Review Article

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# Zebrafish as model for studies in dentistry

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## Abstract

**INTRODUCTION:** Over the last years, zebrafish has gained prominence in the biomedical community. It is currently considered one of the best vertebrate animal models for various types of studies, such as toxicology and developmental biology.

**OBJECTIVE:** The aim of this study was to conduct a literature review on the use of zebrafish in dentistry and whether this animal model could be a viable alternative for performing different types of studies in this area.

**METHODS:** A literature search was performed using the PubMed, Lilacs, Embase, and Dentistry and Oral Sciences Source. The keywords used as search terms were zebrafish and dentistry. The selection criteria were articles published in English that used zebrafish as an animal model in dentistry, oral health, and craniofacial growth/development.

**RESULTS:** The electronic search of literature yielded 421 articles. After the analysis of the abstracts, 29 articles were selected for an in-depth analysis and reading of the full text.

**CONCLUSIONS:** All studies included in this review confirm zebrafish's excellence as an animal model for various types of dentistry studies, as well as assisting and complementing other studies involving mammals.

**Keywords:**
Animal model, dentistry, Zebrafish

## Introduction

Zebrafish (*Danio rerio*) is a freshwater fish native to Southeast Asia.[1] The current use as a model organism occurred from the work of George Streisinger,[2] a pioneer in the use of molecular genetics for the study of embryology of vertebrates, and Kimmel,[3-5] who published detailed descriptions of cell differentiation and organization of the nervous system.

Although mammals are considered the gold standard for developmental toxicity assessment, zebrafish has been increasingly used for *in-vivo* chemical toxicity.[6-8] It represents a viable alternative, considering their small size, high fecundity, embryo optical transparency, rapid embryonic development, and low maintenance cost.[9] Developmental toxicity, reproductive toxicity, cardiovascular toxicity, neurodevelopmental toxicity, and ocular developmental toxicity of hazardous chemicals have been effectively studied using zebrafish model.[10] Considering this, in 2021, a quick search in the PUBMED/NCBI database with "zebrafish" as a keyword resulted in about 42,700 articles.

Zebrafish is currently considered one of the best vertebrate models for developmental biology studies. Despite anatomical and histological differences from mammals, including the lack of organs such as lungs, prostate, and mammary glands, it maintains the general aspects of vertebrate body plan and constituents at anatomical, molecular, and



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physiological levels.[11-13] In addition, zebrafish genome has homologues of 70% of human genes and more than 80% of those associated with human diseases,[13] which allows a more direct extrapolation of findings than studies using invertebrates and supports the significant investment in this species in several areas translational biomedical research areas.

The zebrafish embryo develops rapidly, with primordia of all major organs appearing within 36 h postfertilization, and most of its organs such as brain, heart, and kidneys are functional on the 5th day of postfertilization.[14] Sexual maturity is reached between 3 and 6 months of life.[15]

The visualization of the embryonic development is one of the main advantages of the zebrafish, allowing the monitoring of the development of its various organs with the aid of a stereomicroscope. The relatively large size of the embryo (~650 µm vs. ~90 µm of the mouse embryo) and its transparency allow the use of less invasive real-time techniques without interventions such as surgery or postmortem examinations.[16] Injection of transgenes into the zebrafish can be performed with injection into the cytoplasm, while in the rodent embryo this should be performed in their pronuclei, so the equipment for this type of procedure is less expensive and the technique easier to perform.[17]

This article aimed to review the literature on the use of zebrafish in dentistry and whether this animal model can be a viable alternative for the accomplishment of various types of study.

## Material and Method

An electronic review of the literature was performed using the PubMed, Lilacs, Embase, and Dentistry and Oral Sciences Source databases. The keywords used as search terms were zebrafish and dentistry. The selection criteria were articles published in English that used zebrafish as an animal model for any type of research in dentistry, oral health, and craniofacial growth/development.

Figure 1 shows a flowchart of the article's selection process.

The selection of the articles was carried out independently by two investigators (A.H., H.S.) and double-checked by a second researcher (L.M.) when necessary. Titles and abstracts of potentially relevant articles were analyzed before the full text was obtained.

## Results

The electronic search of literature yielded 421 articles. After the analysis of the abstracts, 35 articles were selected for an in-depth analysis and reading of the full text. Six articles were excluded because they did not use the zebrafish as animal model in the experiments or because they were conference papers, hypothesis, informative consortium, or commentaries. Thus, 29 articles were included in this study [Figure 1].

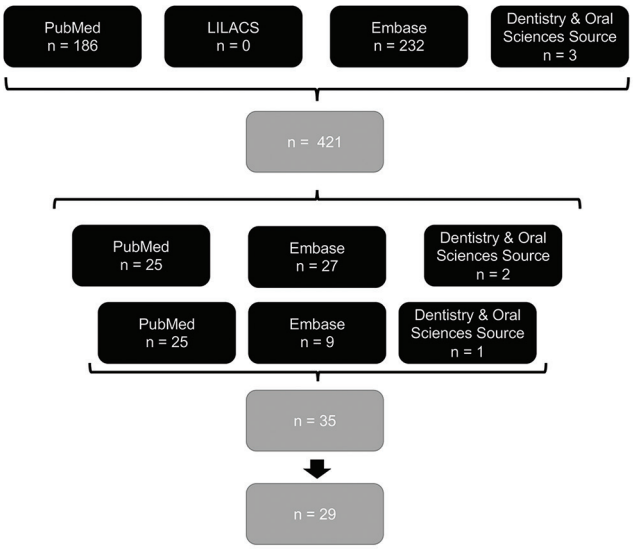


**Figure 1:** Flowchart of the article's selection process

Of these 29 articles, 15 evaluated the expression of specific genes in the development of craniofacial and/or dental anomalies.[18-30] Nine of the papers studied the pathogen mechanism of action in infectious diseases,[31-39] three studies tested the toxicity of materials used in dentistry,[40-42] and two were literature reviews related to zebrafish and the development of craniofacial malformations, especially cleft lip and/or palate.[43,44]

Table 1 summarizes the results of articles selected from the electronic search.

## Discussion

Biomedical research relies on model organisms to study biologic processes conserved between humans and lower vertebrates.[45] Usually, the organism models are small mammals, like rats and mice. Meanwhile, in the past decades, zebrafish has been widely used as an experimental model, considering important advantages over other models[9] and considering the fact that the results can be further validated in mammals.[45] It is an appealing model for toxicological, genetic, and behavioral studies, as well as for testing new therapeutic agents, understanding the mechanism of evolution of several human diseases, and the development under normal and pathologic conditions.

Within oral health and dentistry, the vast majority of studies involve genetics and craniofacial development. Several researchers have investigated the role of specific

**Table 1: Studies using zebrafish as a model animal for research in dentistry found in the databases searched**

| Reference | Objective | Method | Conclusions |
|---|---|---|---|
| Farrugia *et al*. (2020) | To assess the effects of *P. gingivalis* OMVs on the endothelium | OMVs were isolated from wild-type strain W83 and the gingipain-deficient strain ΔK/R-ab. Immunoblotting along with cryo-EM showed gingipain expression in W83 but not ΔK/R-ab-derived OMVs, where gingipains were localized to the cell wall surface. Confluent endothelial cell monolayers infected with either W83 or W83-derived OMV displayed significantly increased dextran permeability over those infected with ΔK/R-ab or its OMV. Moreover, W83-derived OMVs induced significantly more vascular disease in a zebrafish larvae systemic infection model over 72 h compared to those injected with gingipain-deficient OMVs or controls | OMVs from *P. gingivalis* mediate increased vascular permeability, leading to a diseased phenotype both *in vitro* and *in vivo*. Moreover, these data strongly implicate gingipains present on the OMV surface in mediating these vascular events, most likely via a mechanism that involves proteolytic cleavage of endothelial cell–cell adhesins such as PECAM-1 |
| Gebuijs *et al*. (2020) | To investigate the effects of VPA on cartilage and bone formation in the zebrafish larval head during early and late development in which cranial neural crest cells (CNCCs) arise and then proliferate and differentiate, respectively | Zebrafish breeding and husbandry, VPA treatment. Cartilage and bone staining, zebrafish imaging, cartilage and bone analysis, gene-expression analysis | Treatment of larvae with VPA during early and late caused severe malformations in cartilage and mineralized elements in both groups. The observed mortality for the treated larvae was comparable or lower than controls. There was a general growth retardation of the head when we compared the total head length of the treatment groups with the wild types. Treatment with VPA in concentrations similar to clinically relevant levels during early development led to a reduction of cartilage and bone formation in structures derived from CNCCs. Both CNCC formation and differentiation are compromised by VPA depending on the time of exposure, respectively. In the late treatment group, VPA may have disturbed CNCC differentiation and subsequent cartilage and bone formation |
| Hsieh *et al*. (2020) | To verify the feasibility of electrolyzed oxidizing (EO) water as a mouthwash through the evaluation of its *in-vivo* toxicity by embryonic zebrafish and antimicrobial efficacy against *S. mutans* | 1.5–3.0 g of sodium chloride (NaCl), sodium bromide (NaBr), or calcium chloride ($CaCl_2$) was added into an electrolyzer with 300 mL of DD water to produce electrolyzed oxidizing (EO) water. A zebrafish embryo assay was used to evaluate acute toxicity of specimens. Antimicrobial property was conducted with 100 μL microbial count of $1\times10^8$ cfu/mL *S. mutans* to blend with each 10 mL specimen of chlorhexidine (CHX) gluconate or hypochlorous acid (HOCl) for various time points. The concentration of viable microorganisms was assessed according to individually standardized inoculum by a plate-count method | Except for the 0.2% CHX gluconate, all the HOCl specimens and 2.0% CHX gluconate revealed similar antimicrobial properties (>99.9%) against *S. mutans*. The EO water comprising both 0.0125% and 0.0250% HOCl showed>99.9% antimicrobial efficacy but with little *in-vivo* toxicity, illuminating the possibility as an alternative mouthwash for dental and oral care |
| Farrugia *et al*. (2020) | To determine whether *Pg*-induced vascular damage is mediated by gingipains | *P. gingivalis* strains and culture and endothelial cell culture. Generation of zebrafish transgenic lines, endothelial cell invasion quantification and visualization, endothelial cell permeability. *In-vitro* PECAM-1 and VE-cadherin cell adhesion protein expression, *in-vitro* gene expression analysis, *in-vivo* PECAM-1, and VE-cadherin cell adhesion protein expression and *in-vivo* vascular permeability | *In vitro*, human endothelial cells from different vascular beds were invaded by wild-type (W83) but not gingipain-deficient (ΔK/R-ab) Pg. W83 infection resulted in increased endothelial permeability as well as decreased cell surface abundance of endothelial adhesion molecules PECAM-1 and VE-cadherin compared to infection with ΔK/R-ab. When transgenic zebrafish larvae expressing fluorescently labeled PECAM-1 or VE-cadherin were systemically infected with W83 or ΔK/R-ab, a significant reduction in adhesion molecule fluorescence was observed specifically in endothelium proximal to W83 bacteria through a gingipain-dependent mechanism. This study provided crucial evidence for the role of Pg, and gingipains, in affecting endothelial function, reaffirming the likely role of oral microbes in influencing systemic disease outcomes |

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**Table 1: Contd...**

| Reference | Objective | Method | Conclusions |
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| Leu *et al*. (2020) | To isolate and characterize a novel CaS1 scFv mAbs against CaEno1 from a phage display library | A monoclonal antibody (CaS1) was isolated by phage display technology, which recognized the recombinant CaEno1 (rCaEno1) and CaEno1 expressed by *C. albicans* and C. tropicalis. CaS1 attenuated the growth and plasminogen binding activity of *C. albicans*. Effect of CaS1 on *C. albicans* Infection in mice and in Zebrafish Model | CaS1 prolonged survival time and reduced the fungal burden and inflammatory cytokine levels of mice in an *in-vivo* candidiasis model. CaS1 could be a starting point for further development of *C. albicans*-specific therapies |
| Al-Zubidi *et al*. (2019) | To isolate several bacteriophages that target *E. faecalis* strains from the oral cavity of patients suffering root canal infections and to report their therapeutic potential against *E. faecalis* strains forming biofilms and their capacity to eradicate systemic infection in a zebrafish model of infection | Isolation and characterization of bacteriophages targeting *E. faecalis*. Molecular characterization of isolated phages, determination of host range, Genome organization of SHEF2, -4, and -5, identification of enterococcal polysaccharide antigen as the SHEF2 receptor, infection parameters and strain preference of SHEF2. The Ability of SHEF2 to clear *E. faecalis* biofilms was tested. Phage treatment evaluation was performed in a zebrafish model of infection with the clinical strain OS16 | Isolation of phages targeting *E. faecalis* strains, in particular targeting a major virulence determinant of these strains (EPA) and their potential use in treating biofilm infections by testing them in two clinically relevant model infection systems were highlighted. This work strengthens the possibility of developing phages as therapeutics to combat hard-to-treat oral, topical, and systemic infections |
| Swidergall *et al*. (2019) | To investigate whether candidalysin drives endothelial cell activation, immune cell recruitment, and mortality during systemic *C albicans* infections | *C. albicans* was added to the endothelial cells. Cell lysate preparation, immunoblotting, lactate dehydrogenase release, and cytokine and chemokine analysis were performed. Zebrafish *C. albicans* infection model and mouse model were performed. For infection, methylcellulose-embedded, and tricaine-anesthetized dechorionated larvae were injected systemically and viability was assessed by the presence of heartbeat | Candidalysin activates MAPK signaling and cytokine responses in endothelial cells and promotes neutrophil recruitment and mortality during zebrafish and murine models of systemic *C. albicans* infection. During disseminated candidiasis, candidalysin production by *C. albicans* hyphae stimulates a strong proinflammatory response with neutrophil recruitment, which reduces fungal burdens during early infection but later hastens mortality likely related to immunopathogenic effects. Candidalysin production during systemic infections promotes virulence, neutrophil recruitment, and disease and may identify candidalysin-associated damage and immune activation pathways as novel targets to combat disseminated fungal infections |
| Lu *et al*. (2018) | To evaluate the biofilm-forming activity of DST659 and DST693 genotypes and the infectivity of *C. albicans* isolates | *C. albicans* infection model with zebrafish eggs. *Candida* biofilms on zebrafish chorions were semi-quantified by analyzing the photo images and the eggs were evaluated for the survival rate after infection. Retrospective data collected from the enrolled patients included demographics, comorbidities, risk factors, and outcomes | A dominant genotype of clinical *C. albicans*, DST659, possesses a high biofilm-forming activity. a high incidence rate of renal dysfunction is associated with the DST659-infected patients. The renal dysfunction in DST659-infected patients probably could be explained by an extraordinary amount of initial adherence and the fungal mass in patients' kidneys. High biofilm-forming activity was found to be an important factor for the dominance of DST659 genotype in north Taiwan, and that genotype tends to damp the renal function in patients |
| Chiquet *et al*. (2018) | To identify genes that were differentially expressed between wild-type and Crispld2 morphant zebrafish and previously known to play a role in craniofacial development and test the association of single nucleotide variants in the identified genes with nonsyndromic cleft lip with or without cleft palate in our nonhispanic white and Hispanic multiplex and simplex families | Genotyping of the individuals studied. RNA profiling in zebrafish embryos, morpholino injection, and RT-PCR technique. In silico pathway analysis, genotyping, association analysis, and gene network analysis | 249 genes were identified with differentiated expression after Crispld2 knockdown. They also observed that the interaction between 3 of these genes was related to non-syndromic cleft lip and palate (CASP8, FOS, and MMP2) |

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| Reference | Objective | Method | Conclusions |
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| Alifui-Segbaya *et al*. (2018) | To evaluate the biocompatibility of methacrylate for denture bases, splints, retainers, and surgical guides | Lethal, sublethal (eyes development, spontaneous movement, hypopigmentation and edema formation), and teratogenicity outcomes (spinal curvature, caudal malformation, and yolk deformation) were evaluated in zebrafish embryo models | Toxicological data obtained confirmed gradations of toxicity influenced by ethanol treatment, exposure scenarios, and extraction vehicles. Biocompatibility was influenced mainly by the physicochemical characteristics of the materials, which later influenced their residual monomer content before and after immersion in ethanol |
| Watt *et al*. (2018) | To understand and examine the contribution of the Tp53 pathway to the pathogenesis of Acrofacial Dysostosis–Cincinnati type | In a zebrafish model of Acrofacial Dysostosis–Cincinnati type, the role of Tp53 in a polr1a mutant was exanimated. Embryos were prepared for live imaging and skeletal stains, *in situ* hybridization, immunostaining, polysome profiling, protein synthesis assays, and qPCR | Tp53 inhibition suppresses neuroepithelial apoptosis and partially ameliorates the polr1a mutant phenotype. However, complete rescue of cartilage development is not observed due to the failure to improve rDNA transcription and neural crest cell proliferation. Neural crest cell cells appear especially sensitive to alterations in rRNA transcription. Inhibiting Tp53-dependent cell death is not sufficient to completely prevent the pathogenesis of AFDCIN in the zebrafish model because Tp53-independent signaling regulates the proliferation of neural crest cells. Specific functions for Tp53 signaling downstream of polr1a in ribosome biogenesis during neural crest cell and craniofacial development in the pathogenesis of Acrofacial Dysostosis–Cincinnati type were revealed |
| Zhao *et al*. (2018) | To evaluate the effects of various metal alloy shells of porcelain-fused-to-metal crowns on the development of zebrafish embryos and larvae to determine the safety of these materials | Toxicity was assessed based on mortality, hatchability, spontaneous movement, heart rate, malformation, and swimming behavior. Zebrafish embryos and larvae were biologically evaluated; larval behavior was also evaluated | Porcelain-fused-to-metal substrates affect the embryonic development of zebrafish to varying degrees and increasing over time, delaying hatching, increasing mortality, and decreasing spontaneous movement, heart rate, and swimming behavior, suggesting that they may be toxic when used in dental applications, except for Ti and Au–Pd alloys, which showed good biocompatibility and are therefore the most suitable materials for dental crowns |
| Yuan (2017) | To apply whole-exome sequencing to identify the cause of oligodontia in a 9-year-old girl missing 11 permanent teeth | Protein modeling and functional analysis in zebrafish were performed to understand the impact of identified variants on the phenotype. Whole-exome sequencing was performed on DNA samples of the child proband, variation validation, and molecular modeling. Zebrafish husbandry, morpholino injections, alcian blue and alizarin red staining, Western blot, quantitative RT-PCR, and molecular methods and microinjection | A novel compound heterozygous variant in WNT10A is pathogenic for oligodontia. Perturbations of wnt10a expression in zebrafish may directly and/or indirectly affect tooth development recapitulating the agenesis phenotype observed in humans |
| Al Zubidi (2017) | To isolate, characterize and test *in vitro* a range of lytic bacteriophages targeted against *E. faecalis* isolates from oral endodontic infections obtained from labs across Europe. | Five bacteriophages were isolated from concentrated wastewater named (SHEF2, 4, 5, 6, 7) that belong to the Siphoviridae family. Full chromosome sequences of SHEF2, 4, 5 alongside biological evidence revealed that they are lytic bacteriophages which place them as suitable candidates for therapy. The ability of these phages was tested to eradicate biofilm from abiotic surfaces and a novel cross-sectional tooth model while also showing that they can rescue Zebrafish embryos from *E. faecalis* systemic clinical strain infection | The extracellular exopolysaccharide of *E. faecalis* was the bacterial docking target of these phages during the initial stages of phage infection. These or other bacteriophages might be novel adjuncts to current endodontic therapy to eradicate recalcitrant biofilm and antibiotic-resistant *E. faecalis* |

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| Reference | Objective | Method | Conclusions |
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| Liu *et al*. (2017) | To describe a versatile, functional pipeline and apply it to single nucleotide polymorphisms at 1p22, a locus identified in several genome-wide association studies for nonsyndromic cleft lip with or without cleft palate (CL/P) | DNA elements containing the most-highly risk-associated single nucleotide polymorphisms were amplified and tested for their enhancer activity *in vitro*, identifying three single nucleotide polymorphisms with allele-dependent effects. *In-vivo* zebrafish assays tested the tissue-specificity of these enhancers, chromatin conformation capture to test interactions, and genome editing *in vitro* to show allele-specific effects on ARHGAP29 expression and cell migration. Chromatin immunoprecipitation quantitative PCR and CRISPR-Cas9-mediated knockout and knockin | Two single nucleotide polymorphisms affect binding of CL/P-associated transcription factors, and one affects chromatin configuration. These results translate risk into potential mechanisms of pathogenesis. The discovery of potential connections like these motivates further analyses of the gene regulatory networks controlling development of craniofacial tissues. Indeed, despite mounting evidence that ARHGAP29 is the risk gene at 1p22, and that it participates in a network that includes KLF4, MAFB, and IRF6, the role of ARHGAP29 during craniofacial development remains unknown |
| Widziolek *et al*. (2016) | To study the mechanisms of *P. gingivalis* (Pg) systemic pathogenicity | Zebrafish larvae systemic infection model. Zebrafish strains and bacterial culture, microinjection of *P. gingivalis* onto zebrafish embryos, evaluation of gingipain activity, histological processing of zebrafish, labeling of Pg, and fluorescence microscopy | Pg rapidly adheres to and penetrates the zebrafish vascular endothelium causing a dose and time-dependent mortality with associated development of pericardial edema and cardiac damage. Using gingipain knock-out mutants and protease inhibitors the crucial role that these proteases play during systemic infection was identified and the ability of these bacteria to cross the vasculature and disseminate into surrounding tissues *in vivo* was shown. Gingipains are crucially linked to systemic disease and potentially contribute to CVD |
| Watt *et al*. (2016) | To uncover tissue-specific roles for polr1c and polr1d in rRNA transcription, ribosome biogenesis, and neural crest and craniofacial development during embryogenesis | Description of spatiotemporal activity and functional roles of polr1c and polr1d during zebrafish embryogenesis and particularly in craniofacial development. Zebrafish husbandry, phenotype analysis (live imaging, skeletal stain, *in situ* hybridization, immunostaining), molecular analysis (Western blot, qPCR, polysome profiling, image quantification) | polr1c and polr1d mutant zebrafish as models of Treacher Collins syndrome together with a unifying mechanism underlying its pathogenesis and possible prevention. polr1c and polr1d play important functions in rRNA transcription and furthermore that polr1c and polr1d loss-of-function results in tissue-specific phenotypes, including craniofacial cartilage anomalies that mimic TCS. Inhibition of Tp53 function was able to ameliorate cranioskeletal anomalies in polr1c−/− and polr1d−/− mutant zebrafish |
| Leslie *et al*. (2016) | To identify risk factors for nonsyndromic CP | Genome-wide association study of this disorder. A missense variant in GRHL3, replication of the result in an independent sample of case and control subjects. In both the discovery and replication samples, rs41268753 conferred increased risk for CP. In luciferase transactivation assays, p.Thr454Met had about one-third of the activity of wild-type GRHL3, and in zebrafish embryos, perturbed periderm development | This mutation is an etiologic variant for nonsyndromic CP and is one of few functional variants identified to date for nonsyndromic orofacial clefting |
| Liu *et al*. (2016) | To test whether rare missense variants of KLF4 contribute risk for NSCL/P | Human sample genotyping and sequencing. Zebrafish KLF17 and human KLF4 mRNA injection and *in situ* hybridization, RNA isolation and quantitative RT-PCR, cell culture, luciferase reporter constructs, transfections and dual luciferase assay, Immunofluorescent staining, genomic interval manipulation, zebrafish enhancer *in-vivo* reporter assay | Human KLF4 functions like zebrafish Klf17 were found in over-expression assays. IRF6 binds an enhancer of KLF4 that is active in oral epithelium cells, supporting the possibility that KLF4 is directly downstream of IRF6 in a GRN regulating oral periderm differentiation. Rare variants in KLF4 confer risk for NSCL/P. |
| Chen *et al*. (2015) | To establish a zebrafish egg bath infection model as a simple noninvasive model for investigating *C. albicans* pathogenesis | Zebrafish egg bath infection model. *C. albicans* strains with the ability to switch between the yeast and filament forms are those capable of penetrating vital organs and proliferating sufficiently to cause lethal infections | *C. albicans* can form hyphae at a lower temperature. *C. albicans* adhering to the chorion of embryos formed long hyphae/biofilms in egg water, indicating that additional host-related factors from embryos are crucial for superseding the need for increased temperature and other stimuli, such as serum |

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**Table 1: Contd...**

| Reference | Objective | Method | Conclusions |
|---|---|---|---|
| Leslie *et al*. (2015) | To identify mutations and search for the contributions of both rare and common variants as risk alleles for nonsyndromic cleft lip with or without cleft palate. | Genetic sequencing of a sample, zebrafish husbandry, photography, electrophoretic mobility shift assays, luciferase reporter constructs, transfections and luciferase assays, cell culture, murine crosses, immunostaining and mapping of putative transcription factor binding sites | Sequencing of large intervals surrounding genome-wide association studies regions is an effective approach for identifying functional rare and common variants in both coding and noncoding regions. Noncoding regulatory elements play an important role and disruption of these regions by genetic variants is a critical aspect of the pathogenesis of NSCL/P |
| Bloch-Zupan *et al*. (2011) | To report a severe developmental dental defect that results in a dentine dysplasia phenotype with major microdontia, oligodontia, and shape abnormalities in a highly consanguineous family | Homozygosity mapping. Two affected children were found to carry a homozygous mutation in SMOC2. Knockdown of this gene in zebrafish showed pharyngeal teeth that had abnormalities reminiscent of the human phenotype. SMOC2 depletion in zebrafish affected the expression of three major odontogenesis genes | SMOC2 is an early dental developmental gene in human beings and highlighting this protein as potentially useful in regenerative dentistry |
| Gregory-Evans *et al*. (2007) | To insight into the molecular etiology underlying otodental disease | Genomic mapping, blot analysis, quantitative expression, data and histological analysis in a family with otodental disease. Spatiotemporal *in situ* hybridization in zebrafish embryos established for the first time that FADD is expressed during eye development | Haploinsufficiency of FGF3 and FADD (Fas-associated death domain) is the likely cause of the otodental and ocular coloboma phenotypes, respectively FGF3 haploinsufficiency is likely to be the cause of otodental syndrome. FADD haploinsufficiency is likely to be responsible for ocular coloboma in this family |
| Ng *et al*. (2004) | To investigate if Lenz microphthalmia and Oculofaciocardiodental syndromes are likely to result from defects in alternative functions of BCOR, such as interactions with transcriptional partners | Genetic mapping and sequencing for mutation analysis in families with OFCF/ Lenz syndrome. BCOR ortholog zebrafish cloning to examine the pleiotropic effects of BCOR mutations | BCOR is a key transcriptional regulator during early embryogenesis |

genes in nonsyndromic clefts of the lip and palate.[22-24,26,29] Other craniofacial malformations and syndromes have also been extensively studied,[18,21,25,28] as well as dental development disturbances.[19,20,27,46] Craniofacial development is a sensitive process and environmental factors may disrupt neural crest cells' formation, survival, migration, and differentiation.[44] Chiquet *et al*.[29] demonstrated that the knockdown of CRISPLD2 causes a differentiated expression of 249 genes in zebrafish. The interaction between three of these genes (CASP8, FOS, and MMP2) had a significant relationship with nonsyndromic cleft lip and palate. Leslie *et al*.[24] discovered a genome-wide significant association with a missense variant in GRHL3 and replicated the result in an independent sample. In both the discovery and replication samples, it conferred an increased risk for cleft palate, concluding that the mutation is an etiologic variant for nonsyndromic orofacial clefting. Other variants were identified as functional for nonsyndromic cleft lip and/or palate, unraveling the role of some genes in the etiology of malformation.[22,23,26] Due to the multifactorial nature of cleft lip and/or palate, animal models can be used to understanding the full spectrum of this human phenotype.[43] Zebrafish mutants are invaluable in identifying novel candidate genes for this complex disease and testing environmental factors, contributing to understanding the etiology and discovering potential therapeutic remedies for this multifactorial disorder.[43]

The use of pharmaceuticals during pregnancy can lead to craniofacial malformations and the use anti-epileptic drug and mood stabilizer called valproic acid is frequently associated with craniofacial teratogenicity.[44] The use of this anti-epileptic can cause fetal valproate spectrum disorder, with symptoms such as intellectual disability, facial abnormalities (including cleft), and cardiac defects.[47] Recently, Gebuijs *et al*.[30] investigated the effects of valproic acid on cartilage and bone formation in the zebrafish larval head and concluded that the drug disturbed neural crest cells' function leading to defects in cartilage and bone formation. The zebrafish is an excellent model for investigating the genetic and environmental factors, as well as their interaction with craniofacial malformations.[44]

One of the main advantages of using zebrafish to study infectious diseases is the possibility of noninvasive imaging at the cellular level; whereas larval zebrafish can be kept transparent, the fluorescently labeled microorganism after injection into zebrafish larvae can be monitored.[48] The opportunistic pathogen *Candida*

*albicans* is widely studied with the zebrafish model, considering that the understanding of the mechanism of action is essential to develop new therapeutics in infectious diseases. Chao *et al*.[49] demonstrated that *C. albicans* could colonize zebrafish at multiple anatomical sites, causing mortality after being injected into the peritoneal cavities. Chen *et al*.[31] investigated *C. albicans* adhesion factors and Lu *et al*.[34] suggested that a strong biofilm activity of DST659 contributed to a high mortality rate in zebrafish hosts and poor renal function in patients, in the northern Taiwan. Candidalysin is a cytolytic peptide toxin secreted by *C. albicans* hyphae, which induced immune activation and neutrophil recruitment and promoted mortality in zebrafish and murine models of systemic fungal infection.[35] Recent zebrafish research showed that enolase plays an important role in invasive candidiasis and also shows that CaS1 may be potentially useful for the development of immunotherapeutic agents against the infection.[37]

Gram-positive *Enterococcus faecalis*, also an opportunistic pathogen, is frequently responsible for nosocomial infections and represents one of the most common bacteria isolated from root canal infections.[36] The resistance to antibiotics and the capacity to form biofilms cause serious therapeutic problems to infections. Two recent studies isolated characterized the bacteria and tested bacteriophages with therapeutic potential.[33,36] The phages SHEF 2, 4, and 5 were able to rescue zebrafish embryos from *E. faecalis* systemic clinical strain infection.[33] SHEF2 was tested again and cleared a lethal infection of zebrafish when applied in the circulation.[36] The phage described could be used to treat a broad range of antibiotic-resistant *E. faecalis* infections.

*Porphyromonas gingivalis* is a gram-negative anaerobe considered the key oral pathogen for severe periodontitis.[50] *P. gengivalis* frequently enters the bloodstream at oral sites causing a transient bacteremia.[51] In view of this, periodontitis affects the general health status and systemic inflammation. Systemic conditions such as cardiovascular disease,[52] atherosclerosis,[53] and diabetes[54] have been linked to periodontitis. Widziolek *et al*.[32] studied the mechanisms of systemic pathogenicity of Pg. They concluded that zebrafish is a suitable model to study both the host's response to *P. gegivalis* infection and bacterial virulence. Data revealed the first real-time *in-vivo* evidence of intracellular *P. gengivalis* within the endothelium and established that gingipains are linked to systemic disease and potentially contributed to cardiovascular diseases. The outer membrane vesicles produced by *P. gingivalis* mediated increased vascular permeability, leading to diseased phenotype both *in vitro* and *in vivo*.[39] Gingipains presented on this membrane's surface could mediate vascular events, as a mechanism that involves proteolytic cleavage of endothelial cell–cell adhesins, mediating systemic disease.[39] Farrugia *et al*.[38] determined that *P. gengivalis* directly mediate vascular damage *in vivo* by degrading PECAM-1 and VE-cadherin.

The zebrafish is also increasingly used for assessing chemical toxicity and safety.[10] In dentistry, Zhao *et al*.[40] conducted a study where several metal alloys make up the porcelain-fused-to-metal (PFM) crowns, a restoration technique widely used in dentistry, but not yet systematically evaluated *in vivo*. The effects of PFM on the embryonic and larval development of zebrafish were evaluated in order to determine the safety of these materials. Gold–palladium (Au–Pd), silver–palladium (Ag–Pd), nickel–chromium (Ni–Cr), cobalt–chromium (Co–Cr), and titanium (Ti) porcelain crowns were immersed in artificial saliva for 1, 4, and 7 weeks and the leached solutions were collected and used to treat embryos from 4 to 144 hpf. The toxicity parameters evaluated were mortality, spontaneous movement, heart rate, hatchability, malformation, and exploratory behavior. One week of exposure to the five alloys of PFMs was not toxic to zebrafish. Mortality and malformation rates in the Ni–Cr alloy group were increased while spontaneous movement, heart rate, and exploratory behavior were decreased at 4- and 7-week exposures. The Ni–Cr alloy was the most toxic, followed by the Co–Cr and Ag–Pd alloys. The Ti and Au–Pd alloys presented good biocompatibility and were therefore the most suitable for clinical applications.

Alifui-Segbaya *et al*.[41] evaluated the toxicological and teratogenic effects of three types of methacrylates (E-Denture, E-Guard, and Dental SG) used in dentistry for three-dimensional denture bases, splint, restraints, surgical guides, and diagnostic models on initial development of zebrafish. These methacrylate samples were tested in solutions of pure water and solutions with alcohol. The results showed that biocompatibility was influenced by the physicochemical characteristics of the materials, which subsequently influenced their residual monomer content before and after ethanol treatment. Although these materials showed a significant increase in degree of conversion after immersion in ethanol, more than a twofold increase was observed in E-Guard materials. Nevertheless, all methacrylates were unsafe in zebrafish bioassays. With the increasing use of 3D printers and materials on the market, the authors defend that the decision to purchase such products is based not only on economic factors but on those that will provide long-term benefits. According to authors, zebrafish bioassay is a reliable toxicological screening tool that could add to the existing biological evaluation tests in dentistry, where a number of devices for prosthetic treatments are constructed with methacrylates. More recently, Hsieh *et al*.[42] verified the feasibility of electrolyzed oxidizing

water as a mouthwash through the evaluation of its *in-vivo* toxicity by embryonic zebrafish and antimicrobial efficacy against *Streptococcus mutans*. Except for the 0.2% chlorhexidine gluconate, all the hypochlorous acid specimens and 2.0% chlorhexidine gluconate revealed similar antimicrobial properties. The electrolyzed oxidizing water comprising both 0.0125% and 0.0250% hypochlorous acid showed > 99.9% antimicrobial efficacy but with little *in-vivo* toxicity, illuminating the possibility as an alternative mouthwash for dental and oral care.

To date, most studies conducted on zebrafish to date have concluded that the correlation between zebrafish and rodent toxicity is high, thus being a viable alternative to toxicity tests in rodents and other animals.[55]

## Conclusions

This article was designed to provide information on the use of zebrafish as a model organism for studies that require a well-developed vertebrate animal model, especially in dentistry, where this model is still rarely used. All studies included in this review confirm the zebrafish's excellence as an animal model for a variety of study types. The innumerable characteristics of this animal can be useful in the evaluation of potentially toxic substances, besides being able to aid and complement other studies involving mammals.

### Authors contributions/Credit author statement

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

[1]ASCO, [1]HRSS, [2]CSP: methodology, investigation, writing, and preparation.

[3]MRMV: conceptualization, validation, writing – review and editing, and supervision.

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### Conflicts of interest

There are no conflicts of interest.

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