



## Curcumin effects on age-related changes in oral immunity: an *in vivo* study

Ippei Uemura<sup>†</sup>, Natsuko Takahashi-Suzuki<sup>†</sup>, Akari Sano, Shogo Yamada, Akifumi Nakata and Takashi Satoh\*

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 7-Jo 15-4-1 Maeda, Teine-ku, Sapporo, Hokkaido 006-8585, Japan

(Submitted 4 December 2023 – Final revision received 10 March 2024 – Accepted 25 March 2024 – First published online 18 April 2024)

### Abstract

The current study aimed to investigate the effects of ageing on oral immunity using  $\beta$ -defensin (DEFB) 1/2 as a marker and evaluate the effects of curcumin (CUR) on these processes. The study sample included thirty male C57BL/6J mice divided into three groups based on the treatment method used. The young control (YC) and old control (OC) groups received 0.5% methylcellulose-400 (CUR vehicle) orally for 5 days, whereas the CUR group of older mice received a CUR solution suspended in 0.5% methylcellulose-400 (dose: 3.0 mg/kg body). DEFB1/2 and immune indicator levels were measured in the saliva and salivary glands post-treatment. The saliva volume and protein content were significantly reduced in the OC group compared with the YC group. CUR administration restored these parameters, decreased DEFB1 expression in the salivary gland and increased DEFB1/2 secretion and DEFB2 expression. These findings were supported by epigenetic gene regulation and partial cytokine activation from changes in WD40 repeat protein 5, TNF alpha and IL-1beta. CUR can partially restore age-related changes in oral immune responses and promote oral health, thereby preventing frailty in the older population through a nutritional therapeutic pathway.

**Keywords:**  $\beta$ -defensin: Saliva: Salivary glands: Polyphenol: Oral frailty

Frailty has recently been found to directly impact the healthy lifespan of an older individual and acts as a precursor to the need for care<sup>(1,2)</sup>. Frailty can lead to a decline in various physical and physiological declines that can easily lead to health disadvantages, such as weight loss, fatigue, reduced walking speed, decreased grip strength and reduced physical activity<sup>(1,2)</sup>. Similarly, oral frailty is a multifaceted condition with widespread implications for overall health and well-being in the elderly because it implies deterioration of oral tissues, immune response and microbial balance, leading to an increased susceptibility to oral diseases and conditions<sup>(1–4)</sup>. Addressing oral frailty through preventive measures, early detection and appropriate interventions is essential to promote healthy ageing and improve the quality of life of the elderly<sup>(1–4)</sup>. Proactive interventions for oral frailty, characterised by a decline in oral function (e.g. poor oral hygiene, dry mouth, reduced tongue-lip mobility, decreased bite force, low tongue pressure, impaired chewing function and weakened swallowing function), should aim to prevent transition to specialised treatment<sup>(3,4)</sup>. In particular, poor oral hygiene and dry mouth are not only associated with poor oral mucosal immunity but have also been reported to be related to

nutritional status<sup>(5,6)</sup>, and this could likely be attributed to dysregulation of inflammatory and antimicrobial responses associated with down-regulation of regulatory receptors on the mucosal surfaces. Furthermore, evidence also suggests that age-related fluctuations in biological low-molecular-weight compounds can lead to the development of periodontitis and other oral diseases through various underlying molecular mechanisms<sup>(7,8)</sup>. Oral frailty can be addressed through preventive measures, early detection and appropriate interventions as they mainly focus on oral immunological defence mechanisms and their role in maintaining oral health and its resilience<sup>(9,10)</sup>. Therefore, oral immunity, traditionally evaluated using established markers such as IgA and, more recently, oral peptides such as  $\beta$ -defensins, plays an important role in disease status<sup>(9,10)</sup>. Although numerous studies have demonstrated the effects of drugs, certain food items and fluctuations in antigen (e.g. bacteria and viruses) levels on mucosal immunity, very few have examined the effects of ageing to date<sup>(10,11)</sup>.

Oral immunity factors such as  $\beta$ -defensins (DEFB) are typically produced by the gingival tissues and play an important role in innate immunity<sup>(12,13)</sup>. They include subtype 1 (DEFB1),

**Abbreviations:** CFTR, cystic fibrosis transmembrane conductance regulator; CUR, curcumin; DEFB,  $\beta$ -defensin; Myd88, myeloid differentiation factor 88; OC, old control; Pr, parotid glands; Sm, submandibular.

\* **Corresponding author:** Takashi Satoh, email [tsatoh@hus.ac.jp](mailto:tsatoh@hus.ac.jp)

<sup>†</sup> These authors contributed equally to this work

which are constitutively expressed, and subtype 2 (DEFB2) which are induced under certain conditions such as infections or inflammation only<sup>(12–14)</sup>. However, no studies to date have examined variations in DEFB expression and secretion levels in the oral cavity in response to ageing. Defensins are typically induced through pathways that are similar to inflammatory cascades (including toll-like receptors, myeloid differentiation factor 88 and tumour necrosis factor receptor-associated factor 6), with mechanisms regulating transcription levels through DNA and histone modifications also playing a role. However, there is limited evidence on the effects of ageing on these processes<sup>(14–16)</sup>.

Curcumin (CUR) is a polyphenolic compound that has been shown to have anti-inflammatory effects in mice with inflammatory bowel disease, and also immune-regulatory effects in response to drug-induced inflammation in humans<sup>(17–22)</sup>. It increases the expression of antimicrobial peptides stimulated by blood cell components, thus activating innate immunity<sup>(21)</sup>; induces epigenetic gene regulation by inhibiting DNA methyltransferases<sup>(22)</sup>; and exhibits antiaging effects by acting as an antioxidant or Sirtuin modulator<sup>(9,21,23)</sup>. However, despite stimulating various immune responses, polyphenolic compounds such as CUR exhibit poor water solubility and very low absorption rates in the gastrointestinal tract<sup>(24)</sup>. Therefore, their efficacy as nutritional supplements is often limited by their low biological availability, as demonstrated by numerous *in vitro* and *in vivo* studies<sup>(19,25,26)</sup>. However, oral administration of CUR has been shown to affect the gastrointestinal mucosal surfaces through toll-like receptors and similar pathways, regardless of the absorption levels<sup>(27)</sup>. Therefore, we hypothesised that CUR absorption would result in immunomodulatory effects in the gastrointestinal tract.

Although a direct effect of CUR on oral ulcers has not yet been demonstrated<sup>(28)</sup>, an improvement in the oral environment through the regulation of oral flora and promotion of the body's defence mechanisms is expected upon its administration. Therefore, the current study examined changes in DEFB and related cascades in response to ageing and evaluated the effects of CUR on the oral immunity of older mice, with the aim of establishing convenient salivary markers that can inform pre-disease strategies targeted at extending healthy lifespan through dietary habits.

## Experimental methods

### Chemicals

CUR was purchased from Wako Pure Chemical Industries, Ltd. All other reagents were used at the highest grade available and without further purification.

### Animals

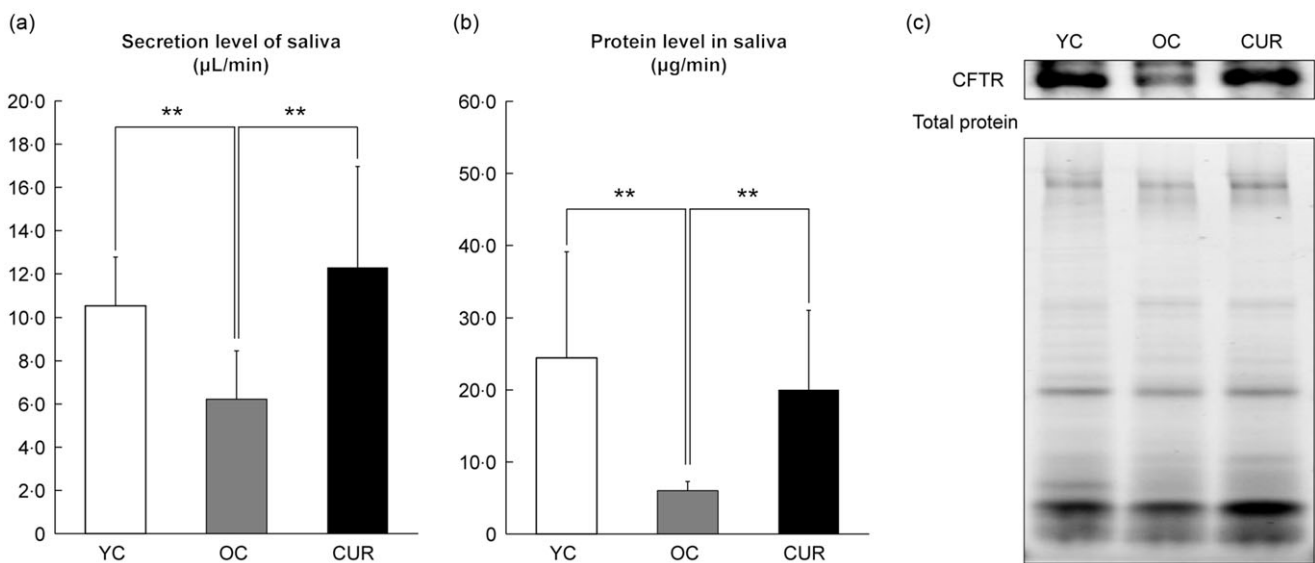
The study sample included thirty male mice (C57BL/6J; weight: 19–40 g; age: 7 or 70 weeks) that were obtained from Jackson Laboratory, Hokkaido, Japan. For the young age group, time of sexual maturity was selected as the criterion. This age range represents a stage in the mice's life cycle where they have

reached sexual maturity but are still considered young adults<sup>(29)</sup>. In contrast, the criterion for the old age group was based on a specific age in weeks. This age corresponds to a point in the mice's lifespan where changes associated with ageing can be reliably detected across various biomarkers in almost all animals<sup>(29)</sup>. For experiments involving older animals, the sample size was set according to older mice, referring to The Mouse Phenome Database (<https://phenome.jax.org/>), to consider the environment, feed and health conditions at the time of rearing. The mice were housed in plastic cages under standard laboratory conditions, including a constant temperature of 23°C ± 2°C and a 12:12-h light–dark cycle, and *ad libitum* access to water and food (standard rodent chow diet) was provided. The cage measured 235 × 353 × 160 mm (width × depth × height), the flooring was Shepherd's® Specialty Blend™ (Shepherd Specialty Papers, Inc.) and there were five animals/cage. The experimental animal protocol was approved by the Committee of the Laboratory Animal Center (No. 2022-011), and all procedures were carried out in accordance with the Guiding Principles for the Care and Use of Experimental Animals at Hokkaido University of Science. CUR (dose: 3.0 mg/kg body) was dissolved in 0.5% methylcellulose-400 and 1 ml/kg was administered orally using a micropipette. To ensure uniformity in body weight, the animals were randomly divided into the young control (YC; age: 7 weeks; *n* 10); old control (OC; age: 70 weeks; *n* 9) and CUR (CUR; age: 70 weeks; *n* 10) groups (online Supplementary Fig. S1). An acclimation period of 1 week was considered in all animal experiments. Herein, 7-week-old mice were acclimatised for 1 week and 70-week-old mice for 70 weeks after purchase at 60 weeks. One 70-week-old mouse died during the acclimatisation period; therefore, the sample size at the start of treatment was *n* 9 in OC. The dose of CUR was set in a way that it did not exceed the acceptable daily intake of CUR established by the WHO<sup>(30)</sup>. The animals were anaesthetised after administration of food reagents for 5 d. A mixture of pharmaceutical-grade medetomidine hydrochloride (Domitor®; NIPPON Zenyaku Kogyo Co., Ltd.); midazolam hydrochloride (Dormicum®; Astellas Pharma Inc.) and butorphanol tartrate (Vetorphale®; Meiji Seika Pharma Co., Ltd.) was created using concentrations of 0.75 mg/kg, 4.0 mg/kg and 5.0 mg/kg, respectively. These drugs were diluted in sterile saline and administered using an intraperitoneal injection. After euthanasia, the submandibular and parotid glands were sampled, stored at –80°C and appropriately processed prior to analysis.

### Saliva collection and measurement

Following intraperitoneal administration of 1 mg/kg of pilocarpine-HCl (Sampiro 1%; Santen Pharmaceutical Co., Ltd.) after anaesthesia<sup>(31)</sup>, the saliva samples were collected strictly from the oral cavity using a micropipette continuously for 10 min. Thereafter, the samples were immediately placed on ice, centrifuged at 10 000 × *g* for 2 min at 4°C and subsequently stored at –80°C until analyses were performed. The DEFB1/2 levels were then detected using Enzyme-Linked Immuno Sorbent Assay (ELISA), whereas bicinchoninic acid assay was used to determine the protein concentration.





**Fig. 1.** The effects of CUR on age-related changes in salivary volume and protein content. The bar graphs show the mean values and standard deviations observed after > 2 independent experiments ( $n=7-10$ ). \* and \*\* indicate statistical significance at a level of  $P < 0.05$  and  $P < 0.01$ , respectively. The results are presented as follows: (a) Saliva secretion level ( $\mu\text{L}/\text{min}$ ), (b) protein levels in saliva ( $\mu\text{g}/\text{min}$ ), (c) expression of CFTR protein by Western blotting. CFTR, cystic fibrosis transmembrane conductance regulator; CUR, curcumin; DEFB, beta-defensin; OC, old control; YC, young control.

### ELISA

DEFB secretion was determined using  $\beta$ -defensin 1 and  $\beta$ -defensin 2 ELISA kits (MyBioSource) as per the manufacturer's instructions. No changes were made to the procedure; however, the saliva samples were diluted 50-fold in phosphate-buffered saline and assayed thereafter.

### Western blotting

The control and treated tissue lysates ( $10 \mu\text{g}$  protein/lane) were separated using 4%–20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad Laboratories Inc., Hercules). The separated proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories Inc.) and analysed using Western Blotting with anti-DEFB1, anti-DEFB2 and anti-cystic fibrosis transmembrane conductance regulator (CFTR) (1:500) antibodies (Santa Cruz Biotechnology). The membrane was blocked for 1 h using 5% EzBlock Chemi (ATTO) and then incubated for 15–18 h using the appropriate primary antibodies at  $4^\circ\text{C}$ . The blots were washed with Tween-20 (Bio-Rad Laboratories Inc.) containing PBS and incubated with appropriate secondary antibodies for 1 h at  $22^\circ\text{C}$ – $25^\circ\text{C}$ . The protein bands were visualised using enhanced chemiluminescence Western Blot detection reagents (Bio-Rad Laboratories Inc.)<sup>(32)</sup>.

### Real-time quantitative PCR

Total RNA was extracted from the tissues using the ISOGEN II reagent (NIPPON Gene Co. Ltd.). Thereafter, single-stranded cDNA was prepared from  $1 \mu\text{g}$  total RNA using ReverTra Ace (Toyobo), and reverse transcription-quantitative polymerase chain reactions were performed using the QuantStudio<sup>TM</sup>5 real-time PCR system (Applied Biosystems) with KAPA SYBR<sup>TM</sup> FAST (NIPPON Genetics Co. Ltd.) as per the manufacturer's protocol.

Online Supplementary Table 1 shows the sequences of the specific primers used.

### Data analysis

All statistical analyses were performed using the statistical software package Excel (Bell Curve for Excel v.3.20). Data were presented as mean  $\pm$  standard deviation and were analysed for statistical significance using ANOVA followed by Tukey's test to compare means of two or more groups and Welch's  $t$  test for between two groups.  $P$  values  $< 0.05$  or  $< 0.01$  were considered statistically significant.

## Results

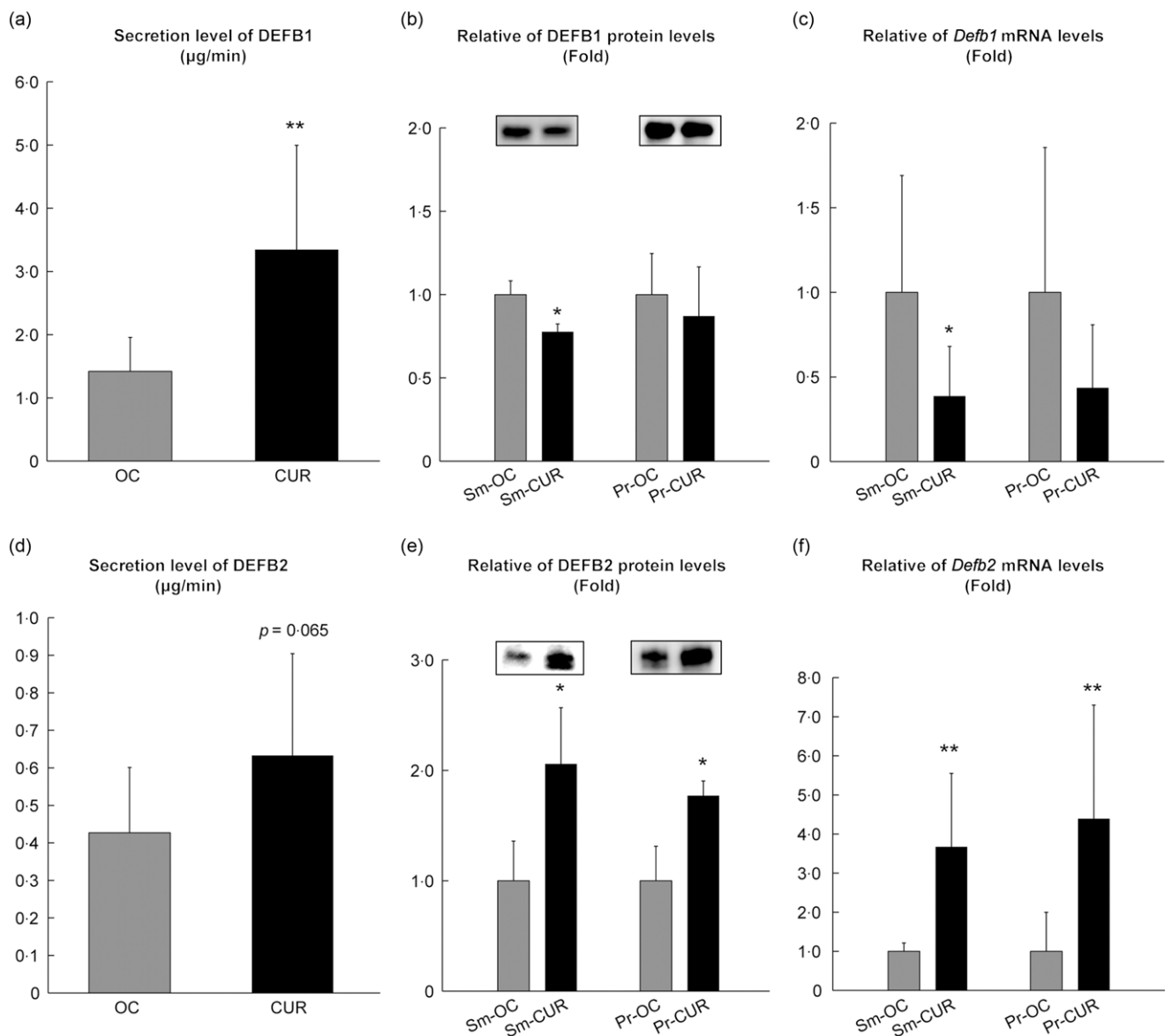
### The effects of curcumin on age-related changes in saliva volume and protein content

The OC group exhibited a significant decrease in salivary volume per minute and salivary protein content compared with the YC group ( $P < 0.01$ ; Fig. 1(a) and (b)). However, administration of CUR in the OC group significantly increased these parameters, suggesting that it exerted a restorative effect on the salivary secretory capacity ( $P < 0.01$ ; Fig. 1(a) and (b)).

The CFTR, responsible for the regulation of pH and salt concentration in the oral mucosa, exhibited significantly decreased expression in the submandibular glands of the OC group compared with the YC group. However, restoration of this decreased expression was observed in the CUR group (Fig. 1(c)).

### Effects of curcumin on DEFB1/2 secretion and expression in old mice

Examination of the effects of CUR on DEFB1/2 in the OC group showed a significant increase in DEFB1 secretion levels in the



**Fig. 2.** Effects of CUR on DEFB1/2 secretion and expression in old mice. The bar graphs show the mean values and standard deviations observed after > 2 independent experiments ( $n = 7-10$ ). \* and \*\* indicate statistical significance at a level of  $P < 0.05$  and  $P < 0.01$ , respectively. The results are presented as follows: (a) DEFB1 secretion level ( $\mu\text{g}/\text{min}$ ), (b) relative to DEFB1 protein levels (Fold), (c) Relative to *Defb1* mRNA levels (Fold), (d) DEFB2 secretion level ( $\mu\text{g}/\text{min}$ ), (e) relative to DEFB2 protein levels (Fold), (f) relative to *Defb2* mRNA levels (Fold). CUR, curcumin; DEFB, beta-defensin; OC, old control; Pr, parotid glands; Sm, submandibular.

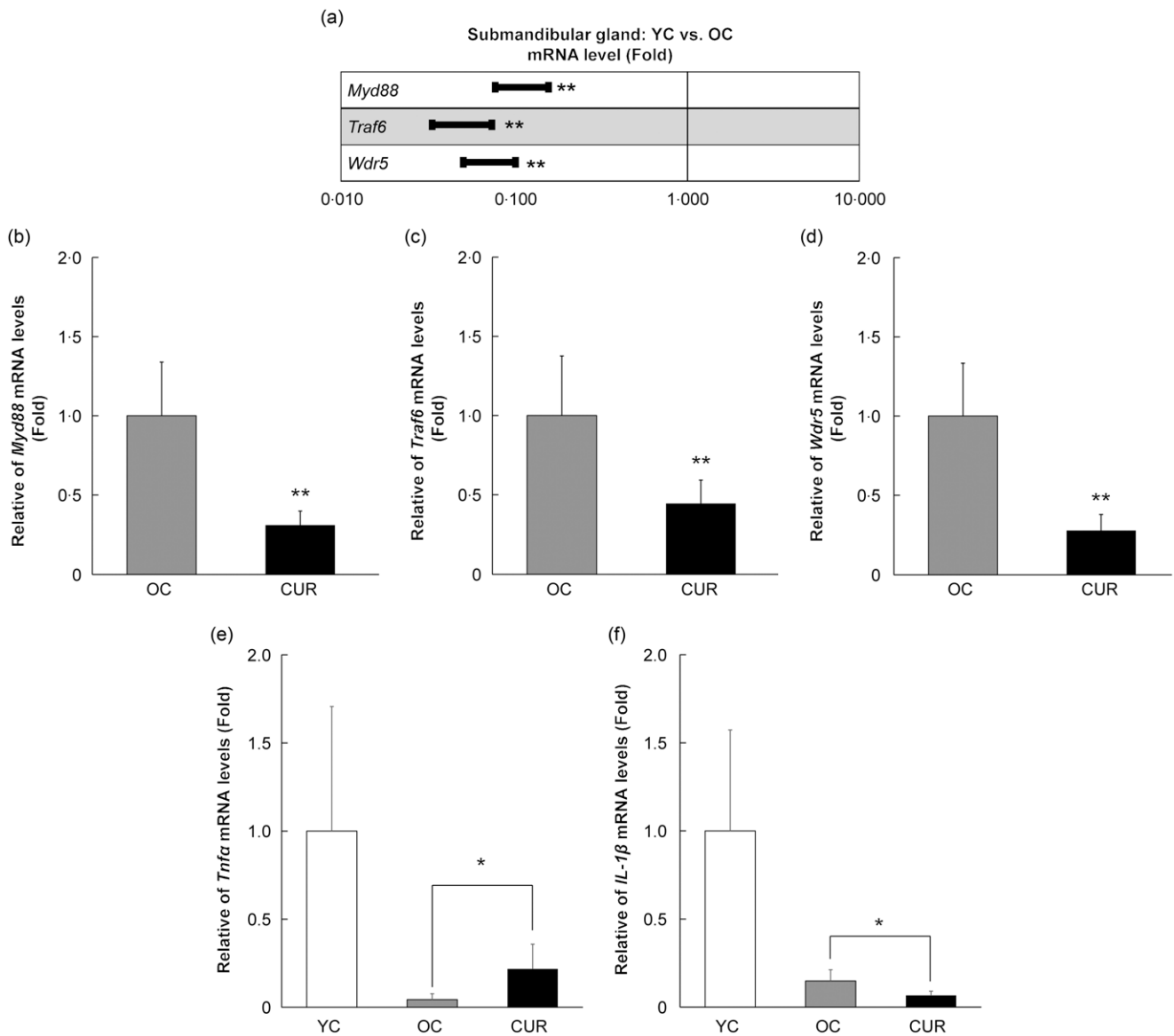
saliva ( $P$  value  $< 0.05$ ; Fig. 2(a)) and a significant decrease in the protein and gene expression levels of DEFB1 in submandibular gland tissues ( $P < 0.05$ ; Fig. 2(b) and (c)). Furthermore, CUR also increased DEFB2 secretion in the saliva ( $P = 0.065$ ; Fig. 2(d)) and protein ( $P < 0.05$ ; Fig. 2(e)) and gene ( $P < 0.01$ ; Fig. 2(f)) expression levels in the submandibular and parotid glands. These findings confirm that CUR induces transcription and translation of DEFB2 in the salivary glands.

#### The effects of curcumin on age-related changes in immunomodulatory capacity

Changes in intracellular signalling, epigenetically related genes, and cytokines were examined to enable elucidation of the

mechanisms underlying the differential effects on constitutively and indelibly expressed DEFB1 and DEFB2. Intracellular signalling variations were assessed by measuring the myeloid differentiation factor 88 (*Myd88*) and tumour necrosis receptor-associated factor 6 (*Traf6*), while epigenetic gene regulation was confirmed by measuring WD40 repeat protein 5 (*Wdr5*). The results showed a significant reduction in gene levels ( $P < 0.01$ ; Fig. 3(a)), indicating age-related effects. Furthermore, CUR significantly decreased *Myd88*, *Traf6* and *Wdr5* levels in the submandibular glands of the OC group ( $P < 0.01$ ; Fig. 3(b)–(d)).

Furthermore, examination of variations in cytokines including tumour necrosis factor- $\alpha$  (*Tnf $\alpha$* ) and interleukin 1 $\beta$  (*IL-1 $\beta$* ), which exhibit similar cascades or DEFB2-inducing abilities in the submandibular gland, showed a significant decrease in



**Fig. 3.** The effects of CUR on age-related changes in immunomodulatory capacity. The bar graphs show the mean values and standard deviations observed after > 2 independent experiments ( $n = 7-10$ ). \* and \*\* indicate statistical significance at a level of  $P < 0.05$  and  $P < 0.01$ , respectively. The results are presented as follows: (a) mRNA variation in the old control group relative to the young control group (Fold), (b) relative to *Myd88* mRNA levels (Fold), (c) relative to *Traf6* mRNA levels (Fold), (d) relative to *Wdr5* mRNA levels (Fold), (e) relative to *Tnfa* mRNA levels (Fold), (f) relative to *IL-1β* mRNA levels (Fold). CUR, curcumin; IL-1β, IL-1beta; Myd88, myeloid differentiation factor 88; OC, old control; *Tnfa*, TNF alpha; *Traf6*, tumour necrosis factor receptor-associated factor 6; *Wdr5*, WD40 repeat protein 5; YC, young control.

expression levels as a result of ageing. CUR administration further decreased *IL-1β* gene expression ( $P < 0.05$ ; Fig. 3(e) and (f)) and restored *Tnfa* gene expression. However, the final *Tnfa* levels observed did not exceed that of the control groups, indicating an immune stimulus that was not inflammatory in nature.

## Discussion

The current study identified age-related changes in oral immunity and examined the effects of CUR administration and its underlying mechanisms. In oral immunity, we focused on

DEFB1/2, which is related to innate immunity, from tissue expression to secretion, as an indicator. Reports stating the effects of CUR on immune responses were used to determine the CUR dosage<sup>(33-35)</sup>. In mice, 100 mg/kg body weight of turmeric extract can suppress ovalbumin-induced anaphylaxis, and even 3 mg/kg body weight of CUR, converted from the fact that CUR contains approximately 3% in the turmeric extract, affected immunity<sup>(33)</sup>. This suggests that the biological activity is sufficient even at acceptable daily intake doses. In particular, the effect of CUR on Th2 cells was discussed in the corresponding report; however, the effect on innate immunity, such as DEFB1/2, was not fully investigated<sup>(33)</sup>; therefore, this study was conducted with CUR dose of 3 mg/kg body weight administered orally.



In this study, CUR restored the reductions in saliva volume/min and salivary protein content observed in the OC group. Decreased saliva volume may lead to decreased oral function, exacerbating frailty, further exacerbating oral health problems, and increasing susceptibility to dental caries, gingivitis, oral mucosal lesions and taste changes<sup>(3,4)</sup>. This may result in weight loss and malnourishment due to decreased food intake<sup>(4)</sup>. Saliva volume is reduced in patients with hyponutrition, suggesting that saliva volume may play a role in the vicious cycle<sup>(5,6,36)</sup>. Therefore, from an oral hygiene perspective, supporting the frailty of the elderly by adequate saliva secretion may not only enhance the mechanical function of digestion and absorption but also help mitigate potential adverse effects<sup>(4-6,36)</sup>. Saliva secretion can be divided into two categories: that controlled by the autonomic nervous system and that secreted during processes, such as mastication<sup>(37)</sup>. The former is present in a certain amount in the oral cavity and is involved in the control of sensory stimuli such as taste, and the saliva measured in this study falls into this category<sup>(37)</sup>. Therefore, amount of saliva can determine age-related abnormalities, leading to a decrease in food intake and progression of frailty, mainly weight loss, due to the loss of enjoyment of taste. However, the restoration of autonomic nervous system-controlled saliva by pilocarpine stimulation by CUR in this study indicates that it can improve taste abnormalities, suggesting the possibility of nutritional intervention for frailty. Moreover, proteins in saliva, which control taste perception, are altered by tasty foods<sup>(38)</sup>, and this study confirms that the amount of immune substances present was also altered by CUR, making food-based oral hygiene interventions for oral frailty a realistic possibility. Because this study was conducted in animals, results commonly observed in humans (such as oral lesions and weight loss) were not obtained. This is a limitation while assessing the association between oral frailty and frailty in terms of food intake, dietary goals, and number of taste receptors, which may be hampered by differences in biological species differences between (laboratory animals and humans)<sup>(3,4,36)</sup>. Therefore, as discussed in detail below, further nutritional interventions may be warranted to investigate the effects of ageing and CUR on the regulators and biomarkers of salivary components. Previous studies suggested that immune senescence associated with ageing predominantly affected adaptive immunity rather than innate immunity<sup>(39)</sup>. Furthermore, examination of the gene expression ratios in various organs through analysis of data from the Human Protein Atlas Project (v.21.1; <https://www.proteinatlas.org/>) showed higher expression levels in the salivary glands, suggesting that DEFB played a significant role in oral immunity (online Supplementary Fig. S2)<sup>(40)</sup>. Therefore, the investigation of age-related alterations in innate immunity, which serves as the initial defence mechanism against antigens, is imperative, to demonstrate the utility of DEFB as a short-term oral immune indicator. The current study examined changes in oral immune function due to ageing by focusing on the quality and quantity of saliva produced.

Notable changes in salivary salt concentration and pH due to ageing have been reported previously<sup>(41)</sup>, with studies examining the pathophysiology of cystic fibrosis demonstrating that increased salt concentration on the mucosal surface and

decreased pH led to a reduction in antimicrobial peptide activity<sup>(42)</sup>. Furthermore, age-related changes in salivary glands, including a decrease in acinar cells, fibrosis and lymphocyte infiltration, have been shown to decrease salivary secretion<sup>(43)</sup>. In the current study, CUR treatment was seen to restore the age-related decrease in CFTR protein expression in the submandibular glands, and this was accompanied by recovery of the salivary flow rate and protein concentration. Although both the submandibular and sublingual glands were examined in the current study, CFTR protein expression recovery was not observed in the former, and this discrepancy could likely be attributed to the fact that the sublingual glands became the predominant source of proteins during saliva collection using pilocarpine<sup>(44)</sup>. Considering the pivotal role of CFTR protein expression in salivary composition, particularly with regard to maintenance of the antimicrobial activity of DEFB proteins, it can be inferred that qualitative changes in salivary components contributed to this process.

Although CUR restored the DEFB1 secretion levels to a certain extent, the overall levels were still relatively low in the salivary gland tissue, potentially due to morphological changes associated with ageing that prevented complete recovery<sup>(45)</sup>. Therefore, evaluation of age-related changes in natural immunity requires examination of variations in DEFB2, which is known to be indelibly expressed and is reflective of immune reactivity. The sublingual gland is highly responsive to immune stimulation, suggesting that the polyphenols may act as ligands and activate the immune system<sup>(11,46)</sup>. Additionally, polyphenols exhibit poor translocation into the cells when existing as aglycones<sup>(24)</sup>. In the current study, the CUR was suspended as an aglycone before administration, suggesting that the observed effects originated from extracellular immune stimulation.

Polyphenols have been shown to modulate immune responses via host defence peptides in various organs and tissues<sup>(11)</sup>, and the mechanisms involved include activation of the MAPK pathway, induction of sphingosine-1-phosphate, inhibition of KEAP1 leading to induction of NRF2, epigenetic gene regulation and metabolic regulation of gut microbiota<sup>(11)</sup>. The current study found that CUR activated the main immunity and inflammatory factors such as MyD88 without inducing inflammation, indicating partial immune stimulation. In addition, they decreased IL-1 $\beta$ , which induced multiple cytokine networks and selectively increased TNF $\alpha$ , which was secondarily induced. These findings were consistent with previous studies that reported DEFB2 induction by TNF $\alpha$ <sup>(47)</sup>, demonstrating restoration of the expression of both genes within the salivary gland. Furthermore, while CUR showed different results compared with its anti-inflammatory effects assessed by TNF $\alpha$  as an indicator in mice, interestingly, the inflammatory state in older individuals, who are at risk for chronic inflammation, appears to operate through a mechanism distinct from that of pathological or drug-induced inflammation<sup>(17,18,48)</sup>. CUR has also been reported to act on G-protein-coupled receptors, suggesting a cascade that is distinct from immune stimulation by immunological ligands<sup>(49,50)</sup>. Evaluation of histone methylation showed inhibition of *Wdr5* gene expression and, consequently, acetylation which controlled the observed transcriptional levels<sup>(51)</sup>.



Although previous studies have used DNA methyltransferase and histone deacetylase enzymes as indicators of epigenetic gene control with polyphenols<sup>(21)</sup>, the effects of Wdr5 were previously unknown. The variations in the effects of CUR could likely be attributed to DNA and histone modifications that regulate the selective recovery of cytokines and result in differential effects of DEFB1 and DEFB2 on the same chromosome. However, the current study was limited by its inability to directly observe methylation/acetylation of histones or methylation at the DNA level. Future studies should aim to examine methylation status in the promoter regions of genes that exhibited altered expression in the current study (e.g. DEFB1 and DEFB2) or examine the symmetrically varied results of IL-1 $\beta$  and TNF $\alpha$  to gain a deeper understanding of epigenetic gene control in the older.

The findings of this study can inform clinical practice by potentially serving as a marker for immune-related diseases. For example, oral flares and fluctuations in the oral environment due to infections require monitoring, similar to autoimmune diseases. The use of saliva samples gained popularity during the novel corona virus infectious disease, emerged in 2019 (COVID-19) pandemic, and this was accompanied by rapid advancements in measurement techniques for viral antigens. However, there are a limited number of markers available for the elucidation of mechanisms underlying the development of autoimmune oral disorders associated with COVID-19<sup>(52)</sup>, and future studies should aim to identify inflammatory markers and examine the possible outcomes of epigenetic gene regulation to facilitate establishment of diagnostic reference values for salivary markers in humans. At the very least, tracking oral health status using salivary molecular markers such as DEFB2 has the potential to facilitate early detection of oral frailty in the general population, even in the absence of medical expertise. This, in turn, could promote self-care practices, including the incorporation of specific food ingredients, to sustain overall health<sup>(9,53,54)</sup>.

In conclusion, the findings of this study showed that salivary secretion declined with age, and the changes in the quality and quantity of saliva may be directly linked to infection control. Moreover, CUR administration in older mice showed suppression of inflammatory signals, epigenetic regulation through suppression of histone methylation and partial induction of inflammatory cytokines in the salivary gland tissues. Increased secretion and expression of DEFB2 helped strengthen the protective response to foreign antigens in older individuals who are susceptible to infection, suggesting that it may be used as a biomarker to monitor the oral environment. These findings confirm the hypothesis that CUR exerts significant effects on the gastrointestinal tract, regardless of absorption. To link the significance of DEFB variation to health effects, attention must be paid to variation in oral bacteria; however, this was not possible in this study. Alternatively, while it has been reported that orthodontic acrylic additives containing CUR have antimicrobial activity against *S. mutans* and *C. albicans* biofilms<sup>(55)</sup>, it is also consistent with the antimicrobial spectrum of DEFB<sup>(56)</sup>, which indicates that this action is mediated by DEFB. Therefore, it is expected that this is DEFB-mediated action. The significance can also be considered from the potential host effects of antimicrobial peptides. We have

previously demonstrated the direct host effects of an antimicrobial peptide,  $\alpha$ -defensin5, on CFTR in the intestinal tract and the utility of polyphenols in cancer therapy<sup>(57–59)</sup>. Further studies to better elucidate the potential host effects of DEFB are required in future. Medical interventions are typically associated with higher risks in older individuals who have lower tolerance to treatment measures. Therefore, the findings of the current study, which showed that innate immunity can be regulated through food components, can prevent progression of disease and provide insight into appropriate dietary habits during the treatment period. CUR is expected to help prevent frailty in the older population by improving the oral environment and partially restoring the age-related decrease in innate immune responses.

### Acknowledgements

We thank Enago ([www.enago.jp](http://www.enago.jp)) for editing the language of the manuscript.

This work was supported by the URAKAMI FOUNDATION (awarded to Natsuko Suzuki) (grant numbers R04208).

I. U.: Conceptualisation, formal analysis, investigation, writing – original draft, writing – review and editing, and visualisation; N. T-S.: conceptualisation, investigation, supervision, writing original draft, writing – review and editing, funding acquisition; A. S.: investigation; S. Y.: investigation; A. N.: investigation, writing – review and editing; T. S.: supervision, writing – review and editing.

The authors declare no conflicts of interest.

### Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114524000801>

### References

1. Fukutomi E, Kimura Y, Wada T, *et al.* (2013) Long-term care prevention project in Japan. *Lancet* **381**, 116.
2. Chen LK, Liu LK, Woo J, *et al.* (2014) Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc* **15**, 95–101.
3. Watanabe D, Kurotani K, Yoshida T, *et al.* (2022) Adherence to the food-based Japanese dietary guidelines and prevalence of poor oral health-related quality of life among older Japanese adults in the Kyoto-Kameoka study. *Br J Nutr* **128**, 467–476.
4. Iwasaki M, Motokawa K, Watanabe Y, *et al.* (2020) Association between oral frailty and nutritional status among community-dwelling older adults: the Takashimadaira study. *J Nutr Health Aging* **24**, 1003–1010.
5. Lin D, Yang L, Wen L, *et al.* (2021) Crosstalk between the oral microbiota, mucosal immunity, and the epithelial barrier regulates oral mucosal disease pathogenesis. *Mucosal Immunol* **14**, 1247–1258.
6. Kotronia E, Brown H, Papacosta AO *et al.* (2021) Poor oral health and the association with diet quality and intake in older people in two studies in the UK and USA. *Br J Nutr* **126**, 118–130.
7. Ebersole JL, Kirakodu S, Novak MJ, *et al.* (2016) Effects of aging in the expression of NOD-like receptors and inflammasome-related genes in oral mucosa. *Mol Oral Microbiol* **31**, 18–32.

8. Ebersole JL & Gonzalez OA (2022) Mucosal circadian rhythm pathway genes altered by aging and periodontitis. *PLOS ONE* **17**, e0275199.
9. Shinkai S, Toba M, Saito T, *et al.* (2016) Immunoprotective effects of oral intake of heat-killed *Lactobacillus pentosus* strain b240 in elderly adults: a randomised, double-blind, placebo-controlled trial - corrigendum. *Br J Nutr* **116**, 1138–1140.
10. Krisanaprakornkit S, Kimball JR, Weinberg A, *et al.* (2000) Inducible expression of human beta-defensin 2 by *Fusobacterium nucleatum* in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in innate immunity and the epithelial barrier. *Infect Immun* **68**, 2907–2915.
11. Tobin I & Zhang G (2023) Regulation of host defense peptide synthesis by polyphenols. *Antibiot (Basel)* **12**, 660.
12. Polesello V, Zupin L, Di Lenarda R, *et al.* (2017) DEFB1 polymorphisms and salivary hBD-1 concentration in oral Lichen Planus patients and healthy subjects. *Arch Oral Biol* **73**, 161–165.
13. Hancock RE, Haney EF & Gill EE (2016) The immunology of host defence peptides: beyond antimicrobial activity. *Nat Rev Immunol* **16**, 321–334.
14. Akira S, Uematsu S & Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* **124**, 783–801.
15. Oladejo AO, Li Y, Wu X, *et al.* (2020) MicroRNAome: potential and veritable immunomolecular therapeutic and diagnostic baseline for lingering bovine endometritis. *Front Vet Sci* **7**, 614054.
16. Palazzo M, Balsari A, Rossini A, *et al.* (2007) Activation of enteroendocrine cells via TLRs induces hormone, chemokine, and defensin secretion. *J Immunol* **178**, 4296–4303.
17. Yue W, Liu Y, Li X, *et al.* (2019) Curcumin ameliorates dextran sulfate sodium-induced colitis in mice via regulation of autophagy and intestinal immunity. *Turk J Gastroenterol* **30**, 290–298.
18. Epstein J, Sanderson IR & MacDonald TT (2010) Curcumin as a therapeutic agent: the evidence from *in vitro*, animal and human studies. *Br J Nutr* **103**, 1545–1557.
19. Guo C, Rosoha E, Lowry MB, *et al.* (2013) Curcumin induces human cathelicidin antimicrobial peptide gene expression through a vitamin D receptor-independent pathway. *J Nutr Biochem* **24**, 754–759.
20. Ming J, Ye J, Zhang Y, *et al.* (2020) Optimal dietary curcumin improved growth performance, and modulated innate immunity, antioxidant capacity and related genes expression of NF- $\kappa$ B and Nrf2 signaling pathways in grass carp (*Ctenopharyngodon idella*) after infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol* **97**, 540–553.
21. Akone SH, Ntie-Kang F, Stuhldreier F, *et al.* (2020) Natural products impacting DNA methyltransferases and histone deacetylases. *Front Pharmacol* **11**, 992.
22. Moghadamtousi SZ, Kadir HA, Hassandarvish P, *et al.* (2014) A review on antibacterial, antiviral, and antifungal activity of curcumin. *BioMed Res Int* **2014**, 186864.
23. Karaman Mayack B, Sippl W & Ntie-Kang F (2020) Natural products as modulators of sirtuins. *Mol* **25**, 3287.
24. Panche AN, Diwan AD & Chandra SR (2016) Flavonoids: an overview. *J Nutr Sci* **5**, e47.
25. Cheng AL, Hsu CH, Lin JK, *et al.* (2001) Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* **21**, 2895–2900.
26. Marczylo TH, Steward WP & Gescher AJ (2009) Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. *J Agric Food Chem* **57**, 797–803.
27. Boozari M, Butler AE & Sahebkar A (2019) Impact of curcumin on toll-like receptors. *J Cell Physiol* **234**, 12471–12482.
28. De Quadros De Bortolli JDQ, Klein M, Teixeira D, *et al.* (2020) Topical application of chamomile and curcumin in oral ulcers: an experimental study in rats. *Oral Surg Oral Med Oral Pathol Oral Rad* **129**, e148.
29. Flurkey K, Curren JM & Harrison DE (2007) The mouse in aging research. In *The Mouse in Biomedical Research*, 2nd ed., pp. 637–672. [JG Fox, MT Davisson, FW Quimby, SW Barthold, CE Newcomer, AL Smith, editors]. Burlington: American College Laboratory Animal Medicine (Elsevier).
30. Nair AB & Jacob S (2016) A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**, 27–31.
31. Nakagawa Y, To M, Saruta J, *et al.* (2019) Effect of social isolation stress on saliva BDNF in rat. *J Oral Sci* **61**, 516–520.
32. Taylor SC & Posch A (2014) The design of a quantitative western blot experiment. *BioMed Res Int* **2014**, 361590.
33. Shin HS, See HJ, Jung SY, *et al.* (2015) Turmeric (*Curcuma longa*) attenuates food allergy symptoms by regulating type 1/type 2 helper T cells (Th1/Th2) balance in a mouse model of food allergy. *J Ethnopharmacol* **175**, 21–29.
34. Chattopadhyay I, Biswas K, Bandyopadhyay U, *et al.* (2004) Turmeric and curcumin: biological actions and medicinal applications. *Curr Sci* **87**, 44–53.
35. Tayyem RF, Heath DD, Al-Delaimy WK, *et al.* (2006) Curcumin content of turmeric and curry powders. *Nutr Cancer* **55**, 126–131.
36. Xu F, Laguna L & Sarkar A (2019) Aging-related changes in quantity and quality of saliva: where do we stand in our understanding? *J Texture Stud* **50**, 27–35.
37. Feron G (2019) Unstimulated saliva: background noise in taste molecules. *J Texture Stud* **50**, 6–18.
38. Martin LE, Gutierrez VA & Torregrossa AM (2023) The role of saliva in taste and food intake. *Physiol Behav* **262**, 114109.
39. Sato K, Kato A, Sekai M, *et al.* (2017) Physiologic thymic involution underlies age-dependent accumulation of senescence-associated CD4+ T cells. *J Immunol* **199**, 138–148.
40. Karlsson M, Zhang C, Méar L, *et al.* (2021) A single-cell type transcriptomics map of human tissues. *Sci Adv* **7**, eabh2169.
41. Nagler RM & Hershkovich O (2005) Age-related changes in unstimulated salivary function and composition and its relations to medications and oral sensorial complaints. *Aging Clin Exp Res* **17**, 358–366.
42. Abou Alaiwa MH, Reznikov LR, Gansemer ND, *et al.* (2014) pH modulates the activity and synergism of the airway surface liquid antimicrobials  $\beta$ -defensin-3 and LL-37. *Proc Natl Acad Sci USA* **111**, 18703–18708.
43. Scott J (1977) Quantitative age changes in the histological structure of human submandibular salivary glands. *Arch Oral Biol* **22**, 221–227.
44. Kiyama J (2000) Characteristics of secretory immunoglobulin A secretion from rat salivary glands. *Jpn J Oral Biol* **42**, 302–314.
45. Scott J, Flower EA & Burns J (1987) A quantitative study of histological changes in the human parotid gland occurring with adult age. *J Oral Pathol* **16**, 505–510.
46. Ogle OE (2020) Salivary gland diseases. *Dent Clin North Am* **64**, 87–104.
47. Tsutsumi-Ishii Y & Nagaoka I (2002) NF-kappa B-mediated transcriptional regulation of human beta-defensin-2 gene following lipopolysaccharide stimulation. *J Leukoc Biol* **71**, 154–162.
48. Rosignoli da Conceição A, Dias KA, Michelin Santana Pereira S, *et al.* (2022) Protective effects of whey protein concentrate admixed of curcumin on metabolic control, inflammation







- and oxidative stress in Wistar rats submitted to exhaustive exercise. *Br J Nutr* **127**, 526–539.
49. Harada N, Arahori Y, Okuyama M, *et al.* (2022) Curcumin activates G protein-coupled receptor 97 (GPR97) in a manner different from glucocorticoid. *Biochem Biophys Res Commun* **595**, 41–46.
  50. Harada N, Okuyama M, Teraoka Y, *et al.* (2022) Identification of G protein-coupled receptor 55 (GPR55) as a target of curcumin. *npj Sci Food* **6**, 4.
  51. Kishimoto S, Uno M, Okabe E, *et al.* (2017) Environmental stresses induce transgenerationally inheritable survival advantages via germline-to-soma communication in *Caenorhabditis elegans*. *Nat Commun* **8**, 14031.
  52. Fitzpatrick SG, Alramadhan S, Islam MN, *et al.* (2023) Increased frequency of oral plasma cell mucositis/plasma cell gingivitis following the COVID-19 pandemic: a 23-year retrospective analysis and review of diagnostic challenges relating to this condition. *Oral Surg Oral Med Oral Pathol Oral Rad* **137**, 153–160.
  53. Morley JE (2020) Editorial: oral frailty. *J Nutr Health Aging* **24**, 683–684.
  54. Diaz-Toro F, Petermann-Rocha F, Parra-Soto S, *et al.* (2022) Association between poor Oral Health and frailty in middle-aged and older individuals: a cross-sectional national study. *J Nutr Health Aging* **26**, 987–993.
  55. Pourhajibagher M, Noroozian M, Ahmad Akhouni MS, *et al.* (2022) Antimicrobial effects and mechanical properties of poly(methyl methacrylate) as an orthodontic acrylic resin containing Curcumin-Nisin-poly(L-lactic acid) nanoparticle: an *in vitro* study. *BMC Oral Health* **22**, 158.
  56. Weinberg A, Krisanaparakornkit S & Dale BA (1998) Epithelial antimicrobial peptides: review and significance for oral applications. *Crit Rev Oral Biol Med* **9**, 399–414.
  57. Uemura I, Takahashi-Suzuki N, Kita F, *et al.* (2024) Regulation of chloride channels by epidermal growth factor receptor-tyrosine kinase inhibitor-induced  $\alpha$ -defensin 5. *Biol Pharm Bull* **47**, 159–165.
  58. Takahashi N, Kobayashi M, Ogura J, *et al.* (2014) Immunoprotective effect of epigallocatechin-3-gallate on oral anticancer drug-induced  $\alpha$ -defensin reduction in Caco-2 cells. *Biol Pharm Bull* **37**, 490–492.
  59. Yasuda G, Kubota A, Okamoto K, *et al.* (2023) Association between  $\alpha$ -defensin 5 and the expression and function of P-glycoprotein in differentiated intestinal Caco-2 cells. *Biopharm Drug Dispos* **44**, 358–364.