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Grading and quantification of dental fluorosis in zebrafish larva

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ABSTRACT

Objective: The prevalence and severity of dental fluorosis in primary teeth are different from permanent teeth. Previous animal models of dental fluorosis mainly focus on juvenile rats, mice and zebrafish. Our experiment aims to set a dental fluorosis model using zebrafish larva and explore the characteristics of the first generation teeth by fluoride treatment.

Materials and methods: After the zebrafish eggs were laid, they were exposed to excess fluoride (19 ppm, 38 ppm and 76 ppm) for five days. The morphological characteristics of first generation teeth were examined by H&E staining, whole-mount alizarin red and alcian blue staining, and scanning electron microscope (SEM) technique.

Results: With whole-mount alizarin red and alcian blue staining, the tooth cusps presented red in normal control. 19 ppm and 38ppmm fluoride resulted in extensive red staining from tooth cusps to the lower 1/3 of teeth. 76 ppm fluoride caused malformed teeth with uneven red staining. H&E staining showed that excess fluoride caused cystic-like changes in 38 ppm and 76 ppm groups. SEM revealed the dose dependent pathological changes in zebrafish enameloid with fluoride treatment. Based on SEM findings, we set 0–4 dental fluorosis index (DFI) score to label the severity of dental fluorosis.

Conclusions: Excess fluoride presented a dose dependent fluorosis changes in the teeth of zebrafish larva. The DFI scores in our experiment reflect dose dependent fluorosis changes in a good way and will benefit the future research of dental fluorosis.

1. Introduction

Dental fluorosis results from the excessive uptake of fluoride during the tooth development. It appears as unnoticeable, tiny white streaks in mild forms, and discoloration or brown markings in severe forms, which are permanent or become darken over time. Dental fluorosis also gives rise the residual organic substances and impairs the mineralization of dental hard tissues (Chen et al., 2006; Tanimoto et al., 2008).

Previously dental fluorosis was studied based on the various animal models, such as mouse, rat (Bronckers, Lyaruu, & DenBesten, 2009) and zebrafish (Bartlett, Dwyer, Beniash, Skobe, & Payne-Ferreira, 2005). The rodent models were more popular in the research of dental fluorosis because their process of enamel development is similar to that of human (Du, Wang, Wang, Wang, & Yang, 2012; Tang, Wang, Du, Yang, & Wang, 2013). The continuously erupted incisors in mice and rats also make it convenience to study the effects of fluoride on amelogenesis in adult animals. Bartlett's group used juvenile zebrafish as a model to study dental fluorosis (Bartlett et al., 2005). They found that the different concentrations of fluoride had effects on juvenile zebrafish teeth after an 8-week fluoride treatment (Bartlett et al., 2005).

According to the human data of dental fluorosis, the children younger than 6–7 years old who have lived in fluoride contamination area for a long time are susceptible to dental fluorosis, as fluoride easily target enamel in the developing and mineralizing process (Dhar and Bhatnagar, 2009). The prevalence and severity of dental fluorosis in primary teeth are different from permanent

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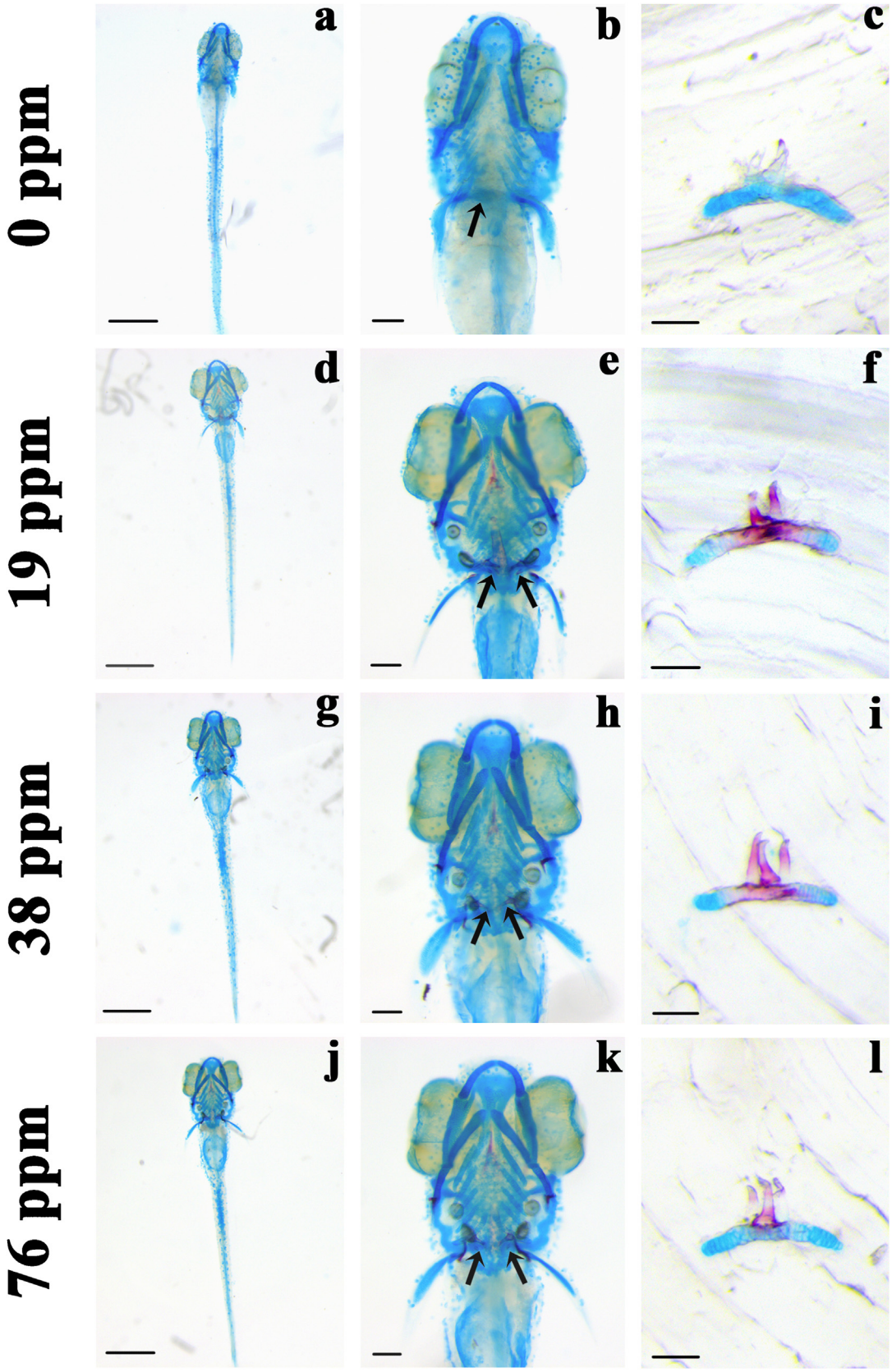

Fig. 1. Alcian blue and alizarin red staining results of cranial bones and teeth of 5dpf zebrafish (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Zebrafish eggs were treated with 0 ppm, 19 ppm, 38 ppm, 76 ppm fluoride for 5 days, then they were collected to perform whole mount alcian blue and alizarin red staining. n = 10 in each group. The intensity and area of red staining were increased in 19 ppm group and 38 ppm group, respectively. a,d,g,j: Whole ventral view. Scale bars = 500 μm. b, e,h,k: Magnification images of head in a,d,g,j. Arrows: pointing to the teeth in cb5. Scale bars = 100 μm. c,f,i,l: Dissected cb5 and teeth. Scale bars = 50 μm. dpf: days post fertilization, cb5: the fifth ceratobranchial arches.