients presented some features of EVs that were different to those seen in healthy individuals. First, the quantity of EVs from vascular endothelial cells in the peripheral blood was greatly elevated in VM patients. Second, the level of EVs was positively correlated with the size of lesions. Third, the miRNA expression profile of circulating EVs from VM patients was significantly different to that from healthy individuals.

## Cysts and tumours

Odontogenic keratocyst (OKC), unlike other odontogenic cysts, is aggressive<sup>18</sup>. Man et al<sup>19</sup> found that the percentage of leucocyte-derived microparticles (LMPs) was higher in inflamed OKCs, which induced the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and matrix metalloproteinase-9 (MMP-9) in OKC fibroblasts by interleukin-15 (IL-15) and other cytokines, and then promoted osteoclastogenesis, revealing a novel mechanism of bone resorption in OKCs. Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer<sup>20</sup>. There has been a great deal of evidence on EVs derived from OSCC cells revealing the mechanism of the development, metastasis and immune escape of OSCC. We previously reported that the level of circulating platelet-derived EVs was positively correlated with the clinical stages of OSCC<sup>21</sup>. Furthermore, circulating EVs may promote the progression of OSCC by inducing angiogenesis<sup>22</sup>. Li et al<sup>23</sup> found that EVs derived from hypoxic OSCC cells can deliver miR-21 to normoxic ones to change their bioactivity and create a hypoxic microenvironment to promote tumour progression and metastasis. Momen-Heravi and Bala<sup>24</sup> also detected a high quantity of oncogenic miRNAs, such as miR-21 and miR-27, in the circulating EVs isolated from OSCC patients. These oncogenic miRNAs carried by tumour-derived EVs can regulate the immune response of monocytes and promote immune escape of OSCC cells by activating the NF- $\kappa$ B pathway<sup>24</sup>. With the exception of EVs derived from tumour cells, those from cancer-associated fibroblasts (CAFs) also induced the migration and invasion of OSCC<sup>25</sup>. Revealing how tumour associated EVs works is of great importance for providing new targets in immune therapies and may help to make early diagnoses and predict patients' prognosis.

### Potential uses and future challenges of EVs in the diagnosis and treatment of oral and craniofacial diseases

#### *Diagnostic prospects of EVs*

Studies have investigated the biological functions of EVs in oral and craniofacial diseases and important points should be taken into account in specific areas. It has been suggested that the abnormality in the cargo or quantity of EVs in body fluids, especially saliva, is closely related to the occurrence, development and prognosis of oral and craniofacial diseases<sup>26</sup>. Salivary EVs may therefore emerge as promising biomarkers for dis-

ease theranostics<sup>27</sup>.

Compared to traditional histopathological analyses, examinations based on salivary EVs offer several advantages. First, the minimally invasive or even non-invasive sampling procedure is safer and more convenient. Second, it is easier to monitor the state of disease and predict the prognosis by simply detecting EVs that contain more information. Third, compared to free nucleic acids, proteins or other conventional biomarkers, EVs present greater specificity and stability<sup>28,29</sup>. However, there are still technical bottlenecks that need to be solved urgently.

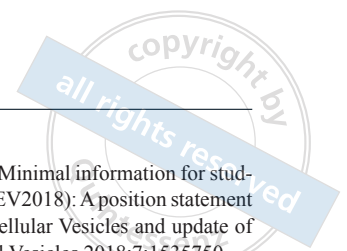
To be biomarkers of liquid biopsy specimens, the specific bioactive molecules (proteins or miRNAs) in EVs associated closely with oral and craniofacial diseases need to be screened and identified. Purification of EVs is difficult due to the complicated composition of tissue fluids. The small particle size and similar physicochemical properties to secreted proteins create a further challenge to EV purification. Methods for EV detection and analysis should also be standardised. Thus, efficient methods for obtaining high purity EVs from tissue fluid such as saliva need to be developed collaboratively by cell biologists, clinicians, material scientists and engineers.

Since salivary EVs have great application prospects in the accurate diagnosis of oral and craniofacial diseases, technical methods and procedures need to be established for the accurate detection and analysis of key biomarkers of EVs from saliva. Further research is required into the specific and sensitive detection of EVs for diagnosis. Detection kits with intellectual properties should be developed to provide new insight to aid the accurate diagnosis of oral and craniofacial diseases.

It has been widely accepted that oral diseases are associated with systemic diseases such as diabetes, cardiovascular disease and malignant tumours<sup>30-32</sup>. Saliva in patients with oral diseases may induce systemic diseases, while systemic diseases may in turn result in changes in saliva composition<sup>33</sup>. The connection between salivary EVs and systemic diseases needs to be elucidated. It is also necessary to provide a simple, effective and noninvasive examination for salivary EVs in the early diagnosis, disease monitoring and efficacy evaluation of systemic diseases.

#### *Prospects of EVs in oral and craniofacial treatments*

Oral pathogens also secrete EVs and release them into oral microenvironments, but few studies have examined their specific roles. Further studies should clarify how EVs released by oral pathogens affect oral infectious



diseases and how potential vaccines for relevant pathogens are developed based on EVs. In vivo studies on the effectiveness and safety of the vaccines may provide new insights into the treatment of oral infectious diseases.

Several kinds of stem cells reside in the oral and craniofacial tissues<sup>34,35</sup>. They present great therapeutic potential in the treatment of oral and craniofacial diseases, such as periodontitis and mandibular bone defects. More importantly, their derived EVs inherit bioactive components and may possess great potential in tissue repair. Avoiding the potential risk of immunogenicity and tumorigenicity in stem cell transplantation, EVs are potential agents in oral tissue regeneration; however, risks still exist regarding their safety and effectiveness. Progress needs to be made concerning the components and biological functions of EVs derived from stem cells, and the evaluation of safety and effectiveness should be considered more precisely for promising applications.

## Conclusion

We overviewed the latest studies on the regulatory roles of EVs in oral and craniofacial diseases. Carrying bioactive molecules, such as miRNAs and proteins, EVs are closely associated with the occurrence and development of oral and craniofacial diseases. Based on current evidence, EVs may emerge as not only promising biomarkers for disease diagnostics, but also potential targets for clinical therapeutics. Nevertheless, great efforts are still required to overcome the practical challenges they present in future applications.

## Conflicts of interest

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

## Author contribution

Dr Jue HUANG drafted the manuscript; Drs Ye LI and Gang CHEN contributed to the conceptualisation, writing and revision of the manuscript.

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