Comparative Toxicity Study of EverX Posterior and SDR Flow+ in Zebrafish Embryo Model

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Abstract: Objective: This study aimed to assess the potentially toxic effects of EverX Posterior and SDR Flow+ dental materials on zebrafish embryos, using various endpoints such as mortality rate, hatching rate, heart rate, and morphological malformations.

Materials and Methods: Zebrafish embryos were exposed to EverX Posterior and SDR Flow+ dental materials from 4 hours post-fertilization (hpf) to 96 hpf. Parameters including survival, hatching rate, heart rate, and morphological malformations were evaluated. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test.

Results: EverX Posterior did not cause mortality in zebrafish embryos, while the mortality rate was higher in the SDR Flow+ group. The hatching rate was similar between the EverX Posterior and control groups, but significantly decreased in the SDR Flow+ group. The heart rate of zebrafish embryos exposed to EverX Posterior was not significantly different from the control group, whereas SDR Flow+ resulted in a significant decrease in heart rate. Morphological analysis revealed normal architecture in the EverX Posterior group, while SDR Flow+ treated embryos exhibited malformations and higher mortality.

Conclusion: The study findings suggest that SDR Flow+ had a slightly toxic effect on zebrafish embryos compared to EverX Posterior. Further investigations are warranted to better understand the underlying mechanisms and refine dental materials for enhanced safety and biocompatibility.

Keywords: Zebrafish embryos, composite materials, toxicity testing, EverX Posterior, SDR Flow+, mortality rate, hatching rate, heart rate, morphological malformation

1. Introduction

Zebrafish toxicity testing is a common approach used to assess the potential toxic effects of various substances, including dental materials, on zebrafish embryos and larvae [1–3]. Zebrafish toxicity testing provides valuable insights into the safety and potential risks associated with dental materials before their use in humans [4–6]. In the context of dental materials, zebrafish toxicity testing involves exposing zebrafish embryos or larvae to extracts or direct contact with dental materials or their components [7–9]. The embryos or larvae are typically exposed to different concentrations of the dental material or its leachates, and various endpoints are evaluated to assess toxicity [10–12].

Endpoints commonly evaluated in zebrafish toxicity testing include mortality rate, developmental abnormalities, organ-specific toxicity, and behavioural changes [13–15]. Morphological analysis, histology, gene expression analysis, and behavioural assays are among the methods used to evaluate these endpoints [16–18]. Zebrafish toxicity testing in dental materials allows for the identification of potential adverse effects, such as developmental defects, tissue damage, or neurotoxicity [19–21]. It helps in understanding the underlying mechanisms of toxicity and can guide the refinement of dental materials to enhance their safety and biocompatibility [22–24]. By using zebrafish as a model organism, researchers can efficiently screen multiple dental materials or formulations, assess their toxicity profiles, and prioritize further testing or development [25–27]. Zebrafish toxicity testing provides a valuable tool in early-stage toxicity assessment and contributes to the development of safer dental materials with minimal risks to human health [28–30].

Zebrafish share similarities with humans in terms of tooth development and regeneration. Researchers have used zebrafish to study the genetic and molecular mechanisms involved in tooth development, regeneration, and repair [31–33]. These studies contribute to a better understanding of dental abnormalities and potential strategies.
for tooth regeneration in humans. However, zebrafish have been extensively utilized as a model organism in dental research to investigate various aspects of oral health and disease [34–36]. The EverX Posterior is a restorative composite resin that utilizes a resin matrix, silanated e-glass fibers, and inorganic particulate fillers. It is specifically designed as a short fiber-reinforced composite to enhance its mechanical properties. To address the challenges associated with adaptation, flowable restorative composites have been developed as an alternative to high-viscosity resin composites. SDR technology, a patented urethane dimethacrylate structure, has been introduced to reduce polymerization shrinkage and associated stress, claiming minimal overall shrinkage (3.5%) [44–46]. In this study, the EverX Posterior and SDR Flow+ toxic effects were investigated in the zebrafish embryos.

2. Materials and Methods

II a. Origin and Maintenance of Zebrafish

Adult zebrafish (Wild type – AB strain, 4 months old) were purchased from a local aquarist (NSK aquarium, Kolathur, Tamil Nadu, India). The male and female fishes were separated and maintained in our facility under the following condition in a 10 L glass tank: 28.5°C, with a 14/10 h light/dark cycle. The fish were fed three times a day, with live brine shrimp (Artemia salina). The fishes were acclimatized for 1 month, later the fishes were utilized for breeding, and embryos were collected and used for the following experiments. The collected embryos are further analyzed under a microscope, unfertilized embryos are discarded, whereas the fertilized embryos are taken in a six-well plate and incubated in an E3 medium [37–39]. II b Fabrication of Samples

Composition of Materials Tested in Study

EverX posterior (everX Posterior GC EUROPE) - Resin matrix, randomly oriented E-glass fibers, and inorganic particulate fillers. The resin matrix contains bisphenol-A-diglycidyl-dimethacrylate (bis-GMA), triethylene glycol dimethacrylate, and polymethylmethacrylate, forming a matrix called semi-interpenetrating polymer network (semi-IPN).

SDR flow+ (SDR; Dentsply-Italy) - The resin matrix contains proprietary modified urethane dimethacrylate resin; TEGDMA; polymerizable dimethacrylate resin; polymerizable trimethacrylate resin; camphorquinone (CQ) photoinitiator; ethyl-4(dimethylamino)benzoate photo accelerator; butylated hydroxytoluene (BHT); fluorescent agent, and UV stabilizer. The filler contains silanated barium-alumino-fluoro-borosilicate glass; silanated strontium alumino-fluoro-silicate glass; surface-treated fumed silicas; ytterbium fluoride; synthetic inorganic iron oxide pigments, titanium dioxide.

A metal mould (2 mm in thickness and 6 mm in diameter) was used to produce ten samples of EverX posterior and SDR Flow+ Composites, for a total of 20 composite disk samples. On the top and bottom of the moulds, a mylar strip was placed, and the cavity was fully filled with composite resin. A thin glass plate was placed over the composite, and using a variable intensity light curing unit (Bluephase NM), the samples were light-cured for 60 seconds. All samples were then finished and polished using Shofu Super Snap Rainbow Technique Kit Ca using a low-speed handpiece (11,000 rpm).

II c Zebrafish embryo toxicity test

For the developmental toxicity assessment studies, 4 hpf embryos were used, and the exposure was carried in a 6-well plate containing untreated larvae as the control, EverX Posterior (EXP) and SDR Flow+ (SDR). Around 15 embryos/well were used with 3 mL of E3 medium. The exposure was non-static and renewed every 24 h with the fresh treatment solution throughout the exposure period (4 hpf to 96 hpf). All the experiments were carried out in triplicates. Parameters such as survival, heart, malformation, and hatching rate were observed during this period, and calculations were presented at the end of 96 hpf [40–43].

II d. Statistical analysis

The data were presented as the mean of triplicates with a standard deviation. GraphPad Prism software (Ver 5.03, CA, USA) was used for statistical analysis. One-way ANOVA was performed and Tukey’s post-hoc test was used to find the level of significance between the control and other groups.

3. Result and Discussion

Zebrafish Embryo toxicity test

III a. Mortality and hatching rate

The EverX Posterior did not cause mortality in zebrafish embryos. As shown in Fig. 1, the mortality rate was found to be more than 10% in and SDR Flow+ group. Additionally, the hatching rate was calculated at 48 hpf. It
was observed that 100% of the zebrafish embryo emerged out of their chorions in the control group. However, a similar hatching rate was observed EverX Posterior group and a significant decrease in the hatching rate was observed in the SDR Flow+ group.

III b. Measurement of heart rate
The heart rate of zebrafish embryos was evaluated at 72 hpf, to assess the toxicity of the treatment group. The atrial and ventricular contractions were counted and recorded under a microscope for 1 min and average heart beats per minute were reported. The result shows that the EXP group did not significantly alter the heartbeat rate of the zebrafish embryos when compared to the embryos from the control (untreated) group, while SDR showed a significant decrease in heart rate (Fig. 2).

III c. Morphological malformation
The EXP-treated zebrafish embryos exhibited normal morphological architecture under the microscope (Fig. 3). No malformations such as yolk sac oedema and bent spine were formed in the larvae. Embryos were observed with normal morphology without any deformities when treated with EXP and SDR was observed with dead embryos.

4. Conclusion
The results show that SDR showed a slightly toxic effect on the embryos compared to the EXP when exposed to the zebrafish embryos.

5. Acknowledgement
Nil

References

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Figure 1.

Fig. 1. The Mortality and Hatching rate of zebrafish embryos was investigated after exposure to different groups of EXP and SDR for 24hrs. The * (p < 0.05) indicates the significant differences between the control (untreated embryos) and treated group.
Figure 2. The heart rate of zebrafish embryos was investigated after exposure to different groups of EXP and SDR for 24hrs. The * ($p < 0.05$) indicates the significant differences between the control (untreated embryos) and treated group.

Figure 3. Microscopic image of embryos after exposure to EXP and SDR. The control was untreated embryos.

Fig. 2. The heart rate of zebrafish embryos was investigated after exposure to different groups of EXP and SDR for 24hrs. The * ($p < 0.05$) indicates the significant differences between the control (untreated embryos) and treated group.

Fig. 3. Microscopic image of embryos after exposure to EXP and SDR. The control was untreated embryos.