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Review Article

Potential Research on Therapeutics Application of Plant-derived Extracellular Vesicles (PDEVs) in Dental Disease in Malaysia

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ABSTRACT

Science witnessed a dramatic growth in studies on extracellular vesicles (EVs). Similar to human, PDEVs serves as a potential tool between cells, transporting signaling molecules to mediate cellular intercellular communication in the form of nano cargoes (40–150 nm) which consist of lipid, protein, and RNA to exhibit pharmacological potential as an anti-inflammatory, antioxidant and anticancer. Moreover, PDEVs are eco-friendly, do not cause cell toxicity, and do not harbor zoonotic. More importantly, highly biocompatible, allows large-scale production. FAO/INFOODS named "FoodEVs". PDEVs easily enter mammalian cells and intermediate plant–animal cross-kingdom plays a role in the homeostatic regulation of the immune system, tissue engineering, and delivery of therapeutic molecules of various origins. This review aims to introduce the potential research opportunity on therapeutics application of plant-derived extracellular vesicles (PDEVs) in dental and oral diseases to support the New Key Economic Areas (NKEA) 2027 target in Malaysia.

Keywords: *Plant-derived extracellular vesicles, therapeutic potential, dental disease, oral disease.*

INTRODUCTION

A dramatic increase in research has been noticed in the last few years, on extracellular vesicles (EVs) as they play roles in various physiological mechanisms with potential therapeutics. Discovering exosomes in animal cells led to evidence of plant exosome existence. Thus, we have witnessed a dramatic growth of published articles related to EVs in foods gaining attention as potential therapeutics.

Natural products have attracted more attention in the treatment of human diseases (Dad et al., 2021) as evidence demonstrated that compounds of natural products have the potential to alleviate human diseases. However, purification difficulties, problems with uncertain target specificity, and poor stability limit the natural products in research for therapeutic applications. The low bioavailability of natural compounds makes it quite challenging for potential therapeutic application whereas, compared to natural products, PDEVSs have higher solubility, a faster breakdown in the bloodstream, It is possible to target specific organs, possess a higher level of permeation through barriers, and low side effects (Bonifacio et al., 2014; Di Gioia et al., 2020; Zhang et al., 2022; Ly et al., 2022; Umar et al., 2022).

The existence of PDEVs was first discovered in carrot cell cultures by Halperin et al. in 1967 and animal-derived exosomes (ADEs) by Trams et al. in 1981. PELNs 50-500 nanometer (nm) in size phospholipid bilayers membrane-enclosed vesicles, contain cargos of mRNAs, proteins, microRNAs (miRNAs), and bioactive lipids (Chevillet et al., 2014; Sharma et al., 2010). Exosomes are released by all types of cells including tumor cells that secrete exosomes and can be detected in a range of biological fluids: plasma, saliva, urine, blood, cerebrospinal fluid, and cell culture media. Cargos molecules derived from donor cells provide an essential intercellular communication system by transferring these biological cargoes to recipient cells also allows to elimination of unwanted products from cells, and immune surveillance. Play roles in regulating gene and protein expression of recipient cells PDEVs play critical roles in regulating cytokine secretion as well as the function of wide varieties of immune cells, thus modulate of various diseases and having the ability to improve diseases state (periodontitis, tumor, inflammatory bowel disease (IBD), diabetes mellitus, etc.) (Trams et al., 1984; Halperin et al., 1967).

Exosome biogenesis: The end-product of endocytic recycling is the most common pathway proposed for the origin of PDEVs and is initiated with the formation of the trans-Golgi network or early endosome. Primary endolytic vesicles and their contents are formed through the invagination of the plasma membrane. Primary endolytic vesicles then process for the conversion to late endosomes. Late endosomes accumulate Intraluminal vesicles formed by the inward budding of the endosomal membrane which gives rise to numerous multivesicular bodies (MVBs). Finally, MVBs fuse into the plasma membrane to release their intraluminal vesicles as exosomes into the extracellular matrix.

Synthetic nanocarriers / mammalian-derived EV vs PDEVs: (Dad et al., 2021; Sundaram et al., 2019; Teng et al., 2018; Di Gioia et al., 2020; Ju et al., 2013)

i. do not cause cytotoxicity on human cells.
ii. lipid bilayer do not contain cholesterol instead enriched in phospholipids such as phosphatidic acid (PA), and phosphatidylcholines (PC). These lipid characteristics provide potential therapeutic advantages PELNs as tissue-targeting drug carriers.
iii. shows stability in the gastrointestinal (GI) tract during the transition through the stomach and small intestine moreover blocks factors that damage while promoting factors that heal them.
iv. low immunogenicity.
v. stable in a wide range of pH levels.
vi. not cross the placental barrier.
vii. ability to cross the blood-brain barrier.
viii. highly efficient uptake at the target site.
ix. strong capacity to deliver a variety of therapeutic agents.
x. Economical in large-scale production.
xi. secondary metabolites of plants are believed to be a potential preventive therapeutic.
xii. the sizes of PDEVS can also be manipulated by selecting an Isolation and purification process and/or a pH-dependent manner.
xiii. involved in interspecies communication between plants and animals.
xiv. autologous EVs of human origin may deliver dangerous molecules as a part of their scavenging mechanism (e.g., including tumor-derived molecules, foreign nuclei acids, and transmissible agents).
i. the large-scale production of human EVs from normal human cells for industrial use also limits their application.
ii. have a more potent anti-oxidant effect, due to their ability to protect anti-oxidants.

Isolation and characterize of PDEVs:

Currently, differential ultracentrifugation plus density gradient centrifugation is commonly used and the “gold standard” for the isolation of plant-derived nanoparticles. The addition of Gradient ultracentrifugation provides a more purified PDEVS. It is suggested to purify PDEVs without possible virus contamination.

Transmission electron microscopy (TEM) is used to characterize plant-derived edible nanoparticles and allows ultrastructural analysis at the subcellular level. However, TEM does not allow to obtain additional further biochemical and mechanical information (Nemidkanam et al., 2022; Chevillet et al., 2014; Sharma et al., 2010).

Atomic force microscopy (AFM) can also be used to characterize the sizes and structures of PDEVs. AFM is a very high-resolution type of scanning probe microscopy (SPM) which allows the resolution on the order of fractions of a nanometer.

Determining the charges and sizes of PDEVs:

Since Dynamic light scattering (DLS) is a non-invasive, highly sensitive, and requires very small volumes of sample, therefore, to determine the size-distribution profiles of PDEVs in suspension It is currently used as a gold standard for quickly and accurately determining the size (Mu el al., 2014).

Recent reports from the literature documented the potential of PDEVs to treat various diseases (e.g., inflammatory bowel diseases, lung inflammation). In-vitro and animal studies reported the therapeutic potential of PDEVs (e.g., ginger-derived exosome-like nanoparticles) in periodontitis. (Mu et al., 2014; Eke et al., 2012; Kassebaum et al., 2014). These represent PDEVs as a valuable tool for extensive use in health care and have been gaining attention as an environment-friendly potential nanomedicine.

Periodontitis (PD) is an infection-driven chronic multifactorial inflammatory disease affecting 9.8% (about 796 million) global adult population affecting over 50% of the worldwide population. It is worth noting that periodontitis is also considered humankind’s 6th most prevalent disease. The pathogenesis of periodontitis is mediated by an interplay between bacterial virulence factors and aberrant immune responses within periodontal tissues. Chronic periodontitis impaired the pluripotency of the periodontal ligament stem cells (PDLSCs) (Eke et al., 2012; Kassebaum et al., 2014; Kinane et al., 2017).

Dental caries known as tooth decay occurs throughout life and is a biofilm-mediated disease that affects both primary and permanent teeth. It is a preventable disease, however, causes considerable economic and quality-of-life burdens. WHO in its global health status report in 2022 documented that, Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth. Even Dental caries is easily preventable however, has not declined significantly over the last thirty years (Pitts et al., 2021; IHME 2019; Bernabe et al., 2020; Kassebaum et al., 2015).

Here we discuss possible PDEVs-based research avenues: (We are interested in collaborating on any potential oro-facial research project using Malaysian PDEVs). In this regard, we have presented our research goal and its potential in Malaysia in two iiDex 2022 conferences (Khan, N.B.LA., 2022i; Khan, N.B.LA., 2022 ii).

Therapeutic potential of PDEVs on PDLSCs in periodontitis research:

Evidence documented that PDEVs treatment significantly upregulated the expression of the genes encoding pluripotent stem cell markers, including SOX2, Nanog, OCT4, and KLF4. This study strongly suggests under physiological conditions, PDEVs play a crucial function in regulating homeostatic regeneration via the induction of stem cells. Oral tissues contain a substantial number of mesenchymal stem cells (MSCs). Most importantly, the above finding may open up a new avenue for studying novel mechanisms in regenerative dentistry maintained by oro-facial mesenchymal stem cells (MSCs), in homeostatic, or injury-induced as well as pathophysiological conditions (periodontitis, cleft-lip/palate, etc) (Ju et al., 2013; Mai et al., 2021).

Therapeutic potential of Secondary metabolite of PDEVs on anti-microbial research:

Secondary metabolite of plant exosomes contain mostly lipophilic molecules e.g., curcuminoids, chlorophylls aconitine, mesaconitine, hypaconitine, naringin, naringenin, Shogaol (Woith et al. 2021) and documented to play roles in anti-microbial activity, most importantly, increased its synthesis during infections (Woith et al. 2021).

BEVs (Bacterial extracellular vesicles) for developing DDSs (Drug delivery systems):

Both in prokaryotic and eukaryotic spontaneously formed membrane vesicles produced Extracellular vesicles (EVs)and mediate intercellular communication. Recent studies showed that bacteria possess properties such as the ability to sense the environment, respond to signals, and extensive cross-talk with both prokaryotic and eukaryotic cells. BEVs (Bacterial extracellular vesicles) are critical for their own defence system against bacteriophages, other microbes, the host's immune system, and environmental stressors. Further, BVEs play an important role in the formation and maintenance of bacterial communities. Both Gram-negative and Gram-positive bacteria produce different types of BEVs through distinct biogenesis mechanisms with discernible composition and content. BEVs are equipped with favorable characteristics for developing DDSs (Drug delivery systems). Moreover, BEVs have the unique ability to carry, protect, and deliver widespread cargo transfer it contains which can be advantageous for enabling DDSs. BEVs is considered to be the potential therapeutics in the human battle against the multi-drug-resistant pathogen. Further, BEVs evidence showed its antifungal activity (Alizadeh et al., 2020; Toyofuku et al., 2019; Mashburn et al., 2005; Zhang et al., 2020; Bitto, et al., 2017; Meers et al., 2018)).

C-Fos immunohistochemistry in PDEVs research:

The immediate early genes (IEGs) encoded protein c-Fos, a transcription factor expressed in most cell types. C-Fos is a functional anatomical marker of activated neurons within the central nervous system. Monitoring the nuclear expression of c-Fos protein s an established reliable anatomical technique for the functional mapping of neuronal activity and be helpful to examine the ability of neurons to react with changes in gene expression to external stimulation under physiological, pathological, and pharmacological challenges. Investigating the molecular mechanisms of PDEVs-induced neuromodulation in CNS nuclei might open up new research avenues for PDEVs and their therapeutic potential in CNS. C-Fos immunohistochemistry could be a potential tool to investigate the neuroanatomical functional site of the PDEVs in animal models of diseases. Kazi et al., 2022; Kazi et al., Kazi et al., 2002; Kazi et al., 2015; Kazi et al., 2016; Kazi et al., 2012; Kazi et al., 2010; Kazi et al., 2007; Li et al., 2020).

Organic Agriculture for PDEVs as therapeutics nanomedicine:

It is noteworthy to mention that, human exosomes share many features with vegetable- and fruit-derived nanovesicles (VFNVs), that including size, charge, and similar lipid composition, Foods derived exosomes has been included in the FAO/INFOODS databases, and been named “FoodEVs”.

However, the stability and biological activities c of critically depends on environmental and its processing factors (e.g., physical parameters, including pH values, temperature). In this regard, specific studies have reported altered morphology of PDEVs of the unpasteurized juice prepared by industrial processes and the absence of PDEVs in the concentrated orange juice.

Evidences showed an increase in the yield and anti-oxidant capacity of PDEVs isolated from organic farms compared to conventional farms based on quantity and quality.

PDEVs has the potential to quickly enter mammalian cells and thus play a critical function in mediating plant–animal cross-kingdom via genetic crosstalk this suggests the potential use of organic PDEVs nanovesicles for medical application in the regulation of fundamental biological processes in the human body.

Malaysia is a treasure of more than 2,000 plants with potential medicinal value. Herbal industry has been identified as a new source of economic growth in 2011 under the agriculture New Key Economic Areas (NKEA) with potential GDP contributions of ranging from RM19 billion to RM28 billion by 2027. Recently, several studies have been focused on PDEVs released from various plants as a nanoplatform for therapeutics and drugs delivery into a target tissue conveniently. Most importantly, clinical trials are currently in progress to test the effectiveness of PDEVs. Whereas, there is little or none PDEVs research conducted as diagnostic and therapeutic tools related to dental disease in Malaysia (Karamanidou et al., 2021; Yang et al., 2018; Berger et al., 2020; Logozzi et al., 2021; Li et al., 2018; Malaysian Investment development Authority (MIDA), 2021).

Finally, the use of PDEVs in nanomedicine has great potential as a therapeutic option in near future. Malaysia's rich resources of medicinal plants can be utilized for fundamental research in health sciences which will lead to significant contribution not only in academic research, but also in the fields of "University - Industry-Organic Agriculture". This has the potential to propel the country into a new era of economic growth (Nemati et al., 2022).

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