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E-mail: jzus_b@zju.edu.cn

Review

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Optogenetics in oral and craniofacial research

Qinmeng ZHANG[1,2*], Luyao SONG[2*], Mengdie FU[1,2], Jin HE[1,2], Guoli YANG[1,2✉], Zhiwei JIANG[1,2✉]

[1]*Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Cancer Center of Zhejiang University, Hangzhou 310000, China*
[2]*Zhejiang University School of Medicine, Hangzhou 310058, China*

Abstract: Optogenetics combines optics and genetic engineering to control specific gene expression and biological functions and has the advantages of precise spatiotemporal control, noninvasiveness, and high efficiency. Genetically modified photosensory sensors are engineered into proteins to modulate conformational changes with light stimulation. Therefore, optogenetic techniques can provide new insights into oral biological processes at different levels, ranging from the subcellular and cellular levels to neural circuits and behavioral models. Here, we introduce the origins of optogenetics and highlight the recent progress of optogenetic approaches in oral and craniofacial research, focusing on the ability to apply optogenetics to the study of basic scientific neural mechanisms and to establish different oral behavioral test models in vivo (orofacial movement, licking, eating, and drinking), such as channelrhodopsin (ChR), archaerhodopsin (Arch), and halorhodopsin from *Natronomonas pharaonis* (NpHR). We also review the synergic and antagonistic effects of optogenetics in preclinical studies of trigeminal neuralgia and maxillofacial cellulitis. In addition, optogenetic tools have been used to control the neurogenic differentiation of dental pulp stem cells in translational studies. Although the scope of optogenetic tools is increasing, there are limited large animal experiments and clinical studies in dental research. Potential future directions include exploring therapeutic strategies for addressing loss of taste in patients with coronavirus disease 2019 (COVID-19), studying oral bacterial biofilms, enhancing craniomaxillofacial and periodontal tissue regeneration, and elucidating the possible pathogenesis of dry sockets, xerostomia, and burning mouth syndrome.

Key words: Lasers; Cell differentiation; Bacterial virulence; Nervous system; Neurophysiology; Behavioral science

1 Introduction

Optogenetics is an emerging technology that combines genetics and optics to precisely activate and control the functions of specific cells in biological tissues, the activities of the spinal cord and peripheral nerves in animals, and even biological behaviors (Cao et al., 2021; Yi et al., 2021; Zhou et al., 2022). Optogenetics has several advantages, including high temporal accuracy, highly accurate stimulation intensity, spatial specificity, and precise spatial and temporal targeting, enabling direct light stimulation in a less invasive manner (Moreno Morales et al., 2021; Yoshii et al., 2021). In many neuroscience studies, targeting optical fibers to locally stimulate cells is far less traumatic to experimental animals than traditional methods (Jackman et al., 2020). Therefore, optogenetics is a powerful tool for studying oral behavior and bacteriology. In particular, optogenetics has been widely used for basic scientific research (e.g., orofacial movement, eating and drinking, licking, and cell differentiation) (Lee et al., 2019; Vajtay et al., 2019; Niyazi et al., 2020; Esmaeili et al., 2021) and in preclinical studies (e.g., trigeminal neuralgia and maxillofacial cellulitis) (Xia et al., 2021; Kc et al., 2022) in recent years (Fig. 1). The unique advantages of optogenetics have inspired many methods and ideas for future research (Pérez et al., 2022). Thus, recent advances in optogenetics in oral and craniofacial research should be reviewed.

Optogenetics was first developed after the discovery of opsin and its encoded microbial rhodopsin proteins, and the adequacy and specificity of opsin expression are the key to optogenetic technology. In

✉ Guoli YANG, guo_li1214@zju.edu.cn
Zhiwei JIANG, jzw0913@zju.edu.cn
* The two authors contributed equally to this work
Guoli YANG, https://orcid.org/0000-0002-1780-8757
Zhiwei JIANG, https://orcid.org/0000-0001-8203-6151

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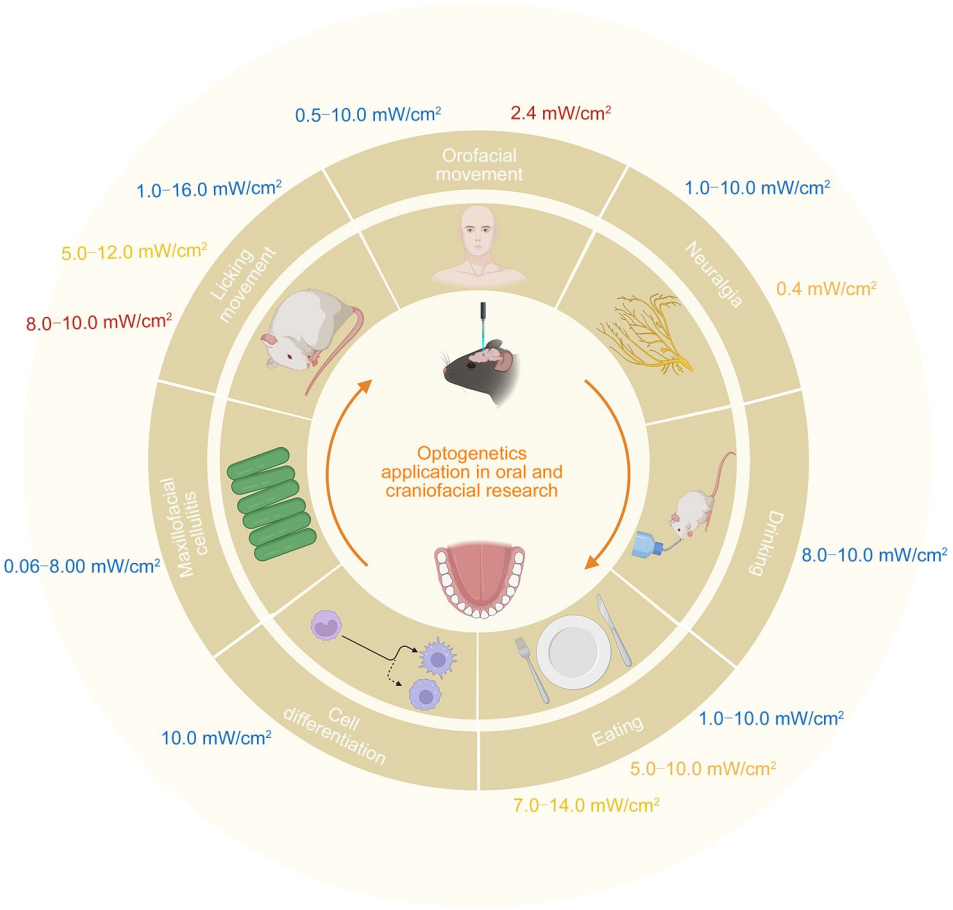

Fig. 1 Optogenetic applications in oral and craniofacial research, including orofacial movement, trigeminal neuralgia, drinking, eating, licking, causative agent of maxillofacial cellulitis, and cell differentiation. The power (mW/cm^2) font color in the outermost circle represents the corresponding light wavelength color (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

1971, Dieter OESTERHELT first found a purplish-red substance containing retinol in the cell membrane of bacteria that could act as a light sensor or photoreceptor, which was named bacteriorhodopsin (BR) (Oesterhelt and Stoeckenius, 1971). In 1977, further research led to the discovery of another protein mediating the chloridion migration of halorhodopsin (HR) (Matsuno-Yagi and Mukohata, 1977). Then, the discovery of channelrhodopsin (ChR), which can be expressed in neurons and affect their physiological function, greatly promoted the development of optogenetics and ChR is considered an ideal medium for the control of neurons through light stimulation. Under light activation, microbial opsin has high spatiotemporal accuracy and can regulate molecular events in living cells and organisms in a targeted manner. At present, microbial opsin is mainly divided into four categories: proton pumps (e.g., BR), ion pumps (e.g., HR), ion channels (e.g., ChR), and sensory rhodopsins (e.g., histidine kinase rhodopsins) (Chowdhury and Yamanaka, 2021). Molecular biology, viral biology, and other approaches have been used to introduce exogenous light-sensitive protein genes into living cells and tissues, making optogenetic tools genetically specific through viral vectors, recombinase-expressing driver animal lines, and anatomical targeting strategies (Bali et al., 2022; Liu et al., 2022) (Fig. 2a). The photosensitive channel proteins in the cell membrane's structure can be controlled by irradiation with specific wavelengths of light. When the photosensitive channel proteins are activated, the cell membrane voltage changes, ultimately leading to the activation or inhibition of neurons (Fig. 2b) (Reshetnikov et al., 2022). Different photosensitive channels are activated by different wavelengths of light (Fig. 2c); therefore, the core technical difference of optogenetics technology is the choice of light-sensitive

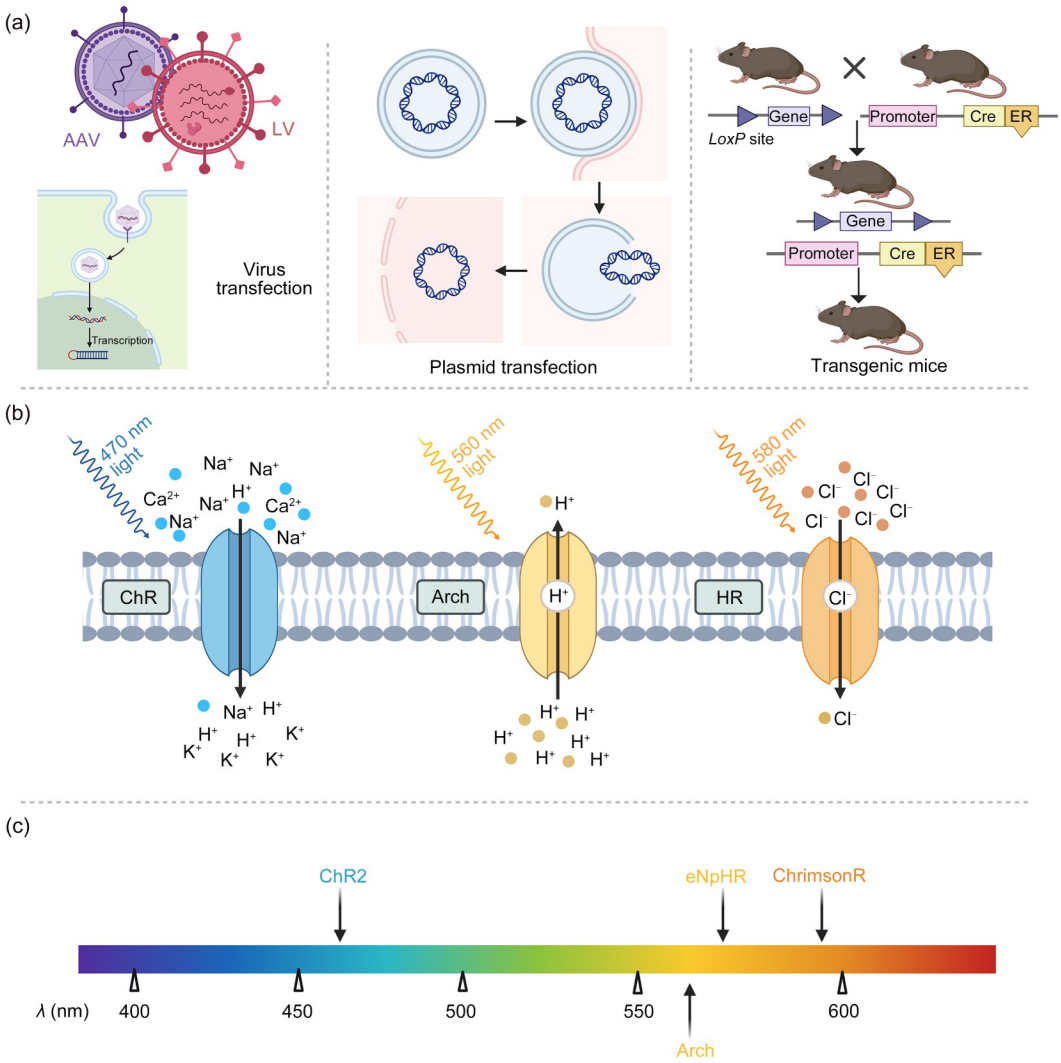

Fig. 2 Classification of optogenetic sensors and excitation wavelengths. (a) Schematic representation of common gene delivery vector technologies used in oral and craniofacial research. (b) Upon excitation of the sensor channelrhodopsin (ChR) with light, a cation channel appears in the middle of the protein and cations flow into the channel. The sensor archaerhodopsin (Arch) moves positively charged protons to the extracellular environment following light activation and hyperpolarizes (inhibits) the cell. An injection of Cl^- into neurons infected with a halorhodopsin (HR) sensor inhibited neuronal activity upon irradiation. (c) Different excitation wavelengths (λ) corresponding to various sensors. AAV: adeno-associated virus; LV: lentiviral vector; *LoxP*: locus of X-over P1; Cre: cyclization recombinase; ER: estrogen receptor; eNpHR: enhanced halorhodopsin from *Natronomonas pharaonis*.

channels (Fenno et al., 2011; Kim et al., 2017; Tremblay et al., 2020).

Optogenetics was first applied in oral and craniofacial research in 2011 to assess drinking behavior (Domingos et al., 2011). Notably, optogenetics has been applied to various fields of oral and craniofacial research in the last decade, including the assessment of orofacial movement, eating, licking, trigeminal neuralgia, bacteria, and cell differentiation, and is increasingly becoming a research hotspot (Fig. 3). Scientists have used various light-sensitive channels to regulate neurons to explain the occurrence of different diseases or behaviors in oral and craniofacial studies (Falkner et al., 2020). Although optogenetics and its related technologies have developed significantly in the last decade, most optogenetic experiments are still in the exploratory stage, and considerably more work is needed before these approaches can be applied in clinical settings. Researchers are therefore committed to improving optogenetic methods to increase the efficiency of

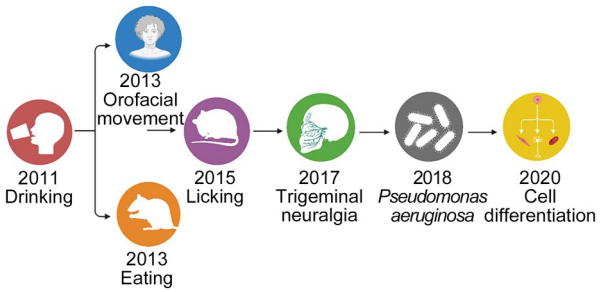

Fig. 3 Timeline of the first optogenetic applications in oral and craniofacial fields. Optogenetics was first applied to assess drinking behavior in 2011. Optogenetic approaches have been applied to assess orofacial movement, eating, licking, trigeminal neuralgia, bacteria, and cell differentiation between 2013 and 2020.

optogenetic stimulation, enhance safety, extend these tools to more clinical conditions, and control neuronal or biological functions through the precise manipulation of optogenetic channels (Zhang et al., 2019; Tsai et al., 2020). In this review, we briefly introduce a variety of optogenetic studies in the oral and craniofacial fields, describe their research protocols and characteristics, present their applications and research achievements, and summarize the advantages of bacterial and neuronal regulation in the field of optogenetics (Table 1). Finally, we conclude with a discussion of limitations and future directions in this field, providing new ideas and guidance for basic research and optogenetics applications.

Table 1 Optogenetic sensors in oral and craniofacial research

Research classification	λ (nm)	Vector	Opsin	Experimental subject	Conclusion	Reference
Orofacial movement	446/637	Transgenic/ AAV2.5	ChR2/ ReaChR	Mice	Optogenetic activation of corticobulbar neurons in the motor cortex evokes orofacial movement.	Mercer Lindsay et al., 2019
Orofacial movement	460	Transgenic	ChR2	Mice	Optogenetic stimulation of denervation-paralyzed whisker pads increases the sensitivity, amplitude, and velocity of whisker pad muscles and reduces fatigue.	Vajtay et al., 2019
Orofacial movement	473	AAV2	ChR2	Mice	Optogenetic activation of the ventrolateral area of the ventromedial hypothalamus-lateral periaqueductal gray pathway activates jaw movement.	Falkner et al., 2020
Orofacial movement	473	AAV1	ChR2	Mice	Optogenetic activation of GABAergic/ glycinergic neurons in the ventral medulla inhibits the activity of tongue protrusion genioglossus and retractor muscles.	Dergacheva et al., 2020
Trigeminal neuralgia	460	Transgenic	ChR2	Mice	Optogenetic stimulation of peripheral sensory neurons in the dorsal root ganglion and trigeminal ganglion produces persistent facial pain and sequelae after trigeminal nerve injury.	Hardt et al., 2019
Trigeminal neuralgia	473	AAV2	ChR2	Rats	Optogenetic stimulation of GABAergic neurons in the nucleus accumbens core modulates the transmission of trigeminal pain signals.	Islam et al., 2021
Trigeminal neuralgia	473	AAV2	ChR2	Rats	Optogenetic stimulation of the motor cortex alleviates neuropathic pain caused by infraorbital nerve injury.	Islam et al., 2020
Trigeminal neuralgia	473	AAV5	ChR2	Mice	Optogenetic activation of neurons in the anterior cingulate cortex modulates trigeminal pain.	Liu et al., 2020
Trigeminal neuralgia	473	AAV2	ChR2	Rats	Optogenetic activation of excitatory neurons in the ventrolateral periaqueductal gray ameliorates pain.	Elina et al., 2021
Trigeminal neuralgia	473	AAV	ChR2	Mice	Optogenetic activation of neurons in the ventrolateral orbital cortex leads to anti-anxiodepressive effects in trigeminal neuralgia.	Sheng et al., 2020

To be continued

Table 1 (continued)

Research classification	λ (nm)	Vector	Opsin	Experimental subject	Conclusion	Reference
Trigeminal neuralgia		AAV5	ChR2	Mice	Optogenetic excitation of A11 neurons leads to pain relief in models of chronic trigeminal neuropathic pain.	Crawford et al., 2021
Trigeminal neuralgia	473/589	AAV	ChR2/ eNpHR	Rats	Optogenetic activation of trigeminal ganglion neurons modulates trigeminal pain.	Kc et al., 2022
Trigeminal neuralgia	470	AAV	ChR2	Mice/rats	Optogenetic activation of the central amygdala-parabrachial pathway inhibits lateral parabrachial neurons, decreasing pain after chronic constriction injury of the infraorbital nerve.	Raver et al., 2020
Trigeminal neuralgia	470	Transgenic	ChR2	Mice	Optogenetic activation of cortical spreading depression causes trigeminal neuralgia.	Harriott et al., 2021
Drinking	473	AAV9	ChR2	Mice	Optogenetic excitation of neurons in the organum vasculosum of the lamina terminalis affects thirst.	Kinsman et al., 2020
Drinking	470	AAV5	ChR2	Mice	Optogenetic stimulation of projections from neurotensin-expressing neurons in the central amygdala to the parabrachial nucleus reinforces and increases ethanol drinking, as well as consumption of sucrose and saccharin solutions.	Torruella-Suárez et al., 2020
Drinking/eating	473/532	AAV2	ChR2/ Arch	Mice	Optogenetic activation and inhibition of glutamatergic neurons modulate food and water consumption.	Gong et al., 2020
Eating	473	AAV1	ChR2	Mice	Optogenetic activation of neurons in the peri-locus coeruleus that express prodynorphin modulates sodium appetite and intake.	Lee et al., 2019
Eating	470	AAV1	ChR2	Mice	Optogenetic stimulation of peptide release from nociceptin/orphanin FQ neurons directly inhibits the ability of these neuronal populations to regulate appetitive behavior.	Hernandez et al., 2021
Eating	465	AAV2	ChR2	Rats	Optogenetic stimulation of nucleus accumbens shell neurons decreases sucrose intake and modifies the lick microstructure.	Chometton et al., 2020
Eating	473/561	AAV2/5	ChR2/ eNpHR	Mice	Optogenetic activation and inhibition of catecholaminergic neurons in the ventrolateral medulla projecting to the paraventricular nucleus of the thalamus modulate feeding behavior.	Sofia Beas et al., 2020
Licking	473	Transgenic	ChR2	Mice	Optogenetic inactivation of the primary tongue-jaw motor cortex leads to an increase in ipsilateral spout licking and a decrease in contralateral spout licking.	Mayrhofer et al., 2019
Licking	473	Transgenic	ChR2	Mice	Optogenetic activation of tongue-jaw-related neurons in the motor cortex increases the fraction of early licks.	Esmaeili et al., 2021
Licking	473	AAV2/9	CoChR	Mice	Optogenetic activation of indirect striatal projection neurons suppresses contraversive licking and promotes ipsiversive licking.	Lee and Sabatini, 2021
Licking	473/563	AAV2/9	ChR2/ Arch	Mice	Optogenetic activation of somatostatin-expressing neurons in the central amygdala and vesicular glutamate transporter 2-positive neurons in the deep mesencephalic nucleus regulates licking behavior.	Zheng et al., 2022

To be continued

Table 1 (continued)

Research classification	λ (nm)	Vector	Opsin	Experimental subject	Conclusion	Reference
Licking	520/532/635	AAV2/8	ChrimsonR	Mice	Optogenetic inactivation of D1- and D2-expressing medium spiny neurons in the ventrolateral striatum results in different licking behaviors.	Chen et al., 2021
Licking	473	Transgenic	ChR2	Mice	Optogenetic perturbation of Purkinje cell activity disrupts behaviors by degrading licking bout rhythmicity and initiating and terminating licking bouts.	Gaffield et al., 2022
Licking	470/593	AAV5	ChR2/Arch	Mice	Optogenetic activation and inactivation of the substantia nigra pars reticulata to the motor thalamus circuit alter licking behavior in mice.	Morrissette et al., 2019
Bacteria	470	Plasmids	bPAC	*Pseudomonas aeruginosa*	Optogenetic activation of bPAC function regulates the ability of cAMP to control bacterial motility and toxicity.	Xia et al., 2021
Bacteria	470	Plasmids	YGS24	*P. aeruginosa*	Optogenetic stimulation of the Gac/Rsm signaling cascade regulates infection and virulence.	Cheng et al., 2021
Cell differentiation	470	LV	ChR2	Stem cells	Optogenetics induces the neurogenic differentiation of human dental pulp cells.	Niyazi et al., 2020

AAV: adeno-associated virus; ChR: channelrhodopsin; ReaChR: red-activatable ChR; GABA: γ-aminobutyric acid; eNpHR: enhanced halorhodopsin from *Natronomonas pharaonis*; Arch: archaerhodopsin; bPAC: bacterial photoactivated adenylate cyclase gene; cAMP: cyclic adenosine monophosphate; CoChR: chloromonas oogama ChR; LV: lentiviral vector.

2 Optogenetics and basic science

2.1 Orofacial movement

Generally, optogenetics shows promise in regulating neurons and muscle cells due to its precise control ability, which may be superior to that of traditional electrical stimulation. On the one hand, optogenetics can influence orofacial activity by regulating muscle cells. The facial nerve innervates facial muscles in addition to chewing muscles and the levator palpebrae superioris, including the auricular muscle, occipitalis muscles, and platysma muscle, which are involved in orofacial movements. In mice, facial neuropathy leads to the loss of whisker pad function and reduced whisker movements. A previous study found that 24 h after facial nerve transection, functional denervation of the whisker pad muscles could be realized by optogenetic stimulation of the facial nerve in transgenic mice. To determine the effect of optogenetics on whisker pad muscles, researchers have used direct optogenetic muscle stimulation in transgenic mice expressing ChR2 in the whisker pad muscles after facial nerve transection. Blue light (460 nm, 8 mW light) can be used to irradiate mice with facial nerve denervation and directly stimulate muscle cells, thus activating the optogenetic sensors ChR2. The sensitivity, amplitude, and speed of the whisker pad muscles were gradually enhanced after 24 h, but fatigue was reduced 48 h after denervation (Vajtay et al., 2019). Therefore, optogenetics is more appropriate than traditional electrical stimulation as a regulatory tool in clinical practice to control cells to recover muscle function after nerve injury or motor neuron degeneration in various neuromuscular systems (Benevides et al., 2022).

On the other hand, optogenetic tools can activate orofacial movements by controlling neurons in the brain. Optogenetics usually combines in vivo and ex vivo physiology and cell type-specific perturbations and explores the mechanism by which the brain pathway coordinates orofacial movement by activating specific photosensitive proteins (Mercer Lindsay et al., 2019; Dergacheva et al., 2020; Falkner et al., 2020). In a mouse experiment, vesicular glutamate transporter 2 neurons exhibited short-latency excitatory postsynaptic currents after exposure to blue light. This technology activated the opsin ChR2, stimulating the ventromedial hypothalamus ventrolateral area-lateral periaqueductal gray pathway, leading to aggressive

behavior and jaw movement (Falkner et al., 2020). This projection-specific control has also been applied to studying postsynaptic inhibition in retractor and genioglossal muscles during sleep (Dergacheva et al., 2020). Moreover, red light penetrates brain tissue more effectively than blue light. To obtain a faster discharge rate, a new ChR2 mutant, red-activatable ChR (ReaChR), has been introduced and successfully applied to studying the effects of the activation of neurons in the orofacial motor cortex on orofacial movement (Mercer Lindsay et al., 2019). Given the critical role of optogenetics in orofacial movements, we believe that this technology shows great promise in treating orofacial muscle injuries and facial paralysis.

2.2 Eating and drinking

Using optogenetics to directly control specific areas of the brain has the advantage of excluding the influence of other factors, so optogenetics can be applied to accurately study eating and drinking behaviors. The anterior peri-locus coeruleus is strongly associated with thirst and eating behaviors, and glutamatergic neurons in the anterior peri-locus coeruleus are multisynaptic convergence areas related to hunger and thirst behaviors. A subset of excitatory neurons expressing prodynorphin in the anterior peri-locus coeruleus are components of the key neural mechanism regulating sodium intake behavior. A previous study confirmed this finding by activating anterior peri-locus coeruleus glutamatergic neurons by irradiating the opsin ChR2 with blue light, resulting in a reduction in food intake. In contrast to ChR2, activation of the optogenetic inhibitory channel protein archaerhodopsin (Arch) with 532 nm light (7–14 mW) led to increased food intake (Gong et al., 2020). The use of optogenetics to activate subsets of prodynorphin index neurons also affects appetite and eating behavior (Lee et al., 2019).

In addition, feeding behavior is mediated by the hypothalamic arcuate nucleus and ventral tegmental area. Optogenetic technology has been used to stimulate nociceptin/orphanin FQ neurons, increasing outward currents and K^+ conductance upon illumination with 470 nm blue light. Irradiation led to ChR2 activation, followed by hyperpolarization and reduced firing, confirming that stimulation of the hypothalamic arcuate nucleus led to an increase in feeding behavior. In contrast, activation of the ventral tegmental area produced the opposite effect and led to decreased eating behavior (Hernandez et al., 2021). Similarly, optogenetic activation of ChR2 in nucleus accumbens shell neurons reduced the activity of rostral ventral pallidum neurons and decreased sucrose intake upon irradiation with blue light (Chometton et al., 2020).

The enhanced HR from *Natronomonas pharaonis* (eNpHR), a chloride pump used to inhibit neural activity, enables high aggregation on the membranes of neurons. Targeted inhibitory regulation of the vasculum of lamina terminalis neurons can be achieved with 561 nm light excitation. This precise method of inhibiting neurons reduces drinking behavior (Kinsman et al., 2020). Further research also showed that eNpHR activation led to reduced glucose deprivation and increased eating behavior by inhibiting catecholaminergic neurons in the ventrolateral medulla-paraventricular nucleus in the thalamus-nucleus accumbens pathway (Sofia Beas et al., 2020). Overall, optogenetics has strong potential as a noninvasive, clinically valuable technique for controlling feeding behavior.

2.3 Licking

Licking movements involve opening the jaw and extending the tongue to realize a specific objective. These movements require that the jaw and tongue muscles coordinate in a reliable and precise manner under the control of brainstem nuclei.

Optogenetics research has revealed the effects of specific brain regions on licking movements by modulating particular neurons. The light-sensitive channel ChR2 was used to activate neurons in the tongue-jaw motor cortex under irradiation with 473 nm blue light (mean power 8–10 mW), which increased early licking (Esmaeili et al., 2021). In contrast to neuron activation optogenetic experiments, one side of the primary tongue-jaw motor cortex was inactivated by stimulating inhibitory neurons expressing ChR2, resulting in increased ipsilateral spout licking and decreased spout licking on the other side (Mayrhofer et al., 2019). In addition, changes in licking movement were studied by optogenetically stimulating indirect striatal projection neurons and the lateral superior colliculus. The results showed that targeted activation of light-sensitive proteins inhibited the ipsilateral lateral superior colliculus but unexpectedly stimulated the contralateral lateral superior colliculus. This result might explain the emergence of ipsiversive licking and reveal thalamic

competition (Lee and Sabatini, 2021). The Purkinje cell is the primary neuron that transmits impulses emanating from the cerebellar cortex (Chang et al., 2020). Researchers have used various experiments to investigate the effectiveness of regulating this cell to modulate licking activity. ChR2 was expressed in all Purkinje cells through hybridization of transgenic mice. In optogenetic experiments, ChR2 stimulation was realized with a continuous-wave laser. At the expected peak of licking, introducing light stimulation interfered with Purkinje cell activity; thus, Purkinje cells in the cerebellum modulate motor events (Gaffield et al., 2022).

ChrimsonR is another red-shifted channelrhodopsin that has been used to activate neurons. ChrimsonR contains the K176R point mutation, which increases the closing speed of the channel and is suitable for use in procedures with high stimulation rates (Goyer and Roberts, 2020). Chen et al. (2021) used laser stimulation in ChrimsonR optogenetic experiments to activate medium spiny neurons expressing D1 and D2 receptors in the ventral striatum, resulting in different licking movements that explained the effects of D1- and D2-expressing medium spiny neurons on licking movements. Optogenetic inhibitory channel proteins have also been applied in research on licking movements. For example, optogenetic technology has been used to inhibit deep mesencephalic nucleus neurons using Arch, which is inhibited by excitation with 563 nm yellow light and affects licking movements (Zheng et al., 2022).

2.4 Cell differentiation

Human dental pulp stem cells are ectoderm-derived mesenchymal stem cells that can form mineralized nodules. They are derived from migrating neural crest cells, and their ability to differentiate has received considerable attention in the fields of tissue engineering and regenerative medicine (Pisciotta et al., 2020). A recent study has shown that human dental pulp stem cells have a remarkably similar immunophenotype to that of bone marrow mesenchymal stem cells and are promising candidates for generating human neuronal cells (Bar et al., 2021).

Since human dental pulp stem cells are readily available and can be isolated from third molars, which are usually considered medical waste, their ability to differentiate has received much attention. An attempt has been made to use optogenetics to modulate the neurogenic differentiation of dental pulp stem cells. Human dental pulp stem cells were infected with a lentivirus carrying human ChR2 (hChR2) (H134R) and were optogenetically activated with 470 nm blue light. Upon stimulation with blue light, ChR2 induced depolarization, resulting in morphological changes in human dental pulp stem cells. The cell body stretched from a fusiform morphology to a neuron-like morphology. In addition, the expression of immature and mature neuronal markers increased, and human dental pulp stem cells viability increased (Fig. 4a) (Niyazi et al., 2020).

Thus, the application of optogenetics to cell differentiation and the control of cell differentiation to specific morphologies holds great promise. Despite the exact mechanism remaining unclear, the result of this experiment suggested that the differentiation of human dental pulp stem cells was a feasible strategy by which to generate neural cells (Niyazi et al., 2020). This method requires additional development as a transplantation therapy for neurodegenerative diseases.

3 Optogenetics and preclinical studies

3.1 Trigeminal neuralgia

Trigeminal neuralgia is the most common form of severe facial pain. Although various treatment options are available, their effects are not ideal. In particular, there is no effective long-term treatment for atypical trigeminal neuralgia (Ma et al., 2020). At present, a major obstacle in the development of new therapies for trigeminal neuralgia is the need to verify the brain areas that are directly related to trigeminal neuralgia to generate potential therapeutic targets. Optogenetics has been combined with behavioral observations to determine whether particular brain areas are involved in the regulation of behavior after trigeminal neuralgia (Chen et al., 2023). These approaches activate or inhibit trigeminal neuralgia by regulating neurons in the pain conduction pathway. These methods can explain the pathological mechanism of trigeminal neuralgia and provide new ideas for clinical treatments.

Some researchers believe that abnormalities instigate trigeminal neuralgia in the afferent neurons of the trigeminal root or ganglion; this is called “Ignition Theory” (Liu et al., 2021). Optogenetic stimulation of

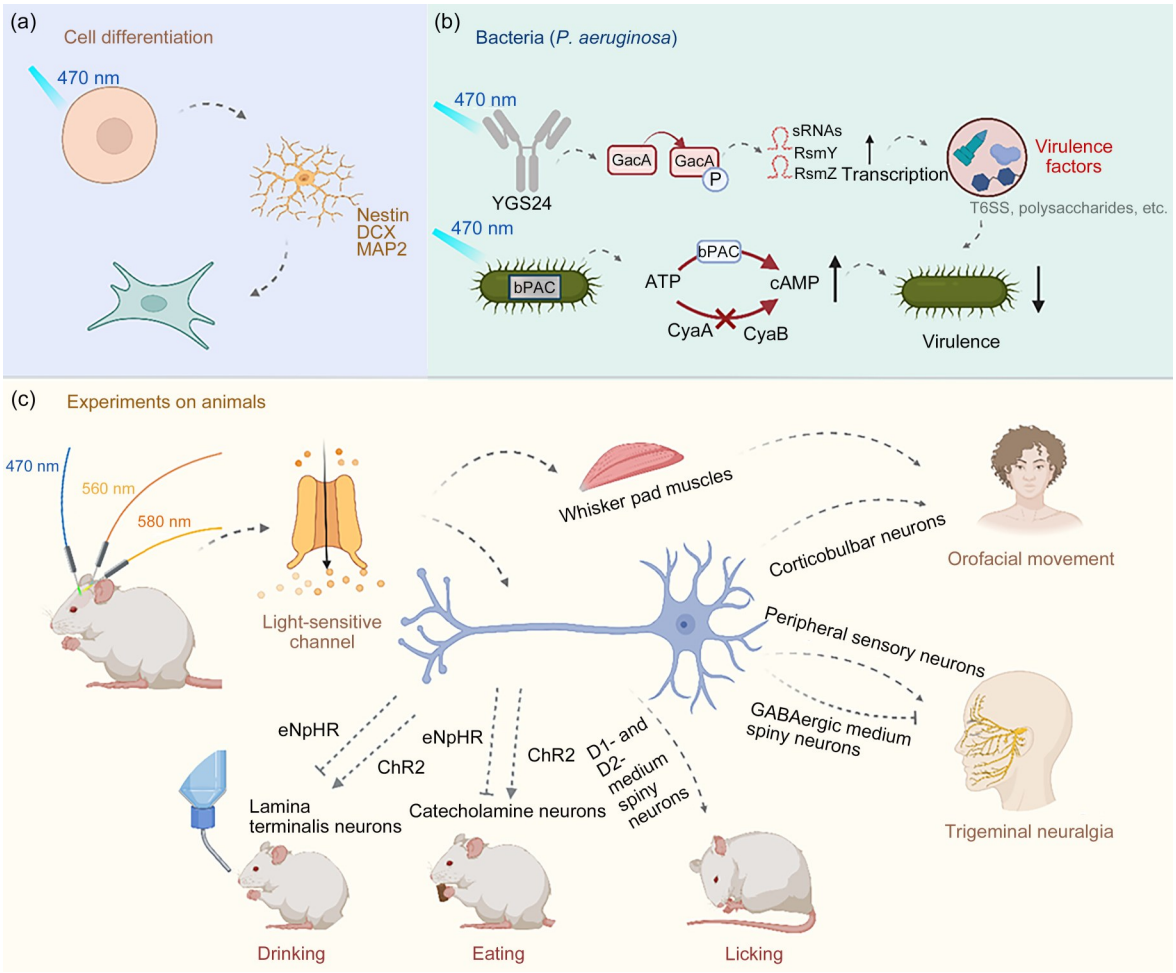

Fig. 4 Diverse mechanisms of optogenetic effects. (a) After optogenetic stimulation of human dental pulp stem cells, the numbers of neural progenitor cells expressing Nestin, immune neurons expressing doublecortin (DCX), and mature neurons expressing microtubule-associated protein 2 (MAP2) increased, and the cell morphology was altered. (b) Optogenetics induces GacS phosphorylation by stimulating light-dependent proteins and activates the transcription of the small RNAs (sRNAs), RsmY, and RsmZ, thereby downregulating acute infection and upregulating genes related to chronic virulence, such as the type 6 secretory system (T6SS), polysaccharides, diguanylate cyclases, hydrogen cyanide, and quorum sensing (QS), affecting the activity of *Pseudomonas aeruginosa*. The bacterial photoactivated adenylate cyclase (*bPAC*) gene was introduced into bacteria to form a light-dependent *P. aeruginosa*, which affected the activity of the bacteria by modulating the level of cyclic adenosine monophosphate (cAMP). (c) Different wavelengths excite different photosensitive proteins and manipulate neurons or cells to cause various maxillofacial activities or sensations. ATP: adenosine triphosphate; CyaA, CyaB: adenylate cyclase; eNpHR: enhanced halorhodopsin from *Natronomonas pharaonis*; ChR: channelrhodopsin; GABA: γ-aminobutyric acid.

peripheral sensory neurons in the dorsal root ganglion and trigeminal ganglion with blue light produced persistent facial pain and sequelae after trigeminal nerve injury (Hardt et al., 2019). Calcitonin gene-related peptide (CGRP) is reported to play an important role in transmission of trigeminal neuralgia and is the most essential neuropeptide expressed in trigeminal neuralgia (Berger et al., 2022). eNpHR was activated by 593 nm yellow light irradiation, and the activity was assessed under light-on and light-off conditions. The results showed that GABAergic inhibition increased and CGRP release decreased in trigeminal ganglion neurons, thereby reducing the effects of trigeminal neuralgia. In contrast, the activation of ChR2 with 473 nm blue light results in persistent hypersensitivity to trigeminal neuralgia (Kc et al., 2022). Further results showed that, after M1 in the trigeminal neuralgia rat model was stimulated by optogenetics, α-CGRP treatment could improve pain, indicating that the central neuromodulation mechanism was

independent of the control of peripheral pain (Islam et al., 2021).

Nevertheless, how neural pathways determine or influence trigeminal neuralgia or which brain nuclei and neurons are involved remains unknown. Optogenetics combined with mouse models of chronic constriction injury of the infraorbital nerve (CCI-ION) can reflect neuronal activity in neural circuits in real time, thus revealing the relationship between these changes and pain behavior (Crawford et al., 2021). Optogenetic tools have been used to manipulate the central amygdaloid nucleus (CeA)-parabrachial pain conduction pathway by activating ChR2, leading to CCI-ION pain relief. Thus, this pathway appears to play a causal role in pain regulation and the development of chronic pain (Raver et al., 2020). Similarly, upon light stimulation, the concentration of GABA neurons in the ventrolateral periaqueductal gray increased, while glutamate release decreased, thus leading to a decrease in excitatory nerve transmission and the regulation of the trigeminal pain pathway in rats (Elina et al., 2021). The anterior cingulate cortex (ACC) is a critical brain region for processing chronic pain (Li et al., 2021). To verify the role of the ACC dopamine receptors D1 and D2 in chronic neuropathic pain, optogenetic stimulation of D1-Cre and D2-Cre mice was used for precise spatiotemporal control of D1 and D2 neurons in the ACC (Liu et al., 2020). D1 and D2 played different roles in pain regulation when ChR2 was activated by blue-light irradiation (Liu et al., 2020). Moreover, some researchers have used 470 nm blue light to induce circuit-specific neuromodulation of neuronal activity in the primary motor cortex layer V to relieve trigeminal neuralgia. This targeted, precise control is similar to the idea of an "optical scalpel," with the advantages of fewer side effects and better therapeutic effects (Islam et al., 2020).

Collectively, the existing research on trigeminal pathways has applied invasive and potentially unharmful techniques. Optogenetics has the advantage of avoiding craniotomy, and proves that there is a clinically congruent causal relationship between trigeminal pain and anxiety behavior (Harriott et al., 2021). By introducing genes encoding light-sensitive transmembrane channels, optogenetic strategies have enabled the precise spatiotemporal control of specific neural populations with different wavelengths of light. Thus, optogenetics could explain the pathogenesis of trigeminal neuralgia and anxiety caused by trigeminal neuralgia (Sheng et al., 2020) and help with the development of potential treatment methods.

3.2 Maxillofacial cellulitis

Maxillofacial cellulitis is an acute inflammation of the perimandibular fascial space tissue. It is one of the most serious infections in the maxillofacial region and can spread to the submandibular, submental, and sublingual spaces simultaneously. In serious cases, the disease can spread and even affect the patency of the respiratory tract or cause systemic poisoning (Dowdy et al., 2019; Sugai and Nishie, 2020). *Pseudomonas aeruginosa* is a common Gram-negative pathogenic bacterium that is the main causative agent of maxillofacial cellulitis (AlBassri et al., 2020). Bacterial infections have become increasingly difficult to treat due to the rapid emergence of antibiotic-resistant bacteria, and new strategies are needed to address the challenge (Prado-Prone et al., 2018). Considering the diffusion property of chemicals, small molecule inducers to regulate the pathogenicity of bacteria may have unintended effects (Morreale et al., 2022). By comparison, light has the advantages of being noninvasive, having low toxicity, and providing superior spatiotemporal resolution (Lindner and Diepold, 2022). Thus, the combination of optogenetic technology and *P. aeruginosa* virulence control has provided scientists with more possibilities for the treatment of maxillofacial cellulitis (Cheng et al., 2021; Xia et al., 2021) (Fig. 4b).

Recently, researchers found that cyclic adenosine monophosphate (cAMP) is an important secondary messenger that controls carbon metabolism, type IVa pili biogenesis, and virulence in *P. aeruginosa* (Berry et al., 2018). Based on this information, a bacterial photoactivated adenylate cyclase (*bPAC*) gene was introduced into bacteria. Upon illumination with 470 nm blue light, *bPAC* cyclizes adenosine triphosphate (ATP) to form cAMP, creating *P. aeruginosa* with light-dependent changes in intracellular cAMP levels. The cAMP concentration increased 6.6-fold after optogenetic activation of bPAC, but cAMP concentrations decreased to normal levels after blue-light irradiation was terminated. This reversible manipulation suggested that blue light could be used to manipulate the motility and toxicity of bacteria (Xia et al., 2021).

In addition to changing cAMP levels, controlling Gac/Rsm from *P. aeruginosa* improved treatment efficacy. GacS is a signal transduction protein in the Gac/Rsm cascade that plays an important role in regulating infection factors. After introducing the YGS24 light-dependent fusion protein into bacteria, optogenetic methods were applied to recombine the GacS of *P. aeruginosa* to control the Gac/Rsm regulatory signal cascade. By regulating Gac/Rsm, light-dependent proteins could fully control virulence after excitation with 470 nm blue light at 120 $\mu W/cm^2$, thus facilitating the development of innovative therapies (Cheng et al., 2021). Given the critical role of *P. aeruginosa* in maxillofacial cellulitis, optogenetic interventions targeting the virulence of *P. aeruginosa* may be a potential therapy for maxillofacial cellulitis.

Thus, optogenetic technology has shown some potential as a future treatment for maxillofacial cellulitis, mainly due to altering the motility and virulence of *P. aeruginosa*. This therapy has not been applied in disease models, and further studies evaluating its safety and feasibility are imperative before applying it to the treatment of maxillofacial cellulitis.

4 Perspectives and limitations

Studies on optogenetics in oral and craniofacial fields have been developed for a decade. Notably, with their characteristic spatiotemporal precision, optogenetics tools can directly apply stimuli and provide visual interpretations of multifaceted diseases and facial movements in oral and craniofacial research (Fig. 4c). Nevertheless, existing studies are limited to small animal experiments and there is a lack of research on large animals such as dogs or gorillas. To date, several drawbacks still exist that hinder the widespread preclinical research on and clinical application of optogenetics, including genetic transfection potential risks, difficulties in cell-specific targeted expression, and the shortcomings of optogenetics itself.

Optogenetics resembles gene therapy, in which it relies on the exogenous expression of genes encoding light-responsive proteins to specific cell populations. Lentiviral transfection and adeno-associated viral transfection are common methods of gene transfection; adeno-associated virus (AAV) in particular is generally used in oral and craniofacial studies. Potential immunogenic reactions are a significant disadvantage of viral gene transfection (Shirley et al., 2020). Furthermore, the variable efficacy of viral transduction, uncertain long-term stability, off-target viral effects, and possible toxicity due to gene product accumulation are non-negligible disadvantages (Wood et al., 2018; Botto et al., 2022). Thus, safety concerns associated with gene transfection have been under the spotlight, and further developed viral vectors with more favorable safety profiles are needed.

Besides the safety concerns of genetic engineering, the difficulties associated with the targeted expression of specific cell types have also hindered the clinical translation of optogenetic technologies. Combining advanced optical methods with genetic engineering for cell-specific targeting enables the manipulation and monitoring of the activity of specific cells, cell populations, or cell types (Mirzapour Delavar et al., 2016). Thus, the emergence of optogenetics allows for the specific, rapid, and direct control of the activity of specific cells, especially neurons, facilitating the study of neural circuits and behavioral correlates (Veres et al., 2023). The most prominent issue is that it is still difficult for optogenetic tools to accurately target specific cells at the right location, especially in neuroscience with highly different kinds of specific neurons (Habibey et al., 2020). Viral delivery or localized light stimuli allow activation or recording from spatially restricted subsets of cells but are not necessarily able to target specific classes of cells within a region (Zgierski-Johnston and Schneider-Warme, 2021). Another important issue is the penetration of light into tissue, and the exploration of more appropriate light expression for specific cell types also needs to be investigated. Light targets different cells with variable safety considerations, specifically activating cell populations with variable expression (Zgierski-Johnston and Schneider-Warme, 2021). Thus, the adequate and specific delivery of light sources to regions of interest also requires safer, more effective and predictable approaches. Replacing light-emitting diode (LED) with a micro-LED or laser is a novel strategy to improve the spatial resolution of a one-photon stimulation system. By using spatial light modulators or holographic projections, two-photon stimulation can be realized, providing improved spatial resolution (Habibey et al., 2020).

In addition, as a new technology, optogenetics itself has some shortcomings. The difficulty of light

penetration in deep tissues greatly hinders the application of clinical optogenetics. The development of excitation light to penetrate deep tissues can be considered a radical solution to the problem. In recent years, newly developed, red-shifted actuators (Oda et al., 2018) and genetically encoded infrared reporters (Monakhov et al., 2020) allow for the use of longer wavelengths of near-infrared (NIR) for excitation, opening up possibilities for new experimental observations and steering. Currently, NIR light has limited application in the oral and craniofacial fields, and with its advantages of low tissue absorbance, low autofluorescence, and reduced light scattering, NIR-responsive proteins may have wider applications in the future (Ntziachristos, 2010). Another noteworthy shortcoming is the immune response that opsins may cause. Future work should attempt to design opsins to evade immune recognition to mitigate the immune response (Maimon et al., 2018). Specifically, most immunogenic peptide fragments were identified and then altered by site-directed mutagenesis or alternative opsin sequence alignment to these regions (Maimon et al., 2018). Focusing on the host and optimizing immunosuppressive drugs, dosage, and time course may be another potentially viable strategy.

In preclinical trials in other fields, optogenetics has been applied to vision restoration (Sahel et al., 2021), cerebellar regulation (Streng and Krook-Magnuson, 2021), and the subcutaneous delivery of therapeutic proteins for a mouse model of experimental autoimmune encephalomyelitis in multiple sclerosis (Audouard et al., 2022). However, it has not been applied to the treatment of diseases in oral and craniofacial research. In the future, it may have potential for the regeneration of craniomaxillofacial and periodontal tissues and for the treatment of dry sockets, taste loss, xerostomia, and burning mouth syndrome (Fig. 5).

5 Conclusions

In this review, we found that optogenetics has been applied in basic science, preclinical studies, and translational studies. Optogenetics uses its unique advantages of being noninvasive and having high temporal-spatial resolution and low toxicity to explain oral and craniofacial movements and diseases. More translational research on optogenetics techniques in large animal experiments and clinical applications is needed in the future.

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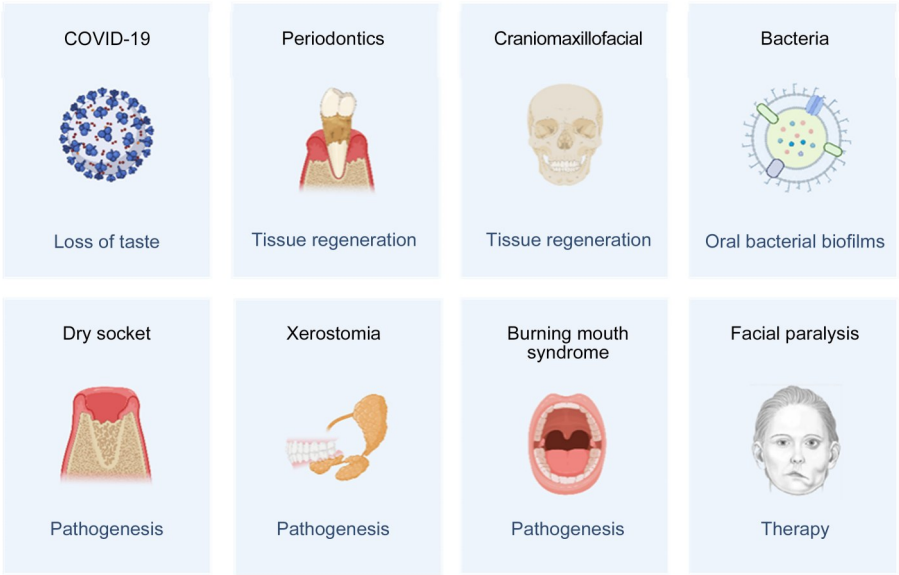

Fig. 5 Possible future research directions in optogenetics mainly including the pathogenesis of taste, oral bacterial biofilm, craniomaxillofacial and periodontal tissue regeneration, dry socket, xerostomia, burning mouth syndrome, and facial paralysis. COVID-19: coronavirus disease 2019.

Author contributions

Guoli YANG and Zhiwei JIANG designed the manuscript. Qinmeng ZHANG wrote the manuscript. Luyao SONG completed the drawing of all the figures. Mengdie FU and Jin HE provided the proofreading of the article. All authors have read and approved the final version.

Compliance with ethics guidelines

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This review does not contain any studies with human or animal subjects performed by any of the authors.

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