EFFECT OF SILVER DIAMINE FLUORIDE SOLUTION ON THE STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Yasmine Monjazebi
Bachelor of Dental Surgery (BDS), University of Sharjah, 2015

Submitted to the Hamdan Bin Mohammed College of Dental Medicine
Mohammed Bin Rashid University of Medicine and Health Sciences
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Endodontics
2022

ABSTRACT

Effect of Silver Diamine Fluoride (SDF) Solution on the Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs)

Yasmine Monjazebi

Supervisors: Dr. Mohamed Jamal, Dr. Iyad Hussein, Dr. Maanas Shah, Dr. Mennatullah Khalil, Dr. Asma Bashir

Background: SDF is a solution containing silver, fluoride, and ammonia. Clinically, it is used to arrest the progression of carious lesions in primary teeth. Recent studies have shown that SDF has indirect effect on pulp tissues by triggering reparative/reactionary dentine formation, similar to pulp capping agents. Such effect could be attributed to mobilization of growth factors from dentine that was subjected to SDF. However, this assumed mechanism of action has not been investigated. Moreover, the effect of SDF on a cellular level has not been studied extensively yet.

Aim: This *in-vitro* study aimed to investigate the effect of SDF on SHEDs at the cellular level, in addition to the ability of SDF to mobilize growth factors from dentine.

Materials and Methods: SDF was diluted into concentrations of 3.8%, 0.38%, 0.038% and 0.0038% by (mesenchymal stem cell) MSC media. SHEDs were seeded and grown over 7 days with MSC media and MSC media supplemented with 38% SDF, along with the diluted concentrations. Cell cytotoxicity and proliferation of SDF were performed in triplicates using CyQuant assay. The CyQuant assay was validated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on SDF concentrations that showed the least

cytotoxicity. Cell differentiation assay was used to investigate the differentiation ability of SHEDs exposed to non-cytotoxic SDF concentration.

The ability of SHEDs to release transforming growth factor beta 1 (TGF β-1) from dentine was also investigated using enzyme-linked immunoassay (ELISA). Groups tested were 38% SDF, 10% Citric acid and phosphate-buffered saline (PBS) that were incubated on dentine discs for 3 days at 37°C.

Results: CyQuant assay revealed that 38%, 3.8% and 0.38% SDF were cytotoxic. Highest cell proliferation rate was detected with 0.0038% SDF. MTT assay confirmed that 0.38% SDF was cytotoxic, while 0.0038% SDF showed no cytotoxicity. Osteogenic differentiation assay revealed no inhibition of differentiation in SHEDs treated with 0.0038% SDF. Highest TGF β-1 release was detected in 10% citric acid, followed by 38% SDF and PBS.

Conclusion: Cell viability and proliferation assay revealed that clinical concentration of SDF (38%) was cytotoxic on SHEDs. 0.0038% SDF promoted cell proliferation and osteogenic differentiation. ELISA experiment showed that dentine exposed to 38% SDF released TGF β-1, indicating that SDF could promote reactionary dentinogenesis.

DEDICATION

I dedicate my thesis work to my institution mentors under whose constant guidance I was able to complete. They always gave me valuable advice and enlightened me with academic knowledge.

DECLARATION

I declare that all the content of this thesis is my own work. There is no conflict of interest with any other entity or organization

Name: Yasmine Monjazebi

Signature:

X _____

Yasmine Monjazebi

ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Mohamed Jamal for his continuous care, for his motivation, immense knowledge, and enthusiasm. His assistance and advice helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank the rest of my thesis committee: Dr. Iyad Hussein, Dr. Maanas Shah, Dr. Mennatullah Khalil and Dr. Asma Bashir for their assistance into the project, their encouragement, and insightful comments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Silver Diamine Fluoride (SDF) is a clear, odorless solution containing ionic silver, fluoride, and ammonia that arrests the progress of carious lesions and prevents the development of future caries. According to *Jong Seto et al.* (2017), it stops 81% of cavitated carious lesions. SDF-treated lesions harden and become resistant to further decay[1]. SDF was first recorded as being used in Japan in 1969 but has recently had a renaissance[2].

Its antimicrobial and remineralization properties have been attributed to its silver and fluoride components[3]. The silver provides the antimicrobial properties of SDF by hindering the growth of cariogenic biofilm of *Streptococcus Mutans* or *Actinomyces Naeslundii* formed on dentine surfaces, thus preventing caries progression and dentine demineralization[4, 5]. Silver ions affect the bacteria's proteins and deoxyribonucleic acid (DNA). They induce mitochondrial failure, inhibit DNA synthesis and bacterial cell wall synthesis. Due to silver ions' bactericidal properties, they cause disruption to the dental plaque biofilm. Dentinal surfaces that are demineralized due to SDF application have significantly reduced cariogenic species growth compared with those that are not treated with SDF[6, 7]. The fluoride contributes to its remineralization properties by converting the demineralized hydroxyapatite crystals into fluorapatite that is harder and more caries resistant. The ammonia acts as a stabilizing agent for the solution. Therefore, SDF may not only achieve its antimicrobial functions by biochemical interactions, but also through its inherent ability to integrate into dentine[1].

The application of SDF is easily adapted for field use. The lesion is isolated, and the solution is painted onto the clean caries lesion and dried. SDF is contraindicated for individuals with a known silver allergy, ulcerative gingivitis, or stomatitis[3, 4].

1.1 Mechanisms of Silver Diamine Fluoride on Arresting Caries

SDF is bactericidal to cariogenic bacteria, mainly *Streptococcus Mutans*, and inhibits the growth of cariogenic biofilms on teeth. According to *Irene Zhao et al.* (2018), dentine surfaces

treated with SDF had significantly less growth of *Streptococcus Mutans* than did those without SDF treatment[4]. Thus, SDF contains a high concentration of fluoride ions (44,800 ppm). A series of chemical reactions take place when placed on carious tooth tissue. This process will promote tooth desensitization by dentinal tubule blockage, bacterial death and remineralization of demineralized enamel or dentine. After SDF treatment, a mineralized surface highly rich in calcium and phosphate is formed on arrested carious lesions, and the mineral loss of demineralized enamel and dentine is reduced. The demineralized tooth surface becomes black and its depth decreases after application of SDF[4].

SDF inhibits collagenases (matrix metalloproteinases and cysteine cathepsins) that play an important role in the enzymatic degradation of collagen and protects dentine collagen from destruction[8, 9]. SDF inhibits the activities of cysteine cathepsins, which are proteolytic enzymes that contribute to dentine collagen degradation[4].

The American Academy of Pediatric Dentistry formed a workgroup and developed guidance and an evidence-based recommendation regarding the application of 38% SDF, that is the most common concentration, to arrest cavitated caries lesions in primary teeth[10]. SDF application does not replace restorations of decayed teeth with dental fillings or crowns. It is rather an effective interim therapy that is used as part of a comprehensive treatment plan, conducted by a dentist within a dental home[1, 11]. Recently, SDF has gained popularity due to its painless, easy, quick and non-invasive way to treat cavities. Studies have found that it is not necessary to remove caries before application of SDF. It provides a more conservative approach as opposed to the traditional dentistry, which relies on the 'drill and fill' technique[9, 12, 13].

2. REVIEW OF THE LITERATURE

Interestingly, recent studies have shown that SDF might have an indirect effect on the dental pulp tissues by triggering reparative dentin/reactionary dentin formation, similar to pulp capping agents such as Calcium hydroxide ($Ca(OH)_2$) and mineral trioxide aggregate (MTA). *Enrique Bimstein* and *Douglas Damm* in 2018 performed a study assessing human primary tooth histology six months after treatment with SDF. The study concluded that the use of SDF on vital carious primary teeth leads to formation of tertiary dentine, which may lead to pulp healing of reversible pulpitis[11].

In a similar study, *Vanegas S. et al.* in 2014 tested the effect of SDF on caries induced in Westar rats. Histological report showed increased predentine thickness in molars from the experimental groups, while in control group the predentine thickness remained unchanged[12].

Korwar et al. in 2015 examined the pulpal response to SDF when used as an indirect pulp capping agent. Histopathological examination revealed a definite incremental line of Owen, which indicated disturbance in cellular function of odontoblasts. Tertiary dentine was evident and revealed a reparative response[14].

2.1 Cytotoxic Effects of SDF:

According to recent research, SDF revealed cytotoxic effects. *Fancher et al.* (2017) investigated the effect of SDF on human gingival fibroblasts cell viability. The study concluded that at 0.01%, SDF was almost 100% cytotoxic to human gingival fibroblasts. SDF treated hydroxyapatite discs was cytotoxic to fibroblasts in vitro 9 weeks after it was applied[15]. Another *in-vitro* study was done by *Garcia-Bernal et al.* (2022) to determine the cytotoxicity of two new commercial SDF products: Riva Star (SDI Dental Limited) and e-SDF (Kids-e-Dental) on mesenchymal stromal cells from human exfoliated deciduous teeth (SHEDs)[16]. SHEDs were exposed to SDF products at different concentrations (0.1%, 0.01% and 0.005%). He reported that Riva Star SDF has better in vitro cytocompatibility on SHEDs than does e-SDF and Riva

Star SDF is more suitable when used in deciduous teeth due to its lower cytotoxicity compared to e-SDF[16].

2.2 Odontoblasts and the Role of Growth Factors:

Odontoblasts are post-mitotic cells, responsible for primary and physiological secondary dentinogenesis. Thus, they do not undergo regular turnover like many cells from other tissues. Growth factors and bioactive molecules are secreted by odontoblasts during primary dentinogenesis, and are integrated within the dentine extracellular matrix[17]. Growth factors are key signaling molecules. They are responsible for controlling and regulating cellular events involved in tissue development, homeostasis and repair[18]. These molecules are bound to protein precursors or binding proteins and sequestered in a protected state within dentine[19]. During tooth development, Odontoblast terminal differentiation occurs in the later bell stage, under the control of the inner dental epithelium and mediated by the dental basement membrane. In caries, the microbial invasion of dentine causes an adaptive response by odontoblasts, in which it culminates in formation of a structurally altered reactionary dentine[20].

It has been established that during dentinogenesis, the odontoblast secret and deposit different growth factors into the dentine matrix. Upon microbial invasion and dentine matrix demineralization, these growth factors are released and trigger the odontoblast to secret new layer of tertiary dentine in the process called reactionary dentinogenesis. These growth factors can also trigger odontoblast-like cell differentiation of perivascular stem cells niche within the dental pulp tissues and formation of new layers of dentine in a process called reparative dentinogenesis[20, 21]. Key growth factors that are present in dentine matrix include:

Table 1. Key growth factors within human dentine matrix[21-25]	
Transforming growth factors beta 1, 2, 3	TGF-β1, TGF-β2, TGF-β3
Bone morphogenetic proteins 2, 4, 7	BMP-2, BMP-4, BMP-7
Insulin-like growth factor binding protein 1	IGFBP-1
Hepatocyte growth factor	HGF
Stem cell factor	SCF
Macrophage colony stimulating factor	M-CSF
Granulocyte macrophage colony stimulating factor	GM-CSF
Nerve growth factor	NGF
Vascular endothelial growth factor	VEGF
Adrenomedullin	ADM
Fibroblast growth factor 2	FGF-2
Platelet-derived growth factor	PDGF
Epidermal growth factor	EGF
Placental growth factor	PGF
Brain-derived neurotrophic growth factor	BDNF
Glial cell line-derived neurotrophic factor	GDNF
Growth/differentiation factor 15	GDF-15

It has been found that chelating agents [such as Ethylenediamine Tetraacetic Acid (EDTA)] and pulp capping agents [such as $Ca(OH)_2$ and MTA] have similar mechanism of action, in which they can mobilize growth factors from the dentine to trigger reactionary dentinogenesis. The binding of growth factors to the dentine matrix provides a robust mechanism that causes protection of these bioactive molecules. It provides an elegant system for signaling regeneration when they are released by bacterial acids during carious attack, or by placement of the pulp capping agents[26-28].

Tomson et al. (2017) in a study done to correlate the effect of pulp capping agents on the release of growth factors from dentine matrix observed a broad range of growth factors released including SCF, M-CSF, GM-CSF, IGFBP-1, NGF and GDNF[29]. A study done by *Goncalves et al.* (2016) concluded that the use of traditional dental materials, like EDTA, facilitate the local release of growth factors from dentine. Findings in the study revealed that EDTA was significantly an effective extractant of TGF-b1, which is most likely attributed to its powerful demineralizing and solubilizing action on hard dental tissues[30].

Another study done by *Alp Abidin et al.* (2020) investigated different dentine conditioning agents as an alternative to EDTA in terms of their ability to extract growth factors from dentine using the enzyme-linked immunosorbent assay (ELISA). Results revealed that alternative dentine conditioning agents extracted higher concentrations of growth factors from dentine compared with EDTA. The highest transforming growth factor beta 1 (TGF β-1) release was observed after 10% citric acid treatment followed by 37% phosphoric acid, and compared with 17% EDTA and 1% phytic acid, there were significant differences observed[31].

As a conclusion, minimal intervention dentistry as a concept within oral healthcare was previously considered a peripheral and unconventional topic but has now moved to center-age of oral healthcare. It represents a patient-centered approach to care, is evidence-based and maintains the development of novel treatment options. One of these is SDF. The use of SDF has recently gained interest. It has changed access to care for the most prevalent human disease. This is especially critical for treatment in young children who are high caries-risk, in which achieving traditional operative dentistry (fillings) is rather difficult. the action of SDF on arresting dentinal caries has been proved by many researchers.

Recent in vitro studies done on the aspect of cellular effects showed increased dentine thickness in the areas where SDF was applied. As mentioned above, animal and histological studies have shown that applying SDF can trigger reactionary dentinogenesis, similar to pulp capping

agents. But the mechanism behind this process have not been explained yet. It is unknown if the SDF have a similar effect on releasing growth factors from dentine matrix as $Ca(OH)_2$ or MTA. Moreover, the direct effect of SDF of dental tissues derived stem cells have not been investigated before.

Stem cells isolated from dental pulp tissues in human exfoliated deciduous teeth (SHEDs) are a type of mesenchymal stem cells, which are highly proliferative pluripotent cells and widely investigated for regenerative treatment. These stem cells have similar characteristics as mesenchymal stem cells with the major difference being that dental pulp stem cells can differentiate into dentine forming odontoblast-like cells[32]. They are very much used as an in vitro research model as well to study diverse kinds of materials, mark different kinds of growth factors on the function of SHEDs and observe its differentiation capacity[33-37].

3. AIM

The aim of this *in-vitro* study is to investigate the effect of SDF on the Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs), and its ability to release growth factors from human primary dentine.

In order to achieve the aim of the study, the following objectives were formulated:

a) Investigate the direct cytotoxic effect of SDF application on SHEDs.
b) Assess the direct effect of SDF application on the differentiation and proliferation capacity of SHEDs.
c) Study the effect of SDF on releasing growth factors from dentine discs of human primary teeth.

4. MATERIALS AND METHODS

4.1 Source of SHEDs and Human Primary Teeth Dentine:

SHEDs and dentine discs were harvested from intact de-identified human primary teeth that were already indicated for extraction as part of a treatment plan. These teeth were collected from healthy patients treated at Dubai Dental Hospital (DDH) that satisfy the below inclusion criteria. These were collected after getting the required MBRU's institutional review board (IRB) approval.

Inclusion criteria:

1) Healthy patients (ASA I)
2) Having primary teeth that are already indicated for extraction as part of a treatment plan
3) The teeth were intact, caries free and have healthy periodontium
4) Patient age: 5-10 years old

SHEDs and dentine discs were harvested from 3 different individuals, to generate biological triplicate for each experiment and to overcome any possible effect of individual genetic background on the results. Furthermore, each experiment was repeated 3 independent times to rule out any experimental bias, random errors and exclude any possible outliers. These teeth were extracted as part of pre-determined treatment plan and for reasons not related to the purpose of this project. Once teeth were extracted, they were kept in a collection media [alpha-MEM (minimum essential medium), supplemented with 1% penicillin and streptomycin] at 4˚C, until they are processed either for SHEDs isolation or dentine discs preparation[38, 39].

4.2 SHEDs Culture and Maintenance:

Each freshly extracted primary tooth was immersed in a tube containing collection medium made of alpha-MEM, supplemented with 1% penicillin and streptomycin (Gibco). The tooth was transferred to the cell culture laboratory at MBRU research facility. Within few hours,

different types of SHEDs were isolated and cultured as described previously[39-43]. Briefly, the dental pulp tissue was micro dissected, minced and incubated in buffer containing alpha-MEM, 3mg/ml collagenase and 4mg/ml dispase (Roche Molecular Biochemicals) for 30 min at 37˚C. To obtain a dispersed cell fraction rich in SHEDs, the solution containing the isolated cells and tissues was filtered through 70um cell strainer (Falcon). Isolated cells were then plated in culture dish containing MSC medium containing alpha-MEM, 10% FBS (Gibco), 1 mM l-alanyl-l-glutamine and 0.1 mM b-mercaptoethanol (Invitrogen), and then incubated at 37˚C in 5% CO2. Culture media was changed every other day. Isolated cells were passaged every 4-5 days using TrypLE™ (LifeTechnologies) and replated in 1:4 (vol/vol) ratio. For all experiments, SHEDs at passages 3 to 5 from 3 different individuals were used. All experiments were done in triplicates.

4.3 Investigating the Effect of SDF on SHEDs:

4.3.1. Cell Viability and Cytotoxicity Assays:

To test the cytotoxicity of the different concentration of SDF on SHEDs, CyQuant assay was used based on standard published protocols[44, 45]. Briefly, SHEDs were seeded in 96 well plate (~10^3cells/well) and allowed to grow overnight. The ready-made clinical SDF solution present in a concentration of 38% (23.6 mM) and it was serially diluted into 3.8% (2.36 mM), 0.38% (0.236 mM), 0.038% (23.6 µM), 0.0038% (2.36 µM) concentrations using mesenchymal stem cell (MSC) media. The media were replaced with MSC media supplement with the different concentrations of SDF and SHEDs were allowed to grow for 2, 5, 7 days. Cells growing in MSC without SDF was used as a control. At the specific time points, the cells were washed with phosphate-buffered saline (PBS) and subjected to freeze thaw cycle (at -80^0C) and 200 µL of the CyQuant dye were added to each well. Cells were incubated at 2-5 min at room temperature and protected from light. The fluorescence was measured using fluorescence microplate reader with ~480 nm excitation and ~520 nm emission. The mean fluorescence level was quantified, and one-way ANOVA was used to determine the presence of any significant

statistical difference between SHEDs growing in MSC media supplement with the different concentrations of SDF and control. The CyQuant assay was done in triplicates and on biological triplicate of SHEDs (total of 9 samples per condition). MTT **(**3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide**)** assay was used for validation of SDF concentrations that showed the least cytotoxicity. The MTT assay was used based on standard published protocols[46, 47]. Briefly, SHEDs were seeded in 96 well plate (~10^4cells/well) and allowed to grow in MSC media for 24hr. Then, the media replaced with MSC media supplemented with the concentrations of SDF that showed the least cytotoxicity in CyQuant assay. The control was cells growing in MSC media without SDF. The viability of cells was checked after 2, 5 and 7 days of exposure to the different concentrations of SDF. MTT (0.5mg/ml) were added to each well at the specific time points and incubated for 4 hours. After incubation, the supernatant was replaced by dimethyl sulfoxide (DMSO) (100ul/well) and absorbance was measured at 560nm in microplate reader. The absorbance was corrected with blank and mean data was analyzed. The MTT assay was done in triplicates and on biological triplicate of SHEDs (total of 9 samples per condition).

4.3.2. Cell Proliferation Assay:

CyQuant assay will be used to investigate the effect of the non-toxic concentrations of SDF (based on 3.3.1 experiment) on SHEDs proliferation using standard published protocols.[43, 44] Briefly, SHEDs will be seeded in 96 well plate (~10^3cells/well) and allowed to grow overnight. The media will be replaced with MSC media supplement with the non-toxic concentrations SDF and SHEDs will be allowed to grow for 2, 5, 7 days. Cells growing in MSC without SDF will be used as a control. At the specific time points, the cells will be washed with PBS and subjected to freeze thaw cycle (at -80^0C) and 200 µL of the CyQuant dye will be added to each well. Cells will be incubated at 2-5 min at room temperature and protected from light. The fluorescence will be measured using fluorescence microplate reader with ~480 nm excitation and ~520 nm emission. The mean fluorescence level will be quantified, and one-way ANOVA

will be used to determine the presence of any significant statistical difference between SHEDs growing in MSC media supplement with SDF to control. The CyQuant assay will be done in triplicates.

4.3.3. Cell Differentiation Assay (Osteogenic Differentiation):

The well established in-vitro mineralization differentiation assay[48, 49] was used to investigate whether the non-toxic SDF concentrations can enhance the odontogenic differentiation of SHEDs. Briefly, SHED was plated at a starting density of $1x10^4$ in MSC media for 24 hr. Then, the media was changed into MSC supplemented with the concentrations that found to be non-toxic based on the precious cytotoxicity experiments. Cells growing in MSC without SDF was used as a control. Once they reached 90% confluency, the mineralization differentiation initiated by incubating cells in mineralization differentiation media (MDM: StemPro Osteogenic differentiation media). After 10 days of culture in MDM, the mineralization was tested using Alizarin red (AR) stain. For AR staining, cells were fixed with 4% paraformaldehyde for 30 min, rinsed twice with distilled water and stained with 2% AR (Lifeline cell technology) solution for 2-3 minutes. Cells were then rinsed 3 times with distilled water and visualized under light microscope for image capturing and analysis. The mean level of AR staining was quantified using image processing software (imageJ, NIH, USA) and one-way ANOVA was used to determine the presence of any significant statistical difference between SHEDs growing in MSC media supplement with different concentrations of SDF and control. The experiment was run in technical triplicates and on biological triplicates (total of 9 samples per condition).

4.4 Investigating the Release of Growth Factors from Human Primary Dentine:

A pilot experiment was designed to investigate the effect of the clinical concentration of SDF on the release of growth factors from the human primary dentine.

4.4.1. Preparation of Dentine Discs:

Sound extracted human primary teeth were collected. Enamel, cementum and soft tissue were then removed, and dentin discs obtained by slicing 1 mm thickness from the remaining dentine. The dentine discs were incubated with the full concentration of SDF, positive control (10% Citric acid) and negative control (PBS) at 37ºC for a period of 3 days. The supernatants were then collected and assessed for growth factors release.

4.4.2. Testing Growth Factors' Release:

To assess whether SDF can release growth factors from dentin, Enzyme-linked Immunosorbent Assay (ELISA) was used to detect the presence of a ligand using antibodies directed against the target protein to be measured. The growth factor that was observed is TGF ß-1. Supernatants collected from the previous experiment was subjected to ELISA using standard published protocol[50]. The experiment was run in technical triplicates and on biological triplicates (total of 9 samples per condition) and absorbance was checked using Hidex plate reader.

4.5 Data Analysis:

In addition to the experiment-specific data analysis approaches detailed above, in general, in those experiments where we want to determine if there is any significant statistical difference between the mean of two groups or more on a continuous dependent variable a t-test or one-way ANOVA will be used, respectively. While in those experiments in which the mean of a continuous dependent variable will be measured in the same group but under two or more conditions, paired-samples t-test or repeated measures ANOVA will be used, respectively. All experiments were repeated three times and the mean of the triplicate was used in the statistical analysis. All statistical analysis was performed with IBM SPSS software. A p value <0.05 indicate statistically significant differences.

Table 2 summarizes different groups in different experiments.

	Control Group	Experimental Group(s)
Cell Viability and Cytotoxicity Assay	Cells growing in MSC media without SDF	Cells growing in MSC media supplemented with various concentrations of SDF: Group 1: Full concentration: 38% (23.6mM), Group 2: 3.8% (2.36mM), Group 3: 0.38% (0.236mM), Group 4: 0.038% (23.6µM), Group 5: 0.0038% (2.36µM)
Cell Differentiation Assay (Odontogenic Differentiation)	Cells growing in MSC media without SDF	Cells growing in MSC media supplemented with non-toxic concentration of SDF
Enzyme-linked Immunosorbent Assay (ELISA)	• Negative control group: Dentine powder incubated with PBS for 14 days • Positive control group: Dentine powder incubated with 10% citric acid for 14 days	Dentine disc incubated with 38% SDF for 14 days

Table 2: Summary of groups in different experiments

5. RESULTS

5.1 SHEDs Isolation and Culture:

SHEDs were isolated and cultured from freshly extracted primary teeth. Figure 1 shows *in-vitro* proliferation of the SHEDs.

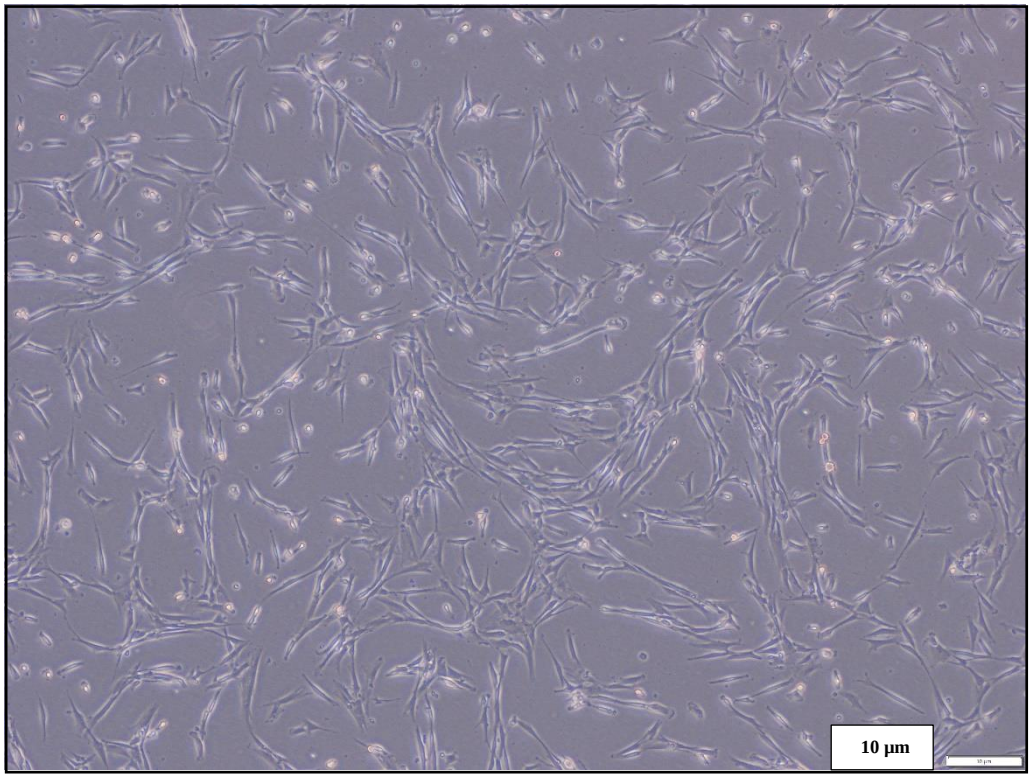

Figure 1 | Light microscopic image of stem Cells from Human Exfoliated Deciduous Teeth (SHEDs) at passage 3

5.2 Effect of SDF on Cell Viability and Cytotoxicity:

The viability of cells was examined after 2, 5 and 7 days of exposure to the variety of concentrations of the SDF solution using the CyQuant assay.

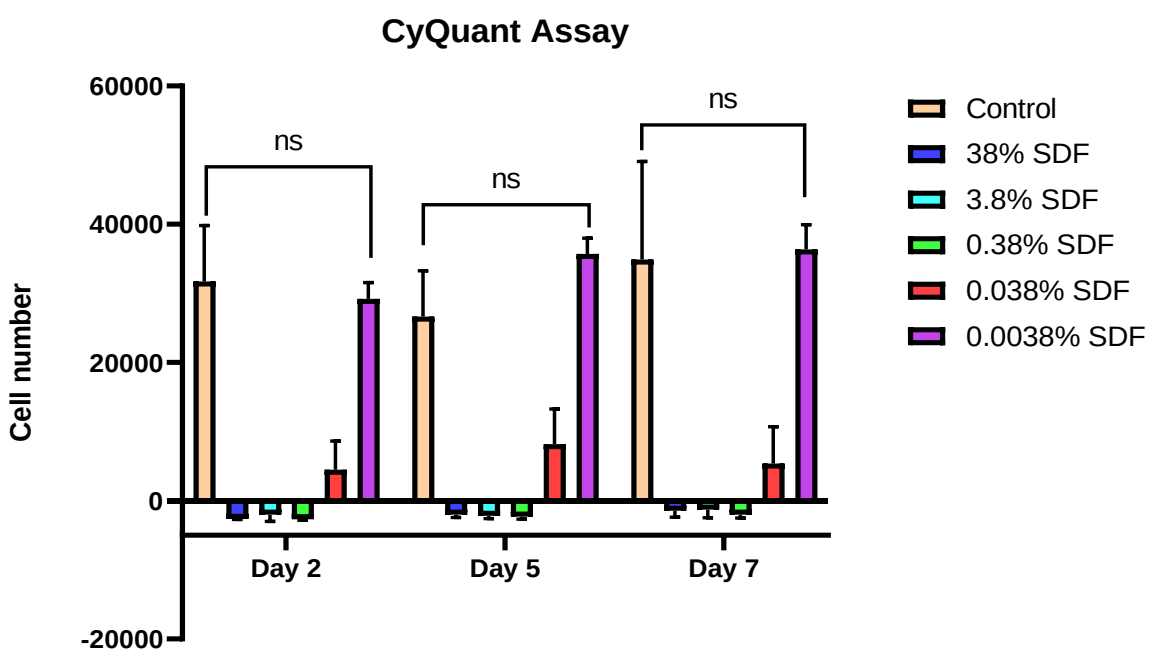

Figure 2 | CyQuant Cell Proliferation Assay. Results revealed that 38%, 3.8% and 0.38% SDF were cytotoxic with no cell proliferation found. Over 7 days, the highest cell proliferation rate was detected with 0.0038% SDF, followed by control and 0.038% SDF.

The bar graph (figure 2) illustrates the findings from the CyQuant assay. Results from the cell proliferation assay revealed that over seven days, 38%, 3.8% and 0.38% SDF were cytotoxic, and no cell proliferation were detected. The two concentrations that showed the least toxicity on the cells were 0.038% and 0.0038% SDF. These two concentrations were viable and promoted cell proliferation. No significant difference was found between the control group and 0.0038% SDF over the course of seven days. At day two, while 38%, 3.8% and 0.38% of SDF were cytotoxic, the control group and SDF concentrations of 0.038% and 0.0038% revealed cell proliferation with no signs of cytotoxicity. The results were similarly noted on day five and seven, in which the number of cells increased in the groups of SDF concentrations of 0.038% and 0.0038%. In addition, more cells were observed in the concentration of 0.0038% SDF in comparison to the control group, but with no significant difference. And likewise, no cells were detected in the other SDF concentration groups.

When the validation was done by the aid of the MTT assay, as shown in figure 3, results concluded that 0.38% SDF was cytotoxic on days 2 and 5, with statistically significant difference (p-value of ≤0.05) compared to the control group. This implies that any concentration above that percentage can be toxic to the cells. However, concentrations of 0.038% and 0.0038% SDF showed no cytotoxic effect. Moreover, there was a higher percentage of cells in the group concentration of 0.0038% SDF compared to the control group, but with no statistically significant difference.

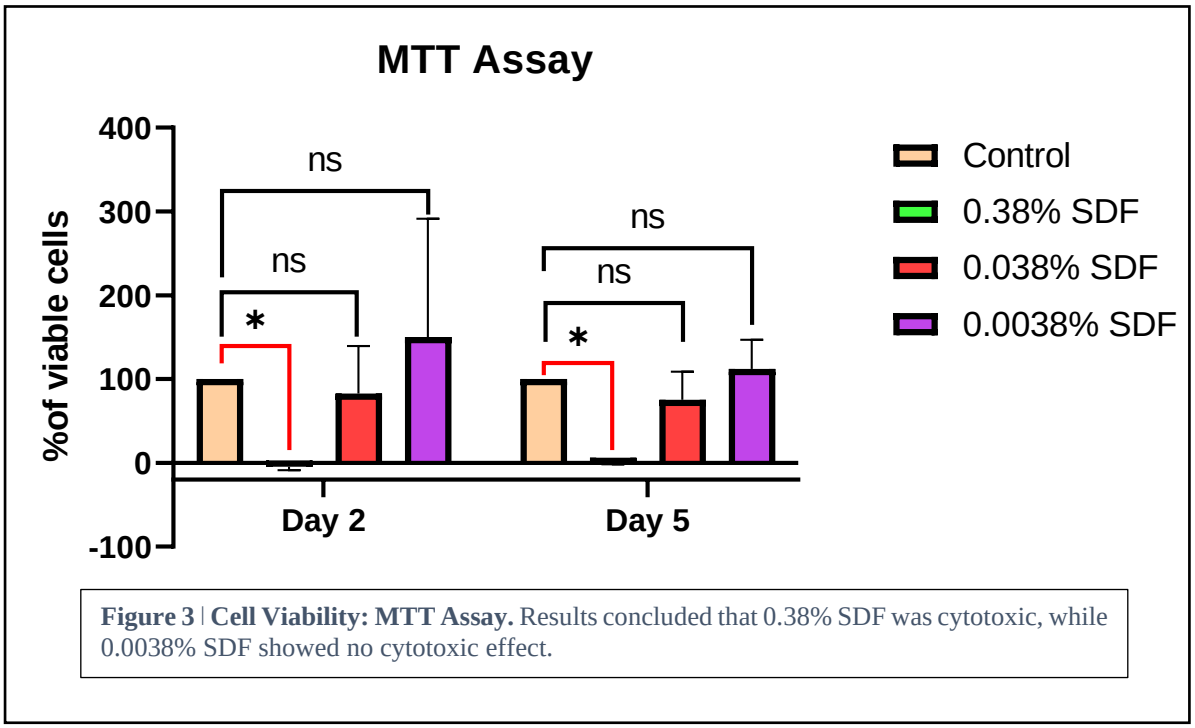

Figure 3 | Cell Viability: MTT Assay. Results concluded that 0.38% SDF was cytotoxic, while 0.0038% SDF showed no cytotoxic effect.

5.3 Effect of SDF on Odontogenic Differentiation:

After determining the non-toxic concentrations of SDF, cell differentiation assay was implemented, in which 0.0038% of SDF was added on the cells for 4 days after being initially cultured. On day 4, in which the cells have reached 90% confluence, the media was changed to osteogenic differentiation media that lasted for 14 days and assessment was made using the Alizarin red stain.

When cells and the extracellular matrix were stained with Alizarin red stain, calcification nodules were detected in both the control group and 0.0038% SDF group, as shown below in figure 4, which is an indication for osteogenic cell differentiation. Less calcification nodules were detected in the 0.0038% SDF when compared to the control. However, when a quantification of nodules was done, no significant difference was noted between the two groups.

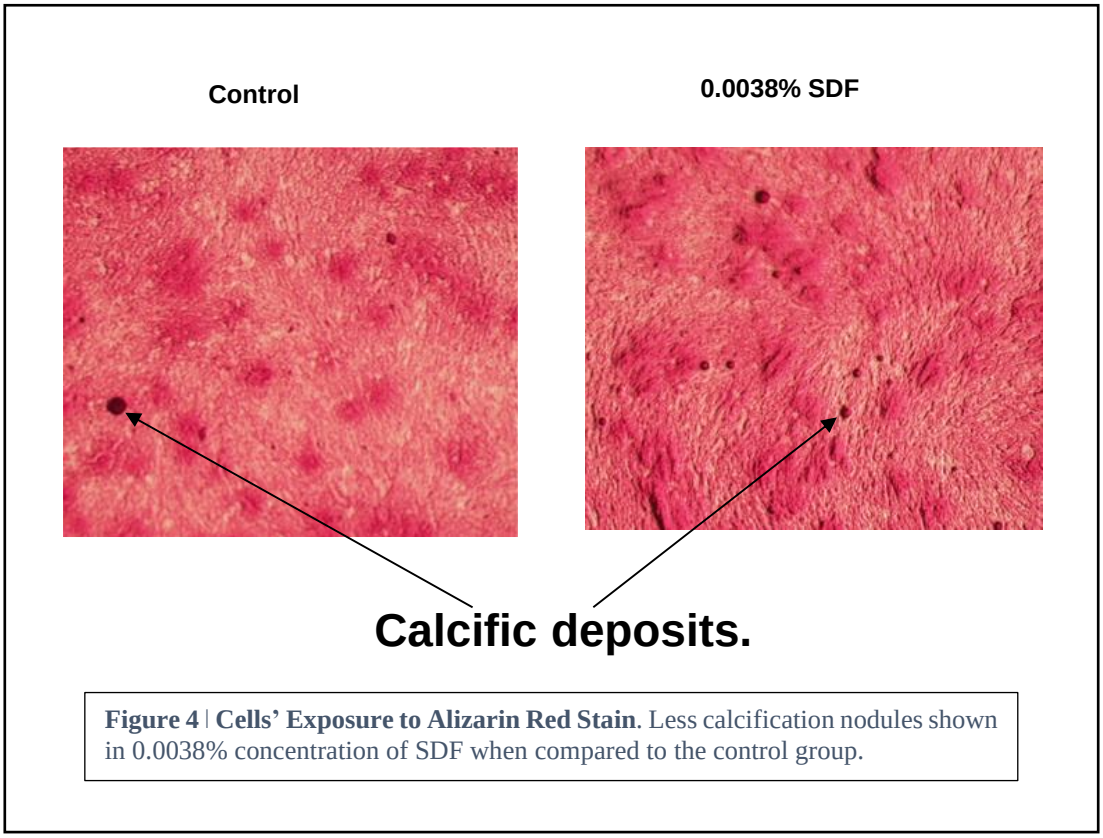

Figure 4 | Cells' Exposure to Alizarin Red Stain. Less calcification nodules shown in 0.0038% concentration of SDF when compared to the control group.

Results from the cell differentiation assay, as shown in figure 5, revealed that the 0.0038% SDF did not inhibit the differentiation of cells into the osteogenic lineage. Nevertheless, no significant difference was noted between the concentration of 0.0038% SDF and the control group.

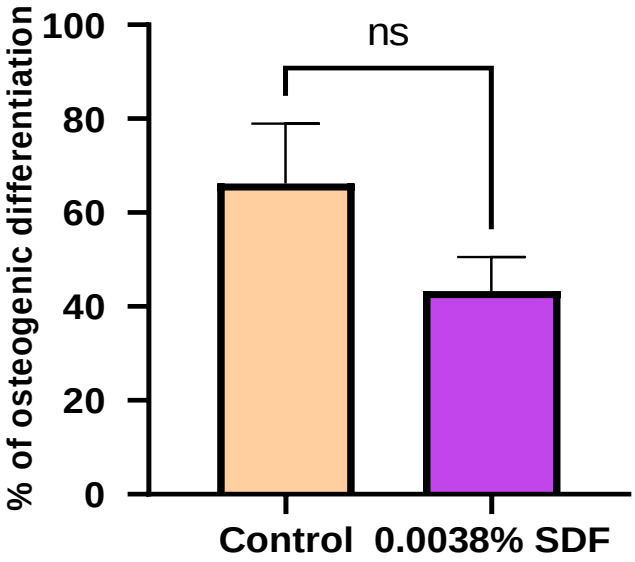

Figure 5 | Cell Differentiation (Osteogenic Differentiation) Assay. Concentration of 0.0038% SDF allowed the differentiation of cells into the osteogenic lineage.

5.4 Release of Growth Factors from Human Primary Dentine:

Figure 6 illustrates the results of ELISA experiment, which concluded that the highest TGF β-1 release was found in 10% citric acid group, followed by 38% SDF and PBS.

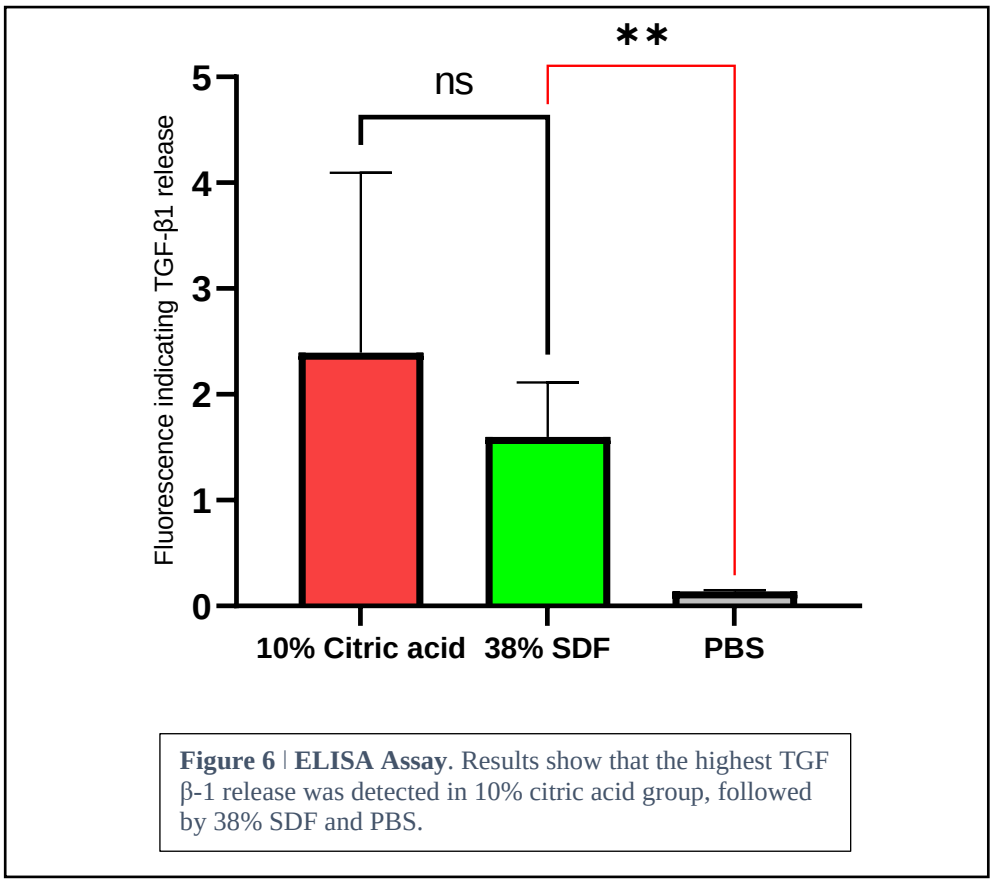

Figure 6 | ELISA Assay. Results show that the highest TGF β-1 release was detected in 10% citric acid group, followed by 38% SDF and PBS.

No significant difference was noted between the two groups of 38% SDF and 10% citric acid. Though when compared to PBS, a significant difference was noted in relation to 38% SDF concentration.

6. DISCUSSION

This *in-vitro* study investigated the effect of SDF at a cellular level, in addition to its ability to mobilize growth factors from human dentine. The study assessed the cytotoxic and proliferative effect of 38% SDF concentration and its dilutions on the stem cells from human exfoliated deciduous teeth or known as SHEDs. Subsequently, this study investigated the effect of the non-cytotoxic concentration of SDF on the differentiation of SHEDs. Furthermore, it evaluated the effect of 38% SDF on the release of the growth factor TGF-β1 from human primary dentine.

The action of SDF on arresting dentinal caries has been proved by many researchers[1, 4, 8, 9]. Recent in-vitro studies done on the aspect of cellular effects showed increased dentinal thickness in the areas where SDF was applied[11, 12, 14]. However, the assumption about the mechanism of action of SDF on its effect on dental pulp and odontoblasts is not clearly explained in the literature. The high concentrations of silver and fluoride in SDF can raise concerns about its biocompatibility to pulpal tissues. Furthermore, researchers have evidently revealed how the process of release of growth factors present in the dentine matrix, which is caused by dental pulp capping agents like EDTA and Calcium Hydroxide, promote pulp tissue repair-associated events[30, 31]. The purpose of this research was to investigate the effect of SDF on the function of SHEDs, and whether it implied the same mechanism of action as the dental pulp capping agents effectively used.

The viability and proliferation of the cells were tested using different concentrations of SDF, and with the CyQuant assay, both cytotoxicity and proliferation were assessed. According to the study done by *Fancher et al.* (2017), his research concluded that at 0.01%, SDF had cytotoxic effects to human gingival fibroblasts[15]. Another study by *Seunggun Kim* (2021)[50] aimed to investigate the effect of diluted 38% SDF solutions (10^{-4} and 10^{-5}) on the viability, alkaline phosphatase (ALP) activity, and morphology of rat pulpal cells and to evaluate the influence of reduced glutathione (GSH) on SDF-induced cytotoxicity. The results

demonstrated that the tested SDF dilutions caused a remarkable cytotoxic effect, while the addition of GSH prevented SDF-induced damage at 6-hour exposure time in the higher dilution of SDF. A study done by *Glenda Rossi* (2017)[51] aimed to investigate the effect of SDF on the dentine-pulp complex using two models: Teeth after SDF application (ex vivo) and experimental animal molars. Brightfield optical microscopy showed SDF sealing the tubules only at the site where it had been placed, with limited penetration beneath. The tubules appeared normal, and the pulp tissue associated to treated caries showed chronic inflammatory infiltrate and formation of tertiary dentine, with no Ag precipitate. In the experimental animal model, pulp histology was not significantly altered in the molar cavities exposed to SDF. The observations using the different techniques on dental tissues suggest that SDF causes minimal adverse effects[51]. Additional systematic review was done by *Ahmed Zaeneldin* (2022) on the effect of silver diamine fluoride on vital dental pulp[52]. A systematic search concluded that direct SDF application on vital pulp can cause pulp necrosis. However, indirect SDF application is generally biocompatible to dental pulp tissue with a mild inflammatory response, increased odontoblastic activity, and increased tertiary dentine formation. This study revealed that certain concentrations of SDF were not toxic and promoted cell proliferation and differentiation. The results of our cell viability and proliferation assays concluded that the clinical concentration of SDF, which was 38%, was cytotoxic on SHEDs, in accordance with the above-mentioned studies. The same was noticed for 3.8%, 0.38% SDF concentration. Both 0.038% and 0.0038% of SDF showed the least cytotoxic effect, with 0.0038% promoting cell proliferation. These results could indicate that 0.0038% is potentially a biocompatible concentration of SDF and can be used in procedures that involve close contact with pulp cells. To test such hypothesis, we investigated whether 0.0038% could inhibit one of SHEDs' function, that is the differentiation towards osteogenic lineage. Our results showed SHEDs grown in 0.0038% of SDF were still able to differentiate and produce calcification nodules, similar to the control group. These results support the hypothesis that 0.0038% of SDF is a potential biocompatible

concentration of SDF. However, such hypothesis faces few challenges and limitations. It is unknown whether 0.0038% SDF can perform other functions, such as the antimicrobial and caries arresting functions, as the clinical concentration of SDF does. *Brian Minavi* (2021) evaluated the substantivity of 3.8% SDF in a bovine dentine model[53]. The objective of his study was to compare the antimicrobial substantivity effect of 3.8% SDF against other commonly used endodontic irrigants such as 2% Chlorhexidine (CHX) and 6.25% Sodium hypochlorite (NaOCl). The diffusion disc assay demonstrated that 38% and 3.8% of SDF inhibited the growth of E. faecalis, and the substantivity of 3.8% SDF was comparable to 2% CHX and was significantly greater than 6.25% of NaOCl compared to the PBS treated samples after 1.5 and 3 weeks of incubation. Further research is warranted to assess whether 0.0038% SDF can still perform caries-arresting, anti-hypersensitivity and antibacterial functions and confirm its potential clinical use.

This study also investigated the ability of SDF to release growth factors from human primary dentine. The purpose of conducting this experiment was to investigate whether the reactionary dentine secreted at area of SDF application is triggered in a similar mechanism to other pulp capping agents such as $Ca(OH)_2$ and MTA. It has been found that pulp capping agents, like $Ca(OH)_2$ and MTA, can mobilize growth factors from the dentine to trigger reactionary dentinogenesis. In *Tomson et al.* (2017) study, a broad range of growth factors' release was observed when correlating the effect of pulp capping agents on the extraction of growth factors[29]. According to *Goncalves et al.* (2016), EDTA was significantly an effective extractant of transforming growth factor beta 1 (TGF β-1), which is most likely attributed to its powerful demineralizing and solubilizing action on hard dental tissues[30]. A further study by *Alp Abidin et al.* (2020) considered different dentine conditioning agents, and results of the study concluded that the highest TGF β-1 release was observed after 10% citric acid treatment followed by 37% phosphoric acid[31]. In our study, we conducted a pilot experiment to investigate the effect of clinical concentration of SDF on the release of growth factor,

specifically TGF β-1, from human primary dentine. Our results showed that the clinical concentration of SDF (38%) released TGF β-1 growth factor when applied to human primary dentine in a level that is similar to citric acid (control). This implies that SDF can potentially promote reactionary dentinogenesis through growth factor release. However, the results of this experiment should be confirmed by investigating the release of other growth factors. In addition, it is crucial to investigate whether 0.0038% SDF concentration can have a similar effect to the clinical concentration of SDF. Furthermore, it is important to compare the amount of growth factors released in response to SDF application from those of $Ca(OH)_2$ and MTA. These experiments are planned in an ongoing project.

Our study concluded that the diluted concentration of SDF (0.0038%) is biocompatible, thereby has the potential to be used in an area close to the pulpal tissue. It will not inhibit the differentiation mechanism of the pulp, and finally, it can promote pulp tissue repair-associated events. These results indicate that the lower concentration of SDF has the potential to be used as a dental pulp capping agent as a new and additional approach to pulp capping treatment. However, the limitation of this finding is that the concentration that provided cell proliferation was when the SDF was diluted to 0.0038%. And therefore, it is unknown whether this can compromise its other relative functions of antimicrobial activity. Also, the study limitation is that it investigated the effect of SDF on stem cells. And for us to prove that it should be used as a pulp capping agent, further study is required on animal models.

7. CONCLUSIONS

The clinical concentration of SDF (38%) was cytotoxic on SHED, while the diluted concentration of 0.0038% SDF promoted cell proliferation. The non-cytotoxic concentration of 0.0038% SDF did not inhibit the differentiation of SHED into osteogenic lineage. The ELISA experiment revealed that dentine exposed to the clinical concentration of SDF released TGF β-1 growth factor, indicating that SDF could promote reactionary dentinogenesis. Further studies are required to validate the growth factor release from dentine, and to test different concentrations of SDF's antimicrobial properties and dentine remineralization potential.

8. REFERENCES

1. Seto J, Horst JA, Parkinson DY, Frachella JC, DeRisi JL. Silver microwires from treating tooth decay with silver diamine fluoride. bioRxiv. 2017:152199.

2. Seifo N, Cassie H, Radford JR, Innes NPT. Silver diamine fluoride for managing carious lesions: an umbrella review. BMC oral health. 2019;19(1):145.

3. Burgess JO, Vaghela PM. Silver Diamine Fluoride: A Successful Anticarious Solution with Limits. Advances in dental research. 2018;29(1):131-4.

4. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, et al. Mechanisms of silver diamine fluoride on arresting caries: a literature review. International dental journal. 2018;68(2):67-76.

5. Crystal YO, Marghalani AA, Ureles SD, Wright JT, Sulyanto R, Divaris K, et al. Use of Silver Diamine Fluoride for Dental Caries Management in Children and Adolescents, Including Those with Special Health Care Needs. Pediatric dentistry. 2017;39(5):135-45.

6. Chu CH, Mei L, Seneviratne CJ, Lo EC. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms. International journal of paediatric dentistry. 2012;22(1):2-10.

7. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans. Australian dental journal. 2005;50(4):242-5.

8. Crystal YO, Niederman R. Evidence-Based Dentistry Update on Silver Diamine Fluoride. Dental clinics of North America. 2019;63(1):45-68.

9. Fakhruddin KS, Egusa H, Ngo HC, Panduwawala C, Pesee S, Samaranayake LP. Clinical efficacy and the antimicrobial potential of silver formulations in arresting dental caries: a systematic review. BMC oral health. 2020;20(1):160.

10. Chen KJ, Gao SS, Duangthip D, Lo ECM, Chu CH. Managing Early Childhood Caries for Young Children in China. Healthcare (Basel, Switzerland). 2018;6(1).

11. Bimstein E, Damm D. Human Primary Tooth Histology Six Months after Treatment with Silver Diamine Fluoride. The Journal of clinical pediatric dentistry. 2018;42(6):442-4.

12. Vanegas S, Godoy A, Urdaneta L, Olávez D, Padrón K, Solórzano E. EFECTO DEL FLUORURO DIAMINO DE PLATA EN CARIES INDUCIDA EN RATAS WISTAR %J Revista Facultad de Odontología Universidad de Antioquia. 2014;26:76-88.

13. Desai H, Stewart CA, Finer Y. Minimally Invasive Therapies for the Management of Dental Caries-A Literature Review. Dentistry journal. 2021;9(12).

14. Korwar A, Sharma S, Logani A, Shah N. Pulp response to high fluoride releasing glass ionomer, silver diamine fluoride, and calcium hydroxide used for indirect pulp treatment: An in-vivo comparative study. Contemporary clinical dentistry. 2015;6(3):288-92.

15. Fancher ME, Fournier S, Townsend J, Lallier TE. Cytotoxic effects of silver diamine fluoride. American journal of dentistry. 2019;32(3):152-6.

16. García-Bernal D, Pecci-Lloret MP, López-García S. The Cytocompatibility of Silver Diamine Fluoride on Mesenchymal Stromal Cells from Human Exfoliated Deciduous Teeth: An In Vitro Study. Materials (Basel, Switzerland). 2022;15(6).

17. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists. 2001;12(5):425-37.

18. Lazar-Molnar E, Hegyesi H, Tóth S, Falus A. Lazar-Molnar E, Hegyesi H, Toth S, Falus AAutocrine and paracrine regulation by cytokines and growth factors in melanoma. Cytokine 12:547-554. Cytokine. 2000;12:547-54.

19. Goldberg M, Smith AJ. CELLS AND EXTRACELLULAR MATRICES OF DENTIN AND PULP: A BIOLOGICAL BASIS FOR REPAIR AND TISSUE ENGINEERING. Critical

reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists. 2004;15(1):13-27.

20. Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. Journal of tissue engineering. 2017;8:2041731417702531.

21. Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot HJTIjodb. Reactionary dentinogenesis. 1995;39 1:273-80.

22. Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ. Quantitation of growth factors IGF-I, SGF/IGF-II, and TGF-beta in human dentin. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1990;5(7):717-23.

23. Cassidy N, Fahey M, Prime SS, Smith AJ. Comparative analysis of transforming growth factor-beta isoforms 1-3 in human and rabbit dentine matrices. Archives of oral biology. 1997;42(3):219-23.

24. Bessho K, Tanaka N, Matsumoto J, Tagawa T, Murata M. Human dentin-matrix-derived bone morphogenetic protein. Journal of dental research. 1991;70(3):171-5.

25. Tomson PL, Lumley PJ, Alexander MY, Smith AJ, Cooper PR. Hepatocyte growth factor is sequestered in dentine matrix and promotes regeneration-associated events in dental pulp cells. Cytokine. 2013;61(2):622-9.

26. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. Archives of oral biology. 2000;45(11):1013-6.

27. Li Z, Liu L, Wang L, Song D. The effects and potential applications of concentrated growth factor in dentin-pulp complex regeneration. Stem cell research & therapy. 2021;12(1):357.

28. McDonald R, Avery D, Hartsfield J. McDonald RE, DR Avery, JK Hartsfield Jr: Acquired and Developmental Disturbances of the Teeth and Associated Oral Structures. In:

Dentistry for the Child and Adolescent, ninth edition. JA Dean, DR Avery, and RE McDonald, eds. Mosby, Inc. St. Louis, MO. Pages 85-125, 2010. 2010. p. 85-125.

29. Tomson PL, Lumley PJ, Smith AJ, Cooper PR. Growth factor release from dentine matrix by pulp-capping agents promotes pulp tissue repair-associated events. International endodontic journal. 2017;50(3):281-92.

30. Gonçalves LF, Fernandes AP, Cosme-Silva L, Colombo FA, Martins NS, Oliveira TM, et al. Effect of EDTA on TGF-β1 released from the dentin matrix and its influence on dental pulp stem cell migration. Brazilian oral research. 2016;30(1):e131.

31. Atesci AA, Avci CB, Tuglu MI, Ozates Ay NP, Eronat AC. Effect of Different Dentin Conditioning Agents on Growth Factor Release, Mesenchymal Stem Cell Attachment and Morphology. Journal of endodontics. 2020;46(2):200-8.

32. Samsonraj RM, Raghunath M, Nurcombe V, Hui JH, van Wijnen AJ, Cool SM. Concise Review: Multifaceted Characterization of Human Mesenchymal Stem Cells for Use in Regenerative Medicine. Stem cells translational medicine. 2017;6(12):2173-85.

33. Naz S, Khan FR, Zohra RR, Lakhundi SS, Khan MS, Mohammed N, et al. Isolation and culture of dental pulp stem cells from permanent and deciduous teeth. Pakistan journal of medical sciences. 2019;35(4):997-1002.

34. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem cell research & therapy. 2019;10(1):68.

35. Hwang NS, Varghese S, Elisseeff J. Controlled differentiation of stem cells. Advanced drug delivery reviews. 2008;60(2):199-214.

36. Willerth SM, Faxel TE, Gottlieb DI, Sakiyama-Elbert SE. The effects of soluble growth factors on embryonic stem cell differentiation inside of fibrin scaffolds. Stem cells (Dayton, Ohio). 2007;25(9):2235-44.

37. Ansari S, Seagroves JT, Chen C, Shah K, Aghaloo T, Wu BM, et al. Dental and orofacial mesenchymal stem cells in craniofacial regeneration: The prosthodontist's point of view. The Journal of prosthetic dentistry. 2017;118(4):455-61.

38. Eubanks EJ, Tarle SA, Kaigler D. Tooth storage, dental pulp stem cell isolation, and clinical scale expansion without animal serum. Journal of endodontics. 2014;40(5):652-7.

39. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(10):5807-12.

40. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(25):13625-30.

41. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet (London, England). 2004;364(9429):149-55.

42. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PloS one. 2006;1(1):e79.

43. Quent VM, Loessner D, Friis T, Reichert JC, Hutmacher DW. Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. Journal of cellular and molecular medicine. 2010;14(4):1003-13.

44. Jones LJ, Gray M, Yue ST, Haugland RP, Singer VL. Sensitive determination of cell number using the CyQUANT cell proliferation assay. Journal of immunological methods. 2001;254(1-2):85-98.

45. Rai Y, Pathak R, Kumari N, Sah DK, Pandey S, Kalra N, et al. Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. Scientific reports. 2018;8(1):1531.

46. Liu Y, Peterson DA, Kimura H, Schubert D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. Journal of neurochemistry. 1997;69(2):581-93.

47. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. Journal of endodontics. 2008;34(6):645-51.

48. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. Journal of endodontics. 2008;34(2):166-71.

49. van Loon AM, van der Veen J. Enzyme-linked immunosorbent assay for quantitation of toxoplasma antibodies in human sera. Journal of clinical pathology. 1980;33(7):635-9.

50. Kim S, Nassar M, Tamura Y, Hiraishi N, Jamleh A, Nikaido T, et al. The effect of reduced glutathione on the toxicity of silver diamine fluoride in rat pulpal cells. Journal of applied oral science : revista FOB. 2021;29:e20200859.

51. Rossi G, Squassi A, Mandalunis P, Kaplan A. Effect of silver diamine fluoride (SDF) on the dentin-pulp complex: ex vivo histological analysis on human primary teeth and rat molars. Acta odontologica latinoamericana : AOL. 2017;30(1):5-12.

52. Zaeneldin A, Yu OY, Chu CH. Effect of silver diamine fluoride on vital dental pulp: A systematic review. Journal of dentistry. 2022;119:104066.

53. Minavi B, Youssefi A, Quock R, Letra A, Silva R, Kirkpatrick TC, et al. Evaluating the substantivity of silver diamine fluoride in a dentin model. Clinical and experimental dental research. 2021;7(4):628-33.

9. APPENDICES

APPENDIX 1: MBRU IRB approval

MBRU
جامعة محمد بن راشد للطب والعلوم الصحية
MOHAMMED BIN RASHID UNIVERSITY OF MEDICINE AND HEALTH SCIENCES

Dec 30, 2020 3:55:14 PM SGT

Yasmine Monjazebi
Endodontics, Pediatric Dentistry

Re: MBRU IRB-2020-16
Initial Effect of Silver Diamine Fluoride Solution on the Stem Cells from Human Exfoliated Deciduous Teeth

Dear Yasmine Monjazebi:

Thank you for submitting clarifications to the observations raised by the Board on the above-referenced study. The Board has reviewed the same and has agreed to approve it.

The approval is valid from December 29, 2020 to the end of the study on the condition that a status report is submitted to the Board by the end of one year from the date of approval or on completion of the project, whichever comes first. Failure to report on the project will be considered as non-compliance with policy.

The project can now commence. Any change in protocol should be notified to the Board.

Sincerely,

Dr Essa Kazim
Chairman

MBRU Institutional Review Board