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Copper-incorporated microvesicles: a new frontier in dentistry and oral surgery?

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Highlights:

- **Innovative CiMs for tissue regeneration**: This review presents groundbreaking insights into copper-incorporated microvesicles (CiMs) as a novel emerging biofunctional material that synergistically enhances tissue repair and regeneration in dental and oral surgery applications, and beyond.
- **Promoting healing with CiMs**: CiMs have the potential to further promote angiogenesis, enhance cell migration, and increase cell proliferation, leveraging the unique anti-microbial and anti-inflammatory properties of copper for improved therapeutic outcomes.
- **Encapsulation strategies to mitigate toxicity**: The article explores innovative methods to encapsulate copper ions within microvesicles, addressing the critical concern of copper toxicity while maximizing its therapeutic benefits in clinical applications.
- **Versatile applications in dentistry**: CiMs can be positioned as a versatile platform for delivering copper-based therapies in treating dental diseases, including dental caries and periodontal disease, as well as enhancing the adhesion of dental materials.
- **Regulatory and market insights for CiMs**: The review discusses potential regulatory barriers and market considerations for introducing CiMs in dental applications, highlighting Chile's unique position as a leading copper producer and the implications for the future of dental biomaterials.

Abstract: The need for effective and safe approaches to promote tissue restoration, repair, replacement, and regeneration has spurred interest in biofunctional materials, including copper and microvesicles, given their potential synergistic effects. Indeed, the recently-introduced combination of copper and microvesicles has been recently proposed as a promising innovative and alternative approach showing promise in enhancing therapeutic effects, thereby, promoting *in situ* tissue repair and regeneration in various fields, such as orthopaedics, dermatology, and dentistry, amongst others. In this article, an overview of Copper-incorporated Microvesicles (CiMs**)** and their potential applications is provided; a promising avenue for addressing unmet needs in dental and oral surgery. Herein, CiMs have been shown to promote angiogenesis, enhance cell migration, and increase cell proliferation, leading to improved tissue regeneration outcomes. Additionally, the anti-microbial, angiogenic, and anti-inflammatory properties of copper enhance the therapeutic potential of microvesicles. The various methods employed to synthesize, formulate, and characterize CiMs and their potential applications in endodontics, implant dentistry, bone regeneration, treatment of gingival and periodontal disease, and prevention of dentin hypersensitivity, to mention a few, are also discussed. Henceforth, ongoing research, development, and innovation (R&D&I) efforts hold promise for further fine-tuning/optimization of formulated CiMs, leveraging the unique physico-chemico-mechanico-biological properties of novel biofunctional materials, suitable for clinical use in oro-dentistry and cranio-maxillo-facial surgery applications, and undoubtedly beyond.

Keywords: copper-incorporated microvesicles; dental; oral surgery; biomaterials; layer-by-layer; self-assembly; polymer; antimicrobial; biocompatibility; tissue regeneration; drug delivery; safety

1. Introduction

Copper is an essential trace mineral that is involved in many physiological processes in the body [1]. It plays a role in the formation of red blood cells, the maintenance of healthy bones and connective tissues, and the function of the immune system [1]. As a metal, it has long been recognized for its versatility and attractive anti-microbial properties [2]. Copper has been used in various applications, from water treatment to medical devices, to reduce the growth of harmful bacteria and other microorganisms, including bacteria, viruses, and fungi, and henceforth, potentially lessen the risk of microbial contamination [2]. The anti-microbial action of copper is thought to be due to several mechanisms, including disrupting the cell membranes of microorganisms, generating reactive oxygen species or ROS that damage their DNA, and interfering with their metabolic processes and nutrient uptake; *i.e.* leading to reduced growth and viability of the bacteria [2]. Recent research, development and innovation (R&D&I) activities has gained copper significant attention mainly due to its potential health benefits, including anti-inflammatory (beneficial for conditions such as arthritis and inflammatory bowel disease), -oxidant (to protect against oxidative stress and damage to cells and tissues), and -cancer properties (regulation of angiogenesis and in radio-pharmaceuticals for imaging applications) [3]. Such has led to the incorporation of copper and copper-based compounds into various products, such as hospital surfaces (surface treatments for high-touch surfaces or objects, in particular), medical devices, water purification systems, and even clothing, to reduce the risk of infections [4]. Indeed, studies have shown that copper surfaces can reduce the number of microorganisms by over 99%, including bacteria such as E. coli, Salmonella, and MRSA (*Methicillin-resistant Staphylococcus Aureus*) [2,4]. All the more, copper has also been shown to possess anti-viral properties with efficacy against viruses such as the influenza virus and the coronavirus (specifically SARS-CoV-2 or Severe Acute Respiratory Syndrome Coronavirus 2) that is responsible for causing COVID-19 [4].

Incorporating or encapsulating bioactive copper into carriers and delivery systems addresses the need to overcome limitations or shortcomings inherent in its use and/or administration. These include issues like poor stability, rapid clearance, off-target effects, and potential toxicity associated with uncontrolled release. Henceforth, by encapsulating copper within specialized carriers, such as liposomes, hydrogels, microparticles or nanoparticles, amongst others, these challenges can be effectively addressed, offering several advantages: (a) enhanced stability: copper encapsulation within a carrier protects it from degradation, ensuring its stability during storage and transportation; (b) controlled release: carriers or matrices can enable the precise control and modulation over the release kinetics of copper, allowing for sustained or triggered delivery as needed, which is critical for therapeutic efficacy and safety; (c) targeted delivery: by engineering carriers and delivery systems with specific targeting moieties, such as surface ligands or antibodies, copper can be delivered selectively to desired cells or tissues, minimizing off-target effects; and (d) reduced toxicity: controlled delivery of copper can mitigate potential toxicity associated with free copper ions, enhancing the safety profile of therapeutic interventions. Herein, microvesicles (50-150 nm) are small, nano-sized (yet, typically larger than exosomes) membrane-bound vesicles that are released (or derived) from various cells into the extracellular environment [5]. They play important roles in cell-to-cell communication and are involved in various physiological and pathological processes [6]. Microvesicles are formed by the outward budding of the plasma membrane and contain a cargo with a variety of biomolecules such as proteins, lipids, and nucleic acids, which can be transferred from the donor cell to the recipient cell, leading to changes in cellular function [7]. Indeed, microvesicles have been implicated in a range of cellular processes, including immune regulation, inflammation, and coagulation [8]. Because of their re-programmable ability/capacity to transfer biological material between cells, microvesicles have emerged as a promising tool for diagnostic and therapeutic applications in various fields, including biomarkers, cancer, cardiovascular diseases, neurodegenerative disorders, cell therapy, tissue engineering, and regenerative medicine [9]. They have also been investigated for their potential use as novel drug delivery vehicles, due to their ability to transport cargo across cell membranes and deliver therapeutic agents to specific target cells and tissues [10]; an ongoing R&D&I effort, just as is in the case of copper and copper-based biomaterials science(s), to fully understand and modulate/control their potential as biomarkers and/or therapeutic targets and/or tools.

Herein, to improve bio-safety and -efficacy, copper-incorporated microvesicles or CiMs has been introduced as a novel class of biofunctional materials that have attracted significant interest in the recent years due to their potential applications in various fields, including dentistry and oral surgery [11]. CiMs have several unique properties that make them well-suited for use in these applications, including their ability to release copper ions, which have potent anti-bacterial [12], anti-inflammatory [13], and tissue-regenerative properties [14]. Henceforth, this critical review article provides an overview of the available and accruing CiMs literature, focusing on application in dentistry, oral and maxillo-facial surgery, and beyond [15–20]. Also, it discusses the distinct and key properties of CiMs, the various methodologies used for syntheses, and their potential employment in sub-speciality areas such as endodontics, implantology, bone regeneration, gum/gingival/periodontal disease therapy, and prevention of dentin hypersensitivity, amongst others. Finally, some of the ongoing R&D&I work in this emerging biofunctional material field alongside the applications and promising future of CiMs in tackling several challenging unmet needs in surgical odontology are highlighted.

2. Copper-incorporated microvesicles

CiMs can be simply defined or described as a type of lipid-based drug delivery system that can be used to deliver a cargo of copper ions (and other biomolecules and compounds) to cells and tissues (Figure 1). Briefly, CiMs can be synthesized from natural lipids, are stable in biological fluids, and can be engineered to target specific cells and tissues [1]. Hence, CiMs have several potential applications (Table 1) in biomedicine, including the treatment of copper deficiency, copper toxicity, and oxidative stress-related diseases; a promising platform for the delivery of copper-based therapies. For example, copper deficiency is a common problem in patients with mal-absorption syndromes, chronic diarrhea, and parenteral nutrition. Copper deficiency is a condition characterized by insufficient levels of copper in the body, which can lead to a range of health problems due to copper's essential role in various physiological processes. CiMs can be used to deliver copper ions to these patients and improve their nutritional status [2]. While copper toxicity is rare, it can be a serious problem that can occur in patients with Wilson's disease, an inherited disorder that causes copper accumulation in the liver and brain. Herein, CiMs can be used to deliver chelating agents that bind to those *extra* copper ions and remove them from the body [3]. Finally, oxidative stress-related diseases, such as Alzheimer's disease and Parkinson's disease, are characterized by an imbalance between reactive oxygen species (RoS) and antioxidant defenses. Herein, CiMs have been demonstrated to deliver copper ions, which can act as co-factors for anti-oxidant enzymes and improve anti-oxidant defenses in these diseases [4]. Thus, designing, formulating, characterizing, optimizing, and translating CiMs (lab to clinic) requires a delicate fine-tuning and controlled balance of characteristics.

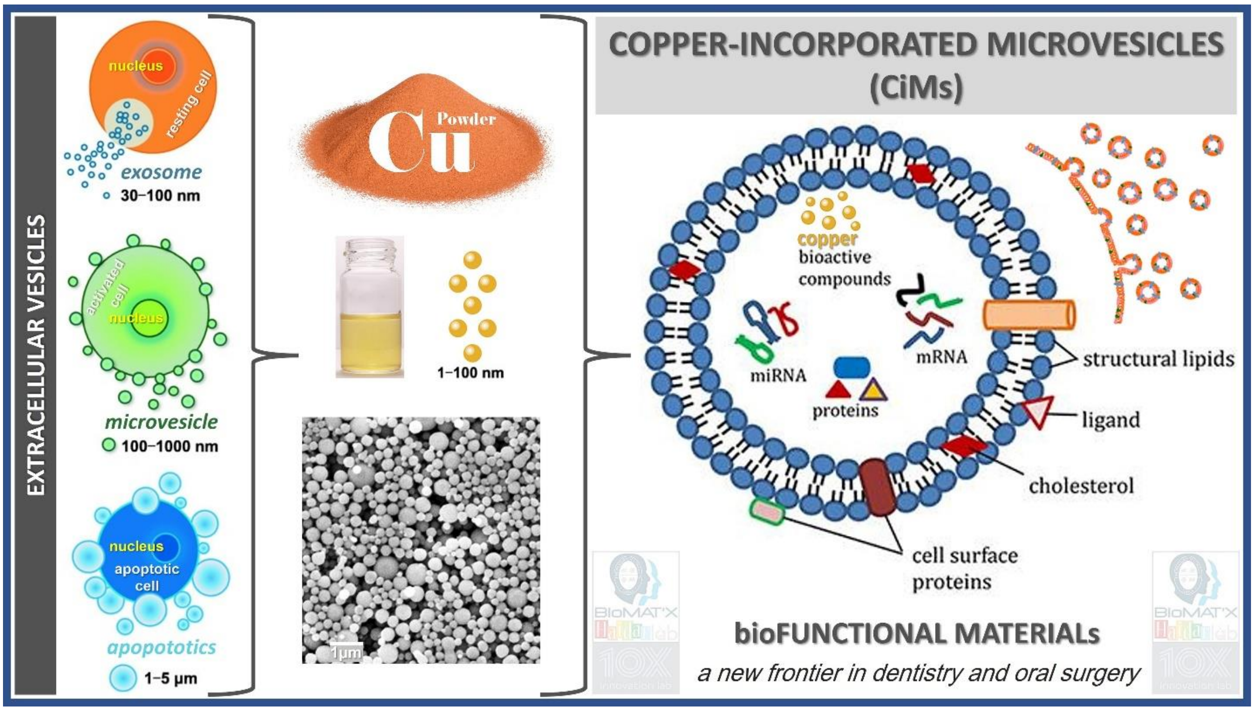

Figure 1. Extracellular vesicles and CiMs as biofunctional materials and drug delivery systems.

3. Advantages and limitations of CiMs

Combinatorial methods, technologies, and systems such as CiMs have several advantages over traditional drug delivery systems. To summarize, CiMs are biocompatible and can be synthesized from natural lipids, which reduces the risk of toxicity and immune reactions. Second, CiMs are stable in biological fluids and can protect their cargo from degradation by enzymes and other biological agents. Third, CiMs can be engineered to target specific cells and tissues by modifying their surface with targeting ligands. Last yet not least, CiMs can cross biological barriers such as the blood-brain barrier and deliver the cargo to the brain [5]. Nevertheless, CiMs also do have some limitations demanding attention. Stability and efficacy can be affected by environmental factors such as temperature, pH, and salt concentration, amongst others. Also, synthesis can be challenging and requires specialized equipment and expertise. Lastly, long-term safety (CiMs toxicity profile) is not well established and requires much more investigation [6]; a current R&D&I topic in our BioMAT'X Laboratory.

Table 1. Potential applications of copper, microvesicles and copper-incorporated microvesicles (CiMs).

Application	Copper	Microvesicles	CiMs
Anti-bacterial and/or-microbial	Potential for antimicrobial use; for synergy with antibiotics; used for targeted delivery of antimicrobials	Used for delivery of antimicrobials	Improved delivery of antimicrobials
Biomedical imaging	Not Applicable (alone)	Used for delivery of imaging agents	Used for delivery of imaging agents
Cancer treatment	Potential as a chemotherapeutic; for synergistic effect with drugs; used for radiation therapy sensitization; used for photodynamic therapy; used for targeted delivery of gene therapy	Used for targeted drug delivery	Improved targeted drug delivery
Cardiovascular disease	Potential for reducing risk	Used for targeted drug delivery	Improved targeted drug delivery
Diabetes	Potential for improving insulin	Not Applicable (alone)	Used for targeted drug delivery
Dental applications	Widely used as in restorative biomaterials	Used for delivery of growth factors, amongst other agents	Potential for improving restorative outcomes
Drug delivery	Poor	Good	Improved
Infectious diseases	Potential for antimicrobial use	Used for delivery of antimicrobials	Improved delivery of antimicrobials
Inflammatory diseases	Potential for reducing symptoms	Used for targeted drug delivery	Improved targeted drug delivery
Neural engineering	Potential for neural stimulation	Used for delivery of neural growth factors	Improved targeted neural growth factor delivery
Tissue engineering	Poor	Good	Improved
Wound healing	Potential for accelerating healing	Used for delivery of growth factors	Improved targeted growth factor delivery
MaxilloFacial and craniofacial surgery	Used for bone regeneration and wound healing	Used for targeted delivery of growth factors and anti-inflammatory agents	Improved targeted delivery of growth factors and anti-inflammatory agents

Cont.

Application	Copper	Microvesicles	CiMs
Oral surgery (intra-oral)	Used for bone regeneration and wound healing	Used for targeted delivery of growth factors and anti-inflammatory agents	Improved targeted delivery of growth factors and anti-inflammatory agents
Orthopedic surgery	Used for bone regeneration and wound healing	Used for targeted delivery of growth factors and anti-inflammatory agents	Improved targeted delivery of growth factors and anti-inflammatory agents
Plastic and reconstructive surgery	Used for wound healing and scar reduction	Used for targeted delivery of growth factors and anti-inflammatory agents	Improved targeted delivery of growth factors and anti-inflammatory agents

4. Synergistic MoA

Briefly, the mechanism of action (MoA) of CiMs relies on the combined properties of copper and microvesicles, both of which contribute to tissue repair and regeneration; a synergy. Copper, known for its antimicrobial and anti-inflammatory effects, promotes wound healing by stimulating angiogenesis, enhancing collagen synthesis, and regulating inflammation. It activates key signaling pathways, such as VEGF for angiogenesis and MMPs for matrix remodeling, to support tissue regeneration. Microvesicles, natural carriers capable of encapsulating copper, ensure targeted delivery and sustained release at the wound site. They also interact with surrounding cells, influencing cellular behavior through signaling pathways like PI3K/Akt, promoting cell survival, proliferation, and migration. The combination of Cu's therapeutic effects with the targeted (carrier) delivery and cellular interactions of microvesicles creates a powerful mechanism for enhancing tissue repair in dental and oral wounds. Ongoing research at our BioMAT'X I+D+i/HAiDAR R&D&I Labs is focused on elucidating the underlying MoA and optimizing this dual action to further advance its potential in oro-dental applications.

5. Dental applications of CiMs

Copper is an essential trace element that plays a vital role in the maintenance of oral and dental health, extending to the cranio-maxillo-facial complex. Indeed, copper ions have been shown to exhibit anti-bacterial, -fungal, and -inflammatory properties, rendering them a promising candidate for the use in management and treatment of oro-dental diseases and conditions [7]. In a 2020 study published in the Journal of Endodontics, Banerjee *et al*. [8] investigated the use of copper nanoparticles (Cu-NPs) as an adjunct to root canal disinfection. Results showed that treatment with Cu-NPs significantly reduced the number of bacteria in infected root canals and improved the success rate of root canal treatment. In 2021, Saquib *et al*. [9] investigated the anti-bacterial properties of copper iodide nanoparticles (CuI-NPs) against *Streptococcus mutans*, a bacterium commonly associated with dental caries. Herein, CuI-NPs effectively inhibited the growth of *S. mutans*, suggesting that they could be used in the development of dental materials with anti-microbial properties. In the same year, Kim and group [10] reported the use of copper-doped titanium dioxide (Cu-TiO2) nanoparticles as an anti-bacterial coating for dental implants. Their results showed that Cu-TiO2 nanoparticles effectively inhibited the growth of *Streptococcus* *sanguinis*, another bacterium commonly found in the oral cavity, and promoted osseointegration of titanium, suggesting a potential use in the further improvement of the success rate of implantology.

On the other hand, research has shown that microvesicles released by mesenchymal stem cells (MSCs) have anti-inflammatory and regenerative properties that could be harnessed to promote healing in the periodontal tissues; *i.e.* soft and hard tissues. In a pre-clinical study recently published in Stem Cells Translational Medicine, Zhao *et al*. [11] isolated microvesicles from MSCs and tested effects on periodontal inflammation and tissue regeneration in a rat model of periodontitis. They found that treatment with MSC-derived microvesicles significantly reduced inflammation and promoted tissue regeneration, when compared to control groups. In another 2020 study appearing in the Journal of Periodontology, Hou *et al*. [12] reported that co-application of MSC-derived microvesicles in a gel form resulted in significant improvement in periodontal tissue healing and bone tissue regeneration, when compared to controls, in a canine/dog model.

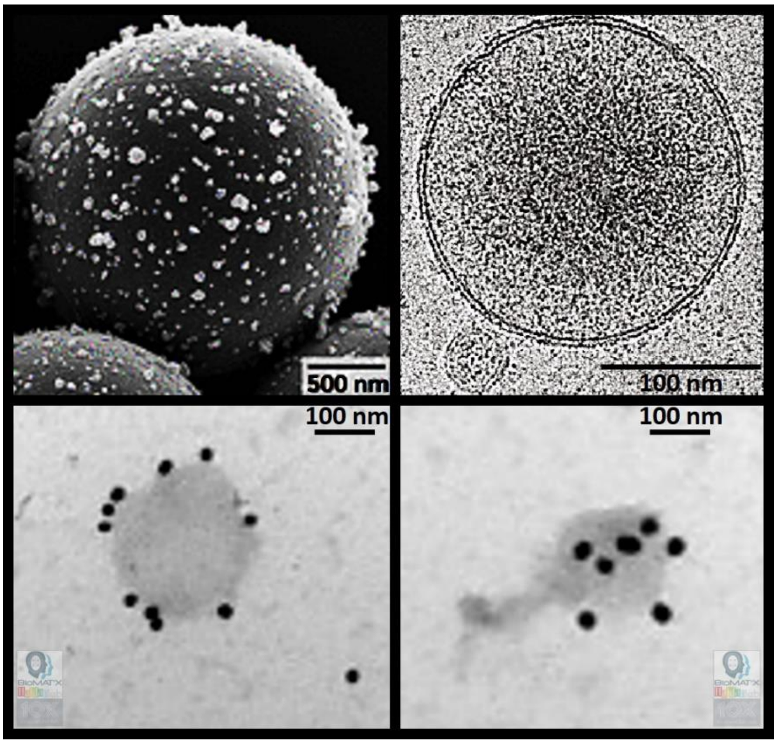

Figure 2. Extracellular vesicles and CiMs as biofunctional materials and drug delivery systems.

The combination (Figure 2) of copper (in nanoparticle format, for example) and microvesicles has been proposed for dental applications as a potential strategy to further enhance the anti-microbial and regenerative properties of dental materials. Copper nanoparticles have been shown to have excellent anti-microbial properties and can effectively inhibit the growth of various oral pathogens, such as *Streptococcus mutans* and *Porphyromonas gingivalis*. However, copper nanoparticles alone may not be sufficient to promote tissue regeneration and repair in dental applications. On the other hand, microvesicles derived from MSCs have been shown to have regenerative and immuno-modulatory properties, and can promote tissue regeneration and repair. Thereby, by combining copper nanoparticles and microvesicles, the resulting composite material may have both strong anti-microbial properties and regenerative properties, making it a promising candidate for dental applications such as periodontal therapy, root canal disinfection and bone repair. Yet, it is important to note that the combinatorial use of copper nanoparticles and microvesicles in dental applications is still in the pre-clinical stage, and more R&D&I is needed to fully understand safety and efficacy before they can be used in clinical settings. Herein, CiMs can be used to deliver copper ions to oral tissues and have several potential applications in dentistry. One potential application of CiMs is in the treatment of dental caries. Dental caries is a common oral disease caused by bacterial biofilm formation on tooth surfaces. Copper ions have been shown to inhibit biofilm formation and kill cariogenic bacteria [13]. CiMs can be used to deliver copper ions to tooth surfaces and inhibit biofilm formation, thus preventing dental caries. Another potential application of CiMs is in the treatment of periodontal disease. Periodontal disease is a chronic inflammatory disease that affects the supporting structures of teeth. Copper ions have been shown to exhibit anti-inflammatory properties and can be used to reduce the inflammation associated with periodontal disease [14]. CiMs can be used to deliver copper ions to periodontal tissues and reduce inflammation, thus improving periodontal health. Finally, CiMs can also be used as a delivery system for dental materials. Dental materials such as restorative materials, cements, and adhesives require adhesion to tooth surfaces to be effective. CiMs can be used to deliver copper ions to tooth surfaces and improve the adhesion of dental materials, thus improving their efficacy [15]. Table 2 summarizes few of the most recent incorporations of CiMs in oro-dentistry.

Table 2. Recent R&D&I related to copper incorporation and use in the oro-dental field.

R&D&I study	Summary of main findings	Reference
Endodontic therapy	Cu-doped phosphate glass with strong anti-bacterial activity against Enterococcus faecalis, a common cause of endodontic infections.	Shetty S, Sekar P, Shetty RM, Abou Neel EA. Antibacterial and antibiofilm efficacy of copper-doped phosphate glass on pathogenic bacteria. *Molecules* 2023, 28(7):3179.
Copper-containing glass ionomer cement (GiC)	The copper ions were found to enhance the mechanical properties and anti-bacterial activity of the GiC, as well as promote the formation of hydroxyapatite, a mineral found in natural teeth.	Aguilar-Perez D, Vargas-Coronado R, Cervantes-Uc JM, Rodriguez-Fuentes N, Aparicio C, *et al.* Antibacterial activity of a glass ionomer cement doped with copper nanoparticles. *Dent. Mater. J.* 2020, 39(3):389–396.
Copper-incorporated implant surface for enhanced osseointegration	Osseointegration is the process by which an implant fuses with bone tissue. Copper ions were found to stimulate osteoblast differentiation and mineralization, as well as inhibit bacterial adhesion and bio-film formation.	Li R, Li S, Zhang Y, Jin D, Lin Z, *et al.* Titanium surfaces with biomimetic topography and copper incorporation to modulate behaviors of stem cells and oral bacteria. *Front. Bioeng. Biotechnol.* 2023, 11:1223339.
Copper-containing composite resin for dental restorations	The polyacrylic acid (PAA)-coated copper iodide (CuI) nanoparticles incorporated into glass ionomer-based materials were found to reduce collagen degradation without an adverse effect on their mechanical properties.	Renn éWG, Lindner A, Mennito AS, Agee KA, Pashley DH, *et al.* Antibacterial properties of copper iodide-doped glass ionomer-based materials and effect of copper iodide nanoparticles on collagen degradation. *Clin. Oral Investig.* 2017, 21(1):369–379.

6. Synergistic effects of copper and microvesicles in CiMs for targeted tissue repair and regeneration

The combination of copper and microvesicles in copper CiMs potentially enhances their therapeutic potential by leveraging the unique properties of both components. Microvesicles, as natural, biocompatible carriers, play a crucial role in encapsulating copper and ensuring its targeted and sustained release at the wound site. They influence key properties such as surface charge and zeta potential, which improve colloidal stability and prevent aggregation. The particle size and distribution of microvesicles also impact cellular uptake, diffusion, and tissue penetration, with their size being an important factor in optimizing drug delivery. Additionally, microvesicles facilitate intercellular communication by interacting with target cells through surface receptors, influencing cellular behavior and promoting tissue regeneration. The biocompatibility of microvesicles is crucial in ensuring that CiMs do not induce unwanted immune responses, while their ability to protect copper ions from oxidation enhances the stability and shelf life of CiMs. Mechanical and rheological properties, such as viscosity and mechanical strength, can also be altered in CiMs, potentially enhancing their stability, drug release profile, and ease of application. On the other hand, copper's antimicrobial and anti-inflammatory properties contribute to tissue repair by regulating inflammation, stimulating angiogenesis, and promoting collagen synthesis. When combined, the synergistic effects of copper and microvesicles—through controlled release, enhanced stability, and targeted delivery—create a potent system for accelerating tissue repair and regeneration, with applications extending beyond dental and oral wounds to chronic wound healing, such as diabetic ulcers, or tissue engineering applications as bone/cartilage regeneration.

These recent developments and studies suggest that CiMs, materials, and surfaces have potential applications in endodontic therapy, dental restorations, and implantology. The incorporation of copper can enhance the anti-bacterial activity, mechanical properties, and biocompatibility of dental materials, and may lead to improved outcomes for patients. A relevant example is a study conducted by Shen *et al*. [7] in which copper-bearing titanium surfaces were tested for their antimicrobial efficacy against oral bacteria. The study found that the copper-bearing surfaces exhibited a significant reduction in bacterial growth compared to the control group, demonstrating the potential of copper ions to inhibit bacterial growth in the oral cavity. CiMs could be used to deliver copper ions to oral tissues and enhance the antibacterial efficacy of dental materials. Another example is a study by Cheng *et al*. [13] in which calcium-phosphate and calcium-fluoride nanocomposites containing chlorhexidine and copper ions were tested for their antibacterial and physical properties. The study found that the addition of copper ions enhanced the antibacterial efficacy of the nanocomposites against cariogenic bacteria. This demonstrates the potential of CiMs to enhance the antibacterial efficacy of dental materials and prevent dental caries. In a study by Cerqueira *et al*. [14], copper and zinc ions were shown to modulate the inflammatory response of human periodontal ligament cells challenged with *Porphyromonas gingivalis* lipopolysaccharide. The study found that copper and zinc ions reduced the expression of pro-inflammatory cytokines, demonstrating their potential to reduce inflammation in periodontal tissues. CiMs could be used to deliver copper and zinc ions to periodontal tissues and reduce inflammation associated with periodontal disease. Finally, a recent review article by Li and Cheng [15] highlights the potential of copper-based biomaterials, including CIMs, for dental applications. The article discusses the anti-bacterial, anti-inflammatory, and remineralization properties of copper ions and their potential to enhance the efficacy of dental materials. The authors suggest that CiMs could be used to deliver copper ions to oral tissues and improve the properties of dental materials, thereby improving their clinical performance. Collectively, these studies illustrate the potential applications of CiMs in dentistry and oral surgery; a quickly-emerging field of R&D&I. To recap once more, CiMs could be used to deliver copper ions to oral tissues and enhance the antibacterial efficacy of dental materials, reduce inflammation associated with periodontal disease, and improve the properties of dental materials, thereby improving their clinical performance.

7. R&D&I update

CiMs for dental, oral, and maxillofacial surgery applications is an active area of study, with many ongoing works exploring the potential of these materials. In addition to the examples discussed above, there are several other ongoing studies and areas of research that are particularly relevant to dental and oral surgery. One area of research is the development of CiMs for use in endodontic applications. Endodontic infections are a common problem in dentistry, and copper ions have been shown to be effective at killing endodontic pathogens. CiMs could be used to deliver copper ions to the root canal system and enhance the antibacterial efficacy of root canal disinfection protocols [16]. Another area of research is the development of CiMs for use in implant dentistry. Implant-related infections are a significant problem, and there is a need for materials that can prevent bacterial colonization and biofilm formation. CiMs could be used to create implant surfaces that are resistant to bacterial colonization, reducing the risk of implant-related infections [17]. It is perhaps noteworthy herein that there is also ongoing R&D&I efforts into the use of CiMs for bone regeneration in oral and cranio-maxillo-facial surgery. Copper ions have been shown to stimulate osteogenesis and angiogenesis; critical processes in bone regeneration. CiMs could be used to deliver the copper ions into bone grafts or to create bio-scaffolds to promote bony regeneration [18]. To re-emphasize, copper ions have also been shown to have anti-bacterial and anti-inflammatory properties, which could be useful in the treatment of periodontal disease. Hence, CiMs could be used to deliver copper ions to the periodontal pocket, where they could reduce bacterial growth and inflammation, and promote tissue regeneration [19]. CiMs could also be used to create dental materials that release copper ions, which could help to reduce or even prevent dentin hypersensitivity, a common dental problem that can cause pain and discomfort in patient, via blocking nerve impulses and reducing fluid flow in the dentin tubules [20]. Such an advancement would greatly impact the dental practise.

Furthermore, the accruing literature includes several studies exploring the use or incorporation of CiMs into dental biomaterials, resins, cements, and other devices. For example, in one study, researchers incorporated CiMs into a dental resin composite material and found that the addition of copper ions improved the material's antibacterial properties and reduced biofilm formation [21]. Another study investigated the use of CiMs in a dental cement material and found that the incorporation of copper ions improved the material's antimicrobial properties and reduced the growth of common oral bacteria [22]. A third study explored the use of CiMs in a dental implant coating material and found that the addition of copper ions improved the anti-bacterial properties of the biomaterial and reduced the risk of implant-associated infections [23]. Moreover, in a study published in the Journal of Dental Research, researchers developed a novel dental adhesive containing copper-doped bioactive glass nanoparticles. They found that the addition of the nanoparticles improved the mechanical properties of the adhesive and increased its anti-bacterial activity against *Streptococcus mutans*, a common cause of tooth decay [24]. Another study investigated the use of CiMs in a dental composite material containing chitosan nanoparticles. Herein, the addition of copper ions to the composite material improved its mechanical properties and also enhanced its antimicrobial activity against a range of oral bacteria [25]. These studies suggest that CiMs have the potential to also be used and/or incorporated within a variety of dental biomaterials and devices to improve their overall anti-microbial properties and reduce the risk of infections. To recap, CiMs have demonstrated the potential to address several unmet needs in dental and oral surgery, including the prevention and treatment of infections (including implant-related), inflammation reduction and control, pain management and enhanced, improved or accelerated soft and hard tissue bio-regeneration. While much work remains to be done to fully understand the potential of these materials and to optimize their properties for specific applications, the ongoing R&D&I in this field looks highly promising, and CiMs could become an important tool or modality for the prevention, management and treatment of oral and cranio-maxillo-facial diseases, and beyond head and face medicine.

8. Technical note

A critical point for the reader to note is the potential toxicity of copper ions, which could limit the use of CiMs in certain applications [26–30]. While copper ions have many beneficial properties, they can also be toxic to cells at high concentrations. Therefore, it is important to carefully balance the concentration of copper ions released by CiMs to ensure that they remain safe for use in the body. Additionally, researchers are exploring ways to encapsulate the copper ions within CiMs to reduce their toxicity and improve their effectiveness. Henceforth, the toxicity of copper ions is an important consideration when developing CiMs for use in dental and oral surgery applications. And, while it is an important consideration, it is also worth noting that the toxicity of copper ions is not a new issue, and researchers have been working to address it for many years. For instance, the use of CiMs as a delivery system for copper ions represents one potential solution to this challenge, as encapsulating the copper ions within the microvesicles could reduce their toxicity while still allowing them to exert their beneficial effects. Herein, and as with any new emerging technology, it is critical to carefully consider both the benefits and the potential risks or drawbacks in order to make informed decisions about their use.

9. Key considerations

To design and develop CiMs suitable for creating a novel dental device or biomaterial, researchers are advised to consider the specific dental application, and design CiMs that can meet those specific and unique requirements. Optimizing the synthesis process is important to ensure that the resulting CiMs have the desired properties [26–30]. Characterizing the properties of CiMs is crucial and should be done thoroughly using techniques such as X-ray diffraction or XRD, scanning/transmission electron microscopy or SEM/TEM, and Fourier Transform Infrared Spectroscopy or FT-IR. Testing the performance of CiMs *in vitro* and *in vivo* is essential to ensure that they are safe and effective for use in the oral environment. Collaborating with experts in the field, including materials science, dentistry, and biomedical engineering, can help ensure the success of the research. By following particular guidelines, researchers can develop and optimize CiMs with the potential to revolutionize the field of oro-dental biomaterials and biodevices. Table 3 highlights such key considerations. In the context of dentistry and biomaterials, copper ion doping and copper nanoparticle loading are two different and distinct methods of modifying the properties of dental materials [31–35]. The copper ion doping method involves incorporating copper ions into the matrix of a dental material, typically through a process known as ion implantation or by using a precursor solution. The copper ions become part of the matrix of the material and can alter its mechanical, antibacterial, or anti-inflammatory properties. Copper ion doping is often used to enhance the mechanical properties of dental materials, as well as to impart antibacterial and anti-inflammatory effects. On the other hand, copper nanoparticle loading is a method that involves incorporating copper nanoparticles onto the surface or within the matrix of a dental material. The nanoparticles can be loaded onto the material through various methods, such as electrospinning or spray-coating. The copper nanoparticles can impart anti-bacterial and mechanical properties to the dental material, as well as improve its biocompatibility. Copper nanoparticle loading is often used to enhance the anti-bacterial properties of dental materials. To summarize, copper ion doping modifies the intrinsic properties of a dental material by incorporating copper ions into its matrix, while copper nanoparticle loading modifies the surface properties of a dental material by depositing copper nanoparticles onto it. Both methods have the potential to improve the properties and performance of dental materials, depending on the specific needs and applications.

Table 3. Key considerations for designing and developing CiMs for dental biomaterials and biodevices.

Property	**Copper**	**Microvesicles**	**CiMs**
Material type	Metal	Biological vesicles	Hybrid material
Size in nm	50-100 microns	50-100 nm	50-100 nm
Surface charge in mv (varies with origin and copper incorporation)**	+2 to +4	−5 to +5	−2 to +2
Biocompatibility	Generally biocompatible	Generally biocompatible	Generally biocompatible
Anti-microbial Properties	Strong	Variable	Strong
Drug delivery	Poor	Good	Improved with copper incorporation
Tissue engineering	Poor	Good	Improved with copper incorporation
Dental applications	Widely used	Under development	Under development
Oral surgery applications	Widely used	Under development	Under development
Mode of action	Oxidative Stress	Various	Oxidative Stress
Limitations	Toxicity, if/when in excess	Short half-life, limited stability	Limited understanding, potential toxicity

** The values provided are general and may vary depending on the specific application or use case.

10. CiMs as biofunctional materials via the layer-by-layer self-assembly technique

Briefly, the Layer-by-Layer (LbL) technique is a method of creating thin films or coatings by alternately depositing positively and negatively charged layers of various materials, such as polyelectrolytes or nanoparticles, onto a substrate or surface (Figure 3). The resulting films can have unique properties and functionalities, depending on the choice and sequence of the deposited layers [36]. To recap, LbL step-wise self-assembly is a versatile and widely used technique for building multi-layer thin films and coatings with precise control over film thickness and composition. It involves the sequential deposition of oppositely charged building blocks, such as polymers, nanoparticles, and biomolecules, onto a substrate or template through electrostatic interactions, hydrogen bonding, and other non-covalent forces. Herein, LbL can be applied to various materials, including metals, ceramics, polymers, and composites, and has been utilized in various fields, such as drug delivery, tissue engineering, and biosensing. Haidar ZS recently discussed the potential of bio-inspired/-functional colloidal core-shell polymeric-based nanosystems, including LbL-based structures, in tissue engineering, bioimaging, and nanomedicine [36]. In this context, LbL is proving a useful and popular technique for the synthesis of CiMs with *controlled* anti-bacterial properties. The LbL self-assembly technique involves the sequential deposition of oppositely charged layers on the surface of the microvesicles, creating a thin film that incorporates copper ions. Studies have shown that CiMs synthesized using this technique exhibit enhanced antibacterial properties compared to non-copper-incorporated microvesicles, making them a promising candidate for dental and other medical applications. For example, Lee *et al*. [37] used the layer-by-layer self-assembly technique to synthesize copper-incorporated microvesicles on the surface of dental implant materials, resulting in enhanced anti-bacterial properties. In addition, Chen and Zhang [38] used the layer-by-layer self-assembly technique to incorporate copper-containing nanoparticles onto electrospun nanofibrous membranes for wound healing, Ren *et al*. [39] synthesized Cu(I)-alginate multilayer films using layer-by-layer self-assembly for antibacterial applications, and Wang *et al*. [40] prepared a copper-doped chitosan/graphene oxide composite film using layer-by-layer self-assembly for enhanced antibacterial activity. These studies demonstrate the potential of CiMs synthesized using LbL self-assembly for a range of dental and medical applications. To recap, the afore-mentioned remarks and those outlined below in Tables 4 and 5 aim to offer expertise-based scientific and technological depth for guiding researchers in the design, development, characterization, optimization and translation of CiMs for creating new dental device(s) and/or biofunctional material(s). The remarks are to take into consideration the various important aspects such as the specific dental application, optimization of the synthesis process, characterization of the properties of CiMs, testing their performance, and collaboration with experts in the field.

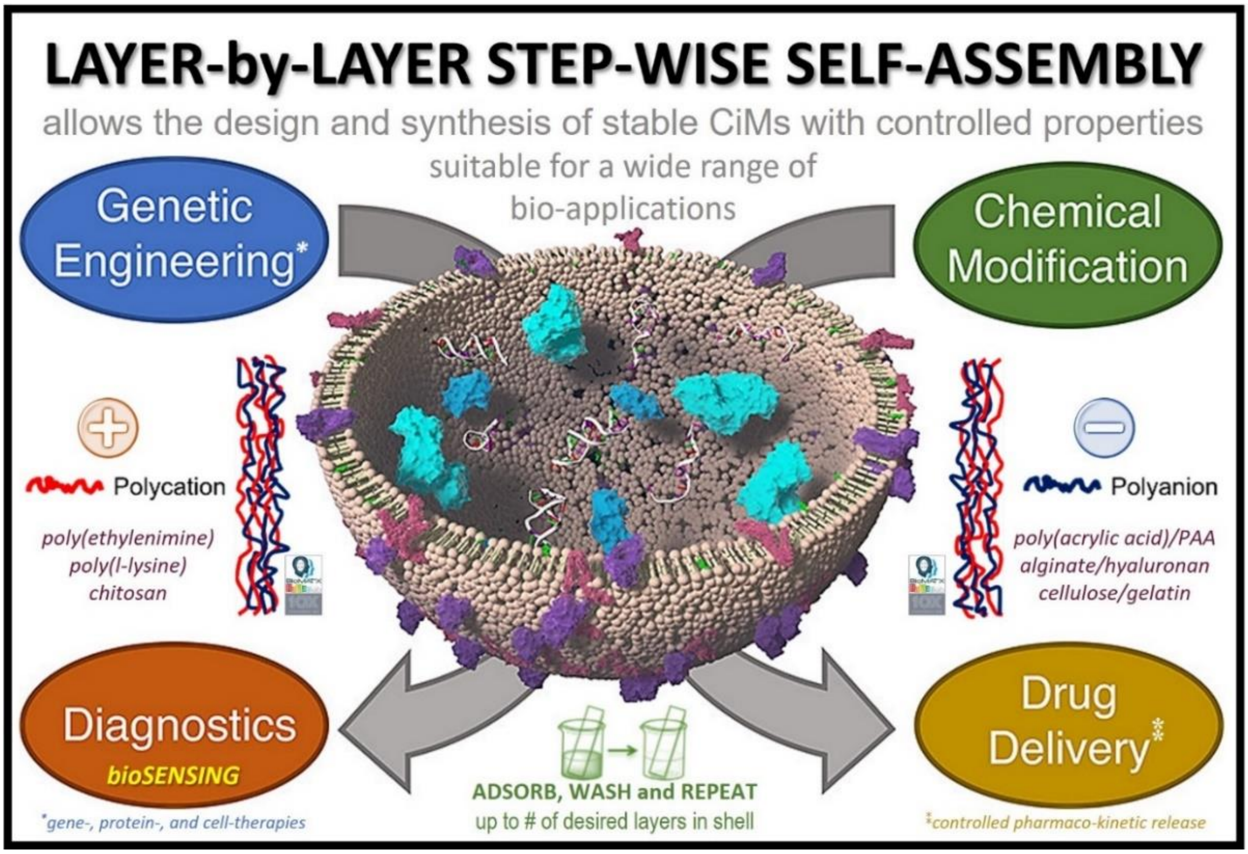

Figure 3. LbL technique and its use in designing and developing controlled CiMs-based systems.

11. Relevant considerations

Copper can be employed in various formats such as nanoparticles, ions, and salts, depending on the specific application. The concentration of copper used also varies depending on the intended use and the type of material being produced. For example, in dental biomaterials, copper nanoparticles have been used at concentrations ranging from 0.1% to 1%, while in copper-infused textiles, copper ions are often used at concentrations of around 50 ppm [41–45]. It is noteworthy that the concentration of microvesicles used in dental materials is not well-established yet, and there is currently no consensus on the optimal concentration. Some studies have used concentrations ranging from 1 μg/mL to 1 mg/mL, while others have used higher or lower concentrations; depending on the specific application and the desired material properties. Table 4 outlines the key considerations for designing and developing CiMs using the LbL self-assembly technique.

Table 4. Key considerations for designing and developing CiMs via the LbL self-assembly technique.

Copper-incorporated microvesicles produced by L-b-L self-assembly build-up
CiMs can be produced by the layer-by-layer (L-b-L) self-assembly technique, which involves sequential deposition of oppositely charged polyelectrolytes onto microvesicle surfaces and subsequent incorporation of copper ions or compounds (including copper nanoparticles and nanocapsules) do have potential biomedical applications. Follows, are some key technical parameters, experimental setup requirements, characterization and optimization steps for the successful translation of CiMs from the laboratory to the clinic:
Critical technical parameters: 1. Microvesicle size: 100-500 nm 2. Surface charge: Slightly -*ve* to reduce non-specific interactions with biological tissues 3. Copper concentration: 1-10 mM 4. Copper release rate: Controlled and sustained release over 24-72 hours 5. Biocompatibility: Non-toxic and non-immunogenic
Model experimental setup: 1. Microvesicle preparation: Sonication, extrusion, or other methods to produce uniform and monodisperse microvesicles 2. Polyelectrolyte deposition: Alternating deposition of polycations and polyanions, typically using poly(allylamine hydrochloride) and sodium alginate 3. Copper incorporation: Addition of copper ions or compounds, such as copper sulfate or copper nanoparticles, during polyelectrolyte deposition 4. Characterization and optimization for translation from lab to clinic: Pre-clinical and clinical analysis of microvesicle size, surface charge, copper content, release rate, cytotoxicity/biocompatibility, and anti-microbial activity, amongst other bio-parameters: a. Microvesicle size and distribution: Dynamic light scattering, electron microscopy, or nanoparticle tracking analysis; b. Surface charge: Zeta potential measurement; c. Copper content and distribution: Atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, or energy-dispersive X-ray spectroscopy; d. Copper release rate (pharmaco-kinetic profile): in vitro release studies using dialysis, fluorescence, or spectrophotometry; e. Biocompatibility: Cell viability assays, in vitro cytotoxicity assays, and in vivo biocompatibility studies; f. Anti-microbial activity: Disk diffusion assays, minimum inhibitory concentration assays, and in vivo efficacy studies; g. Stability and storage: Long-term stability studies under various conditions, including temperature and humidity; h. Scale-up and production optimization: Optimization of production methods, including automation and process scale-up; i. Regulatory compliance: Compliance with regulatory requirements, including Good Manufacturing Practice or GMP and pre-clinical safety evaluations; j. Clinical trials: Conduct of clinical trials to assess safety and efficacy in humans; and k. Commercialization: Strategies for commercialization and market entry, including partnerships with pharmaceutical or medical device companies.

Note: The technical parameters, experimental setup, characterization, and optimization steps may and will vary depending on the specific application, indication or use case.

The incorporation of microvesicles into copper-based formulations for dental applications offers significant advantages beyond the antimicrobial properties of copper alone. Microvesicles serve as biocompatible carriers that encapsulate copper, enabling controlled, sustained release at the site of action, ensuring prolonged antimicrobial effects. This is especially important in dental applications where persistent bacterial colonization in dental caries requires continuous treatment. Additionally, microvesicles facilitate tissue regeneration and repair, promoting healing in areas damaged by caries, which copper alone may not address. As an emerging technology, CiMs combine antimicrobial efficacy with tissue regeneration, offering a promising, comprehensive approach to oral and dental care, awaiting further R&D&I results in the accruing literature, patents, and lab-to-market translation to fully comprehend and apprehend their potential. Table 5 provides a guideline for developing and optimizing LbL-CiMs for a wide range of applications in healthcare.

Table 5. Developing and optimizing LbL-CiMs for a wide range of applications in healthcare—a guideline.

Layer-by-Layer self-assembly technique for copper-incorporated microvesicles
Overview: The layer-by-layer self-assembly technique is a versatile approach for producing copper-incorporated microvesicles with controlled properties and behavior. This technique involves the sequential deposition of oppositely charged polyelectrolytes onto the surface of microvesicles, followed by the incorporation of copper ions or copper-containing compounds to impart desired functionalities.
Key steps: 1. Preparation of microvesicles as a template 2. Deposition of polyelectrolyte multilayers onto the microvesicle surface using the layer-by-layer self-assembly technique 3. Incorporation of copper ions or copper-containing compounds into the polyelectrolyte multilayers to form copper-incorporated microvesicles 4. Characterization of the physical, chemical, and biological properties of the copper-incorporated microvesicles
Properties and behaviors controlled: 1. Size and surface charge of the microvesicles 2. Copper ion release rate and concentration 3. Biocompatibility and cytotoxicity 4. Antimicrobial activity and efficacy against bacterial biofilms 5. Enhanced cellular uptake and targeted drug delivery
Applications: 1. Biomedical imaging 2. Cancer therapy and diagnosis 3. Antibacterial and antimicrobial applications 4. Tissue engineering and regenerative medicine 5. Oral and maxillofacial surgery 6. Drug delivery and controlled release 7. Biosensors and diagnostics
Advantages: 1. Versatile and tunable approach for producing copper-incorporated microvesicles with desired properties and behaviors 2. Biocompatible and non-toxic materials and processes 3. Enhanced stability and bioactivity of incorporated copper ions or compounds 4. Improved targeting and delivery of therapeutic agents 5. Potential for multifunctionality and synergistic effects

Microvesicles have been incorporated into dental materials such as resin composites and cements for their regenerative potential and ability to promote tissue repair [46–48]. Studies have shown that microvesicles derived from stem cells can enhance the biocompatibility and mechanical properties of dental materials, as well as promote pulp regeneration and prevent bacterial growth. For example, one study found that incorporation of stem cell-derived microvesicles into a dental pulp-capping material enhanced cell proliferation and mineralization in vitro, as well as promoting dentin bridge formation and pulp tissue regeneration *in vivo* [49]. Another study showed that microvesicles derived from stem cells can improve the biocompatibility and mechanical properties of resin composites, potentially reducing the risk of postoperative complications and improving the longevity of restorations [50]. To recap, the use of microvesicles in dental materials shows promising potential for enhancing their regenerative and restorative properties, highlighting the need for further R&D&I.

12. Perspective

The current state-of-the-art regarding the combination of copper and microvesicles is still in its early stages. While there have been promising preclinical studies demonstrating the potential of this combination for various biomedical applications, such as wound healing, tissue regeneration, and drug delivery, there is still a need for further research to fully understand the underlying mechanisms and optimize the design of these hybrid systems. Some challenges that need to be addressed include ensuring the stability and biocompatibility of the copper-microvesicle complexes, as well as improving their targeting and cellular uptake efficiency. However, the potential benefits of this approach make it an exciting area of research with promising future prospects. Indeed, as demonstrated throughout this article, CiMs are a promising platform for the delivery of copper-based therapies. They can be synthesized from natural lipids, are stable in biological fluids, and can be engineered to target specific cells and tissues. CiMs have several potential applications in biomedicine, including the treatment of copper deficiency, copper toxicity, and oxidative stress-related diseases. However, their stability, efficacy, and long-term safety profile require further investigation. Nonetheless, CiMs represent an exciting new development in the field of drug delivery and have the potential to improve the treatment of a wide range of diseases. CiMs have several potential applications in dentistry. They can be used to deliver copper ions to oral tissues and treat oral diseases such as dental caries and periodontal disease. CiMs can also be used as a delivery system for dental materials, improving their adhesion to tooth surfaces. The development of CiMs for dental applications represents a promising new avenue for the treatment and prevention of oral diseases. As the field of dental biomaterials and devices continues to evolve, CiMs hold great promise as a versatile platform technology that can be tailored to meet specific dental applications. Ongoing R&D&I efforts can further optimize the synthesis process, characterize the properties of CiMs, and explore their performance in various *in vitro* and *in vivo* models. With further advancements in CiMs biotechnology, researchers may soon be able to develop innovative dental devices and functional biomaterials that can improve patient outcomes and enhance the overall health.

13. Regulatory barriers to market

As with any new medical technology, there will be regulatory barriers to the introduction of CiMs or microvesicle-based therapies for oral and dental applications. Regulatory agencies such as the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA) require extensive testing and clinical trials to demonstrate safety and efficacy before a new product can be approved for clinical human use. Additionally, cost-effectiveness issues may also prose barriers to market entry, for R&D&I to consider. Chile is the World's largest producer of copper, accounting for approximately one-third of global production. The national copper mining industry is a key contributor to its economy, and it has been instrumental in helping Chile become one of the most prosperous nations in South America. Copper is so important to the country that it is sometimes referred to as "Chile's bread and butter". Given Chile's significant role in copper production, it may be well-positioned to take advantage of the benefits of copper-based dental biomaterials and devices. Nonetheless, the aforesaid regulatory, cost and market barriers may still need to be addressed [51–55].

14. Closing remark

With continued, augmented, and collaborative R&D&I efforts, it is very possible that CiMs and microvesicle-based therapies could soon become a valuable and indispensable addition to the oral and dental armamentarium accessible to our patients.

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Conflicts of interests

Author discloses no potential conflict(s) of interest of any shape or form.

Ethical statement

This work is exempt from any Ethics Committee and/or Institutional Review Board approval as it is deemed neither necessary nor required.

References

[1] Lucotti S, Kenific CM, Zhang H, Lyden D. Extracellular vesicles and particles impact the systemic landscape of cancer. *EMBO J.* 2022, 41(18):e109288.

[2] Hordyjewska A, Popiołek Ł, Kocot J. The many "faces" of copper in medicine and treatment. *Biometals* 2014, 27(4):611–21.

[3] Abbastabar M, Sarfi M, Golestani A, Karimi A, Pourmand G, *et al.* Tumor-derived urinary exosomal long non-coding RNAs as diagnostic biomarkers for bladder cancer. *EXCLI J.* 2020, 19:301–310.

[4] Balkrishna A, Kumar A, Arya V, Rohela A, Verma R, *et al.* Phytoantioxidant functionalized nanoparticles: a green approach to combat nanoparticle-induced oxidative stress. *Oxid. Med. Cell Longev.* 2021, 2021:3155962.

[5] Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol. Cell Biochem.* 2010, 345(1–2):91–104.

[6] Guan D, Zhao L, Shi X, Ma X, Chen Z. Copper in cancer: from pathogenesis to therapy. *Biomed. Pharmacother*. 2023,163:114791.

[7] Li R, Li S, Zhang Y, Jin D, Lin Z, *et al.* Titanium surfaces with biomimetic topography and copper incorporation to modulate behaviors of stem cells and oral bacteria. *Front. Bioeng. Biotechnol.* 2023, 11:1223339.

[8] Lina RM, Sindhu R, Bharathwaj VV, Dhamodhar D, Sathiyapriya S, *et al.* Systematic review on copper calcium hydroxide nanoparticles in the treatment of apical periodontitis. *J. Pharm. Bioallied. Sci*. 2023, 15(Suppl 1):S105–S109.

[9] Xu VW, Nizami MZI, Yin IX, Yu OY, Lung CYK, *et al.* Application of copper nanoparticles in dentistry. *Nanomaterials (Basel)* 2022, 12(5):805.

[10] Gkioka M, Rausch-Fan X. Antimicrobial effects of metal coatings or physical, chemical modifications of titanium dental implant surfaces for prevention of peri-implantitis: a systematic review of in vivo studies. *Antibiotics (Basel)* 2024, 13(9):908.

[11] Wang T, Zhou Y, Zhang W, Xue Y, Xiao Z, *et al.* Exosomes and exosome composite scaffolds in periodontal tissue engineering. *Front. Bioeng. Biotechnol*. 2024, 11:1287714.

[12] Lin H, Chen H, Zhao X, Ding T, Wang Y, *et al.* Advances of exosomes in periodontitis treatment. *J. Transl. Med.* 2022, 20(1):279.

[13] Cheng L, Weir MD, Xu HH, Kraigsley AM, Lin NJ, *et al.* Antibacterial and physical properties of calcium-phosphate and calcium-fluoride nanocomposites with chlorhexidine. *Dent. Mater*. 2012, 28(5):573–83.

[14] Yu Y, Li X, Ying Q, Zhang Z, Liu W, *et al.* Synergistic effects of shed-derived exosomes, Cu^{2+}, and an injectable hyaluronic acid hydrogel on antibacterial, anti-inflammatory, and osteogenic activity for periodontal bone regeneration. *ACS Appl. Mater. Interfaces* 2024, 16(26):33053–33069.

[15] Covarrubias C, Trepiana D, Corral C. Synthesis of hybrid copper-chitosan nanoparticles with antibacterial activity against cariogenic Streptococcus mutans. *Dent. Mater. J.* 2018, 37(3):379–384.

[16] Muraguchi K, Minami H, Minesaki Y, Suzuki S, Tanaka T. A study of self-adhesive resin cements for bonding to silver-palladium-copper-gold alloy—effect of including primer components in cement base. *Dent. Mater. J*. 2011, 30(2):199–205.

[17] Mitra D, Kang ET, Neoh KG. Antimicrobial copper-based materials and coatings: potential multifaceted biomedical applications. *ACS Appl. Mater. Interfaces* 2020, 12(19):21159–21182.

[18] Romero-Sánchez LB, Marí-Beffa M, Carrillo P, Medina MÁ, Díaz-Cuenca A. Copper-containing mesoporous bioactive glass promotes angiogenesis in an in vivo zebrafish model. *Acta. Biomater.* 2018, 68:272–285.

[19] Kumar R, Nagesh S, Mani SP. Preparation and assessment of antimicrobial effect of strontium and copper co-substituted hydroxyapatite nanoparticle-incorporated orthodontic composite: a preliminary in vitro study. *Cureus* 2023, 15(10):e47495.

[20] Ghamor-Amegavi EP, Yang X, Qiu J, Xie L, Pan Z, *et al.* Composition control in biphasic silicate microspheres on stimulating new bone regeneration and repair of osteoporotic femoral bone defect. *J. Biomed. Mater. Res. B Appl. Biomater.* 2020, 108(2):377–390.

[21] Munir A, Marovic D, Nogueira LP, Simm R, Naemi AO, *et al.* Using copper-doped mesoporous bioactive glass nanospheres to impart anti-bacterial properties to dental composites. *Pharmaceutics* 2022, 14(10):2241.

[22] Marovic D, Haugen HJ, Negovetic Mandic V, Par M, Zheng K, *et al.* Incorporation of copper-doped mesoporous bioactive glass nanospheres in experimental dental composites: chemical and mechanical characterization. *Materials (Basel)* 2021, 14(10):2611.

[23] Marovic D, Bota M, Tarle F, Par M, Haugen HJ, *et al.* The influence of copper-doped mesoporous bioactive nanospheres on the temperature rise during polymerization, polymer cross-linking density, monomer release and embryotoxicity of dental composites. *Dent. Mater.* 2024, 40(7):1078–1087.

[24] Li Y, Lu J, Shi J, Zhang L, Mu H, *et al.* Carboxymethyl chitosan nanoparticle-modulated cationic hydrogels doped with copper ions for combating bacteria and facilitating wound healing. *Front. Bioeng. Biotechnol.* 2024, 12:1429771.

[25] Xu VW, Nizami MZI, Yin IX, Niu JY, Yu OY, *et al.* Copper materials for caries management: a scoping review. *J. Funct. Biomater.* 2023, 15(1):10.

[26] Vitaliti A, De Luca A, Rossi L. Copper-dependent kinases and their role in cancer inception, progression and metastasis. *Biomolecules* 2022, 12(10):1520.

[27] Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* 2005, 12(10):1161–208.

[28] Antsotegi-Uskola M, Markina-Iñarrairaegui A, Ugalde U. New insights into copper homeostasis in filamentous fungi. *Int. Microbiol.* 2020, 23(1):65–73.

[29] Dou Y, Lee A, Zhu L, Morton J, Ladiges W. The potential of GHK as an anti-aging peptide. *Aging Pathobiol. Ther.* 2020, 2(1):58–61.

[30] Kumar V, Pandita S, Singh Sidhu GP, Sharma A, Khanna K, *et al.* Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. *Chemosphere* 2021, 262:127810.

[31] Fernandes G, Renné WG, Visser MB, Sabatini C. Esterase inhibition and copper release from copper iodide dental adhesives - an in vitro study. *J. Adhes. Dent.* 2020, 22(3):265–274.

[32] Türk S, Altınsoy I, Efe GÇ, Ipek M, Özacar M, *et al.* Biomimetic synthesis of Ag, Zn or Co doped HA and coating of Ag, Zn or Co doped HA/fMWCNT composite on functionalized Ti. *Mater. Sci. Eng. C Mater. Biol. Appl.* 2019, 99:986–998.

[33] Xu VW, Yin IX, Niu JY, Yu OY, Nizami MZI, *et al.* The anti-caries effects of copper tetraamine fluoride on enamel: An in vitro study. *J. Dent.* 2024, 151:105446.

[34] Wang P, Yuan Y, Xu K, Zhong H, Yang Y, *et al.* Biological applications of copper-containing materials. *Bioact Mater* 2020, 6(4):916–927.

[35] Kargozar S, Mozafari M, Ghodrat S, Fiume E, Baino F. Copper-containing bioactive glasses and glass-ceramics: from tissue regeneration to cancer therapeutic strategies. *Mater. Sci. Eng. C Mater. Biol. Appl.* 2021, 121:111741.

[36] Haidar ZS. Bio-inspired/-functional colloidal core-shell polymeric-based nanosystems: Technology promise in tissue engineering, bioimaging and nanomedicine. *Polymers* 2010, 2(3):323–352.

[37] Li Y, Luo W, Liu Y, Lu Y, Geng W, *et al.* Copper-containing titanium alloys promote the coupling of osteogenesis and angiogenesis by releasing copper ions. *Biochem. Biophys. Res. Commun.* 2023, 681:157–164.

[38] Lee KH, Farheen R, Arshad Z, Ali M, Hassan H, *et al.* Optimized Cu-doping in ZnO electro-spun nanofibers for enhanced photovoltaic performance in perovskite solar cells and photocatalytic dye degradation. *RSC. Adv.* 2024, 14(22):15391–15407.

[39] Tidim G, Guzel M, Soyer Y, Erel-Goktepe I. Layer-by-layer assembly of chitosan/alginate thin films containing Salmonella enterica bacteriophages for antibacterial applications. *Carbohydr Polym* 2024, 328:121710.

[40] Zong J, He Q, Liu Y, Qiu M, Wu J, *et al.* Advances in the development of biodegradable coronary stents: a translational perspective. *Mater. Today Bio.* 2022, 16:100368.

[41] Renné WG, Lindner A, Mennito AS, Agee KA, Pashley DH, *et al.* Antibacterial properties of copper iodide-doped glass ionomer-based materials and effect of copper iodide nanoparticles on collagen degradation. *Clin. Oral Investig.* 2017, 21(1):369–379.

[42] Niccoli-Filho W, da Silveira MC, Vieira ND, Riva R. Effects of copper vapor laser radiation on the root canal wall of human teeth: a scanning electron microscope study. *Photomed. Laser. Surg.* 2005, 23(3):317–23.

[43] Torres-Rosas R, Torres-Gómez N, García-Contreras R, Scougall-Vilchis RJ, Domínguez-Díaz LR, *et al.* Copper nanoparticles as nanofillers in an adhesive resin system: An in vitro study. *Dent. Med. Probl.* 2020, 57(3):239–246.

[44] Xia C, Cai D, Tan J, Li K, Qiao Y, *et al.* Synergistic effects of N/Cu dual ions implantation on stimulating antibacterial ability and angiogenic activity of titanium. *ACS Biomater. Sci. Eng.* 2018, 4(9):3185–3193.

[45] Manziuc M, Kui A, Chisnoiu A, Labuneț A, Negucioiu M, *et al.* Zirconia-reinforced lithium silicate ceramic in digital dentistry: a comprehensive literature review of our current understanding. *Medicina (Kaunas)* 2023, 59(12):2135.

[46] Kotan G, Uysal BA. Effects of boric acid combined with injectable platelet rich fibrin on the mineralized nodule formation and the viability of human dental pulp stem cells. *Tissue Cell* 2024, 90:102508.

[47] Han P, Johnson N, Abdal-Hay A, Moran CS, Salomon C, *et al.* Effects of periodontal cells-derived extracellular vesicles on mesenchymal stromal cell function. *J. Periodontal Res.* 2023, 58(6):1188–1200.

[48] Fernandes TL, Cortez de SantAnna JP, Frisene I, Gazarini JP, Gomes Pinheiro CC, *et al.* Systematic review of human dental pulp stem cells for cartilage regeneration. *Tissue Eng. Part B Rev.* 2020, 26(1):1–12.

[49] Camussi G. Exosomes and microvesicles: from stem cell biology to translation in human diseases. *Stem Cell Rev. Rep.* 2021, 17(2):303–304.

[50] Tan F, Li X, Wang Z, Li J, Shahzad K, *et al.* Clinical applications of stem cell-derived exosomes. *Signal Transduct. Target. Ther. 2* 024, 12;9(1):17.

[51] National Statistics Institute (INE) of Chile. Copper Production in Chile. 2021, Available: https://www.ine.gob.cl/statistics/economic/mining/inventories-of-copper-mining (accessed on 20 July 2024)

[52] Akhtar H, Alhamoudi FH, Marshall J, Ashton T, Darr JA, *et al.* Synthesis of cerium, zirconium, and copper doped zinc oxide nanoparticles as potential biomaterials for tissue engineering applications. *Heliyon* 2024, 10(7):e29150.

[53] Liu H, Tang Y, Zhang S, Liu H, Wang Z, *et al.* Anti-infection mechanism of a novel dental implant made of titanium-copper (TiCu) alloy and its mechanism associated with oral microbiology. *Bioact. Mater.* 2021, 8:381–395.

[54] Pierre C, Bertrand G, Pavy I, Benhamou O, Rey C, *et al.* Antibacterial electrodeposited copper-doped calcium phosphate coatings for dental implants. *J. Funct. Biomater.* 2023, 14(1).
[55] Abdulghafor MA, Mahmood MK, Tassery H, Tardivo D, Falguiere A, *et al.* Biomimetic coatings in implant dentistry: a quick update. *J. Funct. Biomater.* 2024, 15(1).