Ref: Ro J Stomatol. 2024;70(2)
DOI: 10.37897/RJS.2024.2.13

Cytotoxicity comparison between fluoride varnish and SDF in pulpal stem cells using MTT assay: an *in vitro* study

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ABSTRACT

Background and objectives. SDF and fluoride varnish have garnered attention for their ease of application and potential to address ECC, particularly in young children with limited cooperative abilities for traditional dental procedures. But the cytotoxicity of these materials are not yet studied extensively.

Materials and methods. Extracted human primary molars of comparable size and with no visible cavitated carious lesions were collected. The teeth were stored for less than 30 days in 0.1% thymol solution at 23°C prior to the experiment. Each tooth was coated with fluoride varnish and SDF respectively. MTT assay was performed and cytotoxicity is evaluated.

Results. The materials used in this study - SDF and fluoride varnish showed significantly lower cell viability compared to the control group. There was significantly higher cell viability in SDF samples compared to fluoride varnish.

Conclusions. Within the limitations of the study, it is concluded that the cell viability of SDF is higher when compared with the fluoride varnish.

Keywords: Cytotoxicity, fluoride varnish, SDF, MTT assay

Abbreviations

SDF – Silver Diamine Fluoride
MTT assay – 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

INTRODUCTION

Early Childhood Caries (ECC), often referred to as nursing caries, is a highly prevalent and concerning oral health issue affecting infants and young children worldwide. ECC is characterized by the rapid and severe decay of primary teeth in children aged six years and younger [1]. This condition poses significant challenges to the oral health and overall well-being of affected children, making it a topic of paramount importance in the field of dentistry [2].

ECC can lead to substantial pain, discomfort, and impaired function, negatively impacting a child's ability to eat, sleep, and communicate effectively [3]. If left untreated, it may result in tooth loss, misalignment of permanent teeth, and, in severe cases, can even affect a child's nutritional status and quality of life [4]. The etiology of ECC is multifactorial, involving dietary habits, oral hygiene practices, bacterial colonization, and caregiver behaviors [5].

While ECC prevention and management strategies have focused on promoting proper oral hygiene, dietary guidelines, and regular dental check-ups, there is a growing interest in the use of dental materials to mitigate the risk and progression of ECC. Sil-

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Article History:
Received: 7 March 2024
Accepted: 7 May 2024

ver diamine fluoride (SDF) and fluoride varnishes have emerged as promising candidates in this context due to their demonstrated efficacy in caries prevention and management [6-8].

SDF, a silver-based solution containing fluoride, has shown remarkable potential in arresting dental caries by promoting remineralization and inhibiting further demineralization [9]. SDF is a non-invasive treatment and is especially beneficial for young children or individuals who may have dental anxiety. SDF can halt the progression of tooth decay. It is particularly useful in managing cavities in their early stages, preventing them from causing further damage. SDF has minimal side effects. Some patients may experience temporary staining of the treated tooth, but this is usually cosmetic and not harmful.

Fluoride varnishes provide a localized fluoride reservoir at the tooth surface, bolstering enamel remineralization and demineralization inhibition [6]. Fluoride varnish is highly effective at preventing cavities (dental caries). It helps remineralize weakened enamel and makes teeth more resistant to acid attacks from bacteria. Fluoride varnish is safe for both children and adults. It can be applied to deciduous teeth and permanent teeth to protect against dental caries.

Both of these materials have garnered attention for their ease of application and potential to address ECC, particularly in young children with limited cooperative abilities for traditional dental procedures. But the cytotoxicity of these materials are not yet studied extensively. The aim of the study is to compare the cytotoxicity of fluoride varnish and SDF in pulpal stem cells using MTT assay.

MATERIALS AND METHODS

Extracted human primary molars of comparable size and with no visible cavitated carious lesions were collected from the Department of Paediatric and preventive dentistry from a private institution located in Chennai. The study was approved from the institutional scientific and research committee (IRB number - SRB/SDC/PEDO-2205/22/085). The teeth were stored for less than 30 days in 0.1% thymol solution at 23 °C prior to the experiment. Each tooth was coated with fluoride varnish and SDF respectively.

For evaluating the cytotoxicity, a cell culture medium consisting of penicillin-streptomycin solution, trypsin, EDTA, fetal bovine serum (FBS) and heat-inactivated horse serum (HS) was used. Pulpal stem cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 µg/ml). They were then kept at 37°C in an atmosphere containing 5% CO2. After two passages, the cells were plated at the density of 5000 per well in a 96-well microplate for the methyl-thiazole-tetrazolium (MTT) assay. The wells were grouped into three different groups - fluoride varnish (VOCO Profluorid varnish), SDF (Kids e) and control (cell culture).

Cell viability assay and optical density (OD) of the groups were evaluated as follows: cellular viability was assessed by the reduction of yellow tetrazolium MTT [2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to Formosan which is purple in colour. The MTT solution was reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular Formosan could be solubilized and quantified by spectrophotometric means. MTT was dissolved in phosphate-buffered saline (PBS) and added to the culture at final concentration of 0.5 mg/ml. After incubation for 2 h at 37°C, the media were carefully removed and 100 µL DMSO was added to each well, and the OD values were determined by spectrophotometry at 490 nm with a microplate reader. Results were expressed as percentages of control.

RESULTS

The materials used in this study - SDF and fluoride varnish showed

significantly lower cell viability compared to the control group. (Table 1) and (Figure 1) There was significantly higher cell viability in SDF samples compared to fluoride varnish.

TABLE 1. Cell viability (%) of the tested materials

	Average	Group average	Standard deviation	% Cell viability
SDF	0.168167	0.171972	0.020444	78.15647
Fluoride varnish	0.132583	0.173875	0.013007	61.6189
Control	0.215167	0.215167	0.012532	100

DISCUSSION

In our recent research, we delved into the biological effects of a commercially available Silver Diamine Fluoride (SDF) and fluoride varnish when tested in vitro. Specifically, we conducted a comparative assessment of their impact on the cell viability of pulpal stem cells. Our research results decisively reject the null hypothesis, signifying that both the application of SDF and fluoride varnish does indeed have an effect on pulpal stem cells.

It is worth noting that the cytotoxicity of commercially available SDF and fluoride varnishes has not received substantial attention in the existing literature. There is a noticeable dearth of research

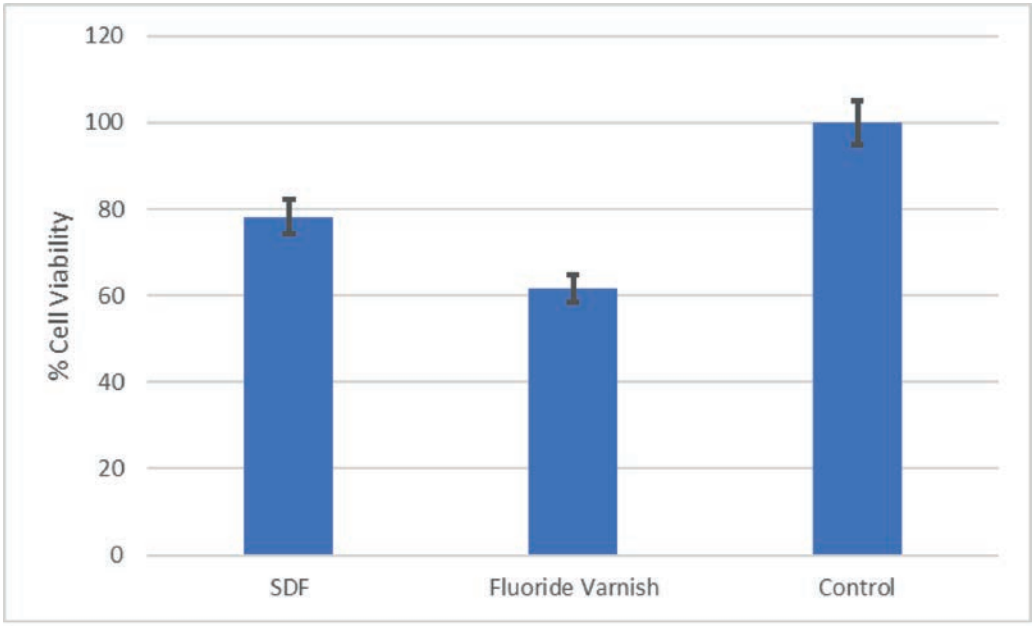

TABLE 1. Cell viability between SDF, fluoride varnish and control groups

that meticulously scrutinizes the potential harm posed by the ingredients contained in SDF and fluoride varnishes to oral tissues.

Our findings underscore the necessity for a deeper comprehension of the biological repercussions of SDF and fluoride varnishes on pulpal stem cells. This underscores the urgency for further exploration in this domain. It is crucial to persist in investigating the cytotoxicity and possible adverse impacts of SDF and fluoride varnish to ensure their safety and optimal utilization in dental care, particularly with regard to pulpal stem cells.

Biocompatibility stands as a critical prerequisite for dental products, guaranteeing their safety and compatibility with oral tissues [10]. The International Organization for Standardization (ISO) has established stringent standards to guide in vitro biocompatibility assessments for dental materials [11]. Notably, ISO 7405 and ISO 10993-5 outline the comprehensive protocols for evaluating the biocompatibility of dental products.

While numerous studies have assessed the biocompatibility of various dental materials, it's important to acknowledge that each material may exhibit distinct characteristics and interactions with oral tissues. Consequently, comprehensive evaluations, conducted in accordance with ISO standards, are imperative to ensure that dental materials conform to the requisite biocompatibility criteria. Adhering to ISO standards and conducting rigorous biocompatibility investigations enables dental manufacturers and researchers to evaluate the potential risks and benefits associated with dental materials. This information is vital for making informed decisions concerning the safety and suitability of dental products for clinical use [12].

Presently, there exist only two studies that analyze the cytocompatibility of SDF: Kim et al [13] assessed the cytotoxicity of these products using rat pulpal cells, while Fancher et al [14] employed human gingival fibroblasts. A crucial consideration when selecting resources for cytotoxicity testing is the cells and tissues most likely to be affected by the tested agent. Prior research has indicated that human cell lines tend to be more sensitive to such tests compared to animal cell lines like L929, emphasizing the importance of avoiding SDF contact with pulpal stem cells [15]. Nonetheless, there remains a potential risk of accidental soft tissue contact, making this information invaluable.

Kim et al.'s findings revealed that all tested SDF dilutions exhibited notable cytotoxicity, but the introduction of reduced glutathione offered protection, mitigating cytotoxicity. This suggests that the composition of SDF can influence cytotoxicity [13]. Fancher et al., on the other hand, assessed the cytotoxicity of other SDFs, such as Advantage Arrest, and concluded that this particular product was cytotoxic to gingival fibroblasts at a concentration of 0.01% [14].

According to research by Pagano and colleagues, during varnish application, residual monomers are released into oral tissues, posing potential toxicity risks [16]. Several studies have reported that ingredients like ethyl acetate and cetylpyridinium chloride found in products such as Cervitec F and Fluor Protector S exhibit cytotoxic effects on cells and contribute to the development of bacterial resistance [17]. These findings have raised concerns about the safety and efficacy of these ingredients. Therefore, a growing consensus among researchers advocates for the consideration of alternative ingredients or

formulations to replace ethyl acetate and cetylpyridinium chloride in dental products. This transition towards safer and more effective alternatives is pivotal to safeguard patient well-being and mitigate potential risks linked to cytotoxicity and bacterial resistance.

It is essential to acknowledge that the application of fluoride compounds can influence the healing process, either by expediting or retarding it [18]. This encompasses the investigation of cellular migration using this technique by previous authors, specifically examining the migration of various oral cells, such as human mesenchymal stem cells from the apical papilla, human periodontal ligament stem cells, and human pulp fibroblasts.

Numerous studies have concluded that both SDF and fluoride varnish exhibit cytotoxic effects in the oral cavity. However, to date, no studies have directly compared the cytotoxicity of SDF and fluoride varnish in the oral cavity. Our study addresses this gap by comparing the cytotoxic effects of both SDF and fluoride varnish on pulpal stem cells. Remarkably, our findings indicate that SDF exhibits higher cell viability in comparison to fluoride varnish.

One limitation of our current study is the absence of trials designed to evaluate the ions produced by different desensitizers and their potential impact on biocompatibility. This lack of investigation into the specific compositions of the generated ions could be considered a shortcoming of our research. In fact, earlier studies have reported that low sodium fluoride levels expedite wound healing by promoting cell migration and proliferation.

CONCLUSION

Within the limitations of the study, it is concluded that the cell viability of SDF is higher when compared with the fluoride varnish.

Conflict of interest: none declared

Financial support: none declared

Author's contribution:
Conceptualization – Manisha Bala Rathy and Mahesh Ramakrishnan
Methodology - Manisha Bala Rathy
Validation - Mahesh Ramakrishnan
Formal analysis - Manisha Bala Rathy
Investigation - Manisha Bala Rathy
Resources - Manisha Bala Rathy
Data curation - Manisha Bala Rathy
Writing - original draft preparation - Manisha Bala Rathy
Writing - review and editing - Mahesh Ramakrishnan
Visualization - Manisha Bala Rathy
Supervision - Mahesh Ramakrishnan
Project administration - Mahesh Ramakrishnan
All authors have read and agreed to the published version of the manuscript.

Acknowledgements:
All authors have read and agreed to the published version of the manuscript

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