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Enhanced teeth whitening by nanofluidic transport of hydrogen peroxide into enamel with electrokinetic flows

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ABSTRACT

Tooth whitening, a routine procedure in dentistry, is one of the examples of medical procedures that are limited by the challenge of delivering molecules into various types of nanoporous tissues. Current bleaching methods rely on simple diffusion of peroxides into enamel nano channels, therefore requires sufficient contact time with peroxides. In-office treatments often involve enamel etching or light activation which often results in patient sensitivity and potential soft tissue damage.

Objective. To demonstrate a robust method to transport hydrogen peroxide to greater depths into enamel nanopores through nanofluidic flows driven by electrokinetics, with the intention to increase efficacy while reducing treatment time.

Methods. Freshly extracted human teeth were subjected to electrokinetic flow treatment with hydrogen peroxide under different electric fields with varying operation times. Pre- and post-operative shade matching was done using a photospectrometer.

Results. It is demonstrated that the operation time for the same concentration of hydrogen peroxide can be shortened by 10 times. The proposed method showed significant improvements in whitening effects over control groups and thus offers promising clinically-viable chairside applications with efficacy.

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Significance. The demonstrated nanofluidic transport of hydrogen peroxide into enamel has a potential to be applied for enhancing tooth whitening, compared to simple diffusion, without heating the hard dental tissues.

1. Introduction

Tooth discoloration can be due to extrinsic or intrinsic causes. Extrinsic causes may include frequent intake of coffee, tea, tobacco, red wines and dyes in the food. Intrinsic discoloration is associated with systemic conditions such as medication use, congenital conditions, trauma or insult to the tooth during development, or simply as a result of aging [1]. With increased concern and awareness for dental health and esthetic needs, tooth whitening has become one of the most frequently requested dental procedures by the public [2–4]. Enamel nanopores, usually 2–6 nm in diameter, act as capillaries through which bleaching agents diffuse, and as a result, current bleaching methods require sufficient contact time with peroxides [5,6]. Treatment options include both in-office treatment and at-home treatment. In-office treatments are more effective than at-home systems but involve enamel etching or light activation, which often contributes to patient sensitivity and also even soft tissue damage [7–11]. While recent whitening agents have been improved with a use of potassium nitrates and fluorides to reduce sensitivity, patients still report intra- and post-operative sensitivity, and current whitening systems are limited by efficacy and long treatment time to achieve visible whitening [9,12].

To overcome the difficulty of diffusion through the nanometer-sized pores [13], a novel method of enhancing peroxide transport into teeth is highly desirable. Recently, electric fields have been used to transport calcium to re-mineralize enamel, and in a previous study by our group, electrokinetic flow (EKF) has been used to deliver both positive (Ca^{2+}, K^{+} and Na^{+}) and negative ions (such as F^{-}) into the enamel sections to significantly greater depths (~1 mm) than by diffusion alone [14,15]. Electrokinetic flow (EKF) is motion of liquids driven by an electric field through nanoporous materials [16]. If electrokinetic flow can be utilized to transport commercially available bleaching agents (hydrogen peroxide or carbamide peroxide) more efficiently through enamel pores, then it may be possible to improve tooth whitening procedures, with shorter treatment time, and greater whitening efficacy than current methods.

The purpose of this study is to demonstrate that hydrogen peroxide can be effectively transported through nanopores of the enamel layer by electrokinetic flow. The dependence of nanofluidic transport of hydrogen peroxide into the enamel *via* electrokinetic flow on varied applied voltage and treatment time are studied. Pre- and post-operative whitening effects are measured quantitatively using a photospectrometer. Our findings showed that electrokinetic flows are able to carry hydrogen peroxide through enamel nanopores and improve the whitening effects while shortening treatment time by 10 times compared to currently available treatment.

2. Methods

2.1. Enamel slab sample preparation

Enamel groundsection samples are prepared following the procedure in Refs. [14,15]. The orientation of enamel prisms was checked under polarizing microscopy. All the pieces of enamel ground sections (n = 10) were cut under optical microscope. The dentin parts were removed from the samples by using a stainless-steel blade and the direction of the infiltration was marked following the enamel-dentin orientation to make sure infiltration following the enamel rod orientations. All the samples have average length 1.1–1.3 mm and pre-soaked in deionized (DI) water to reach the equilibrium. The microfluidic sample is prepared by embedding a thin section of enamel (thickness: 100 μm) in UV glue (Norland 68) on a glass slide. The UV glue is cured for 15 min (Spectroline UV Transilluminator). The two ends of enamel with UV glue are cut off by razor blade to ensure that the enamel rod ends are exposed to air. Two reservoirs are created on each end of exposed enamel using epoxy glue, Fig. 1a. In order to visualize the infiltration of different molecules by fluorescence microscopy, the left reservoir is filled with hydrogen peroxide solution 30% (w/w) in water (from *Sigma, USA*) with hydrogen peroxide fluorescence assay (from *Abcam, USA*). It produces fluorescent signals when there is hydrogen peroxide present. This solution was placed in the left reservoir, where the deionized water was placed in the right reservoir, Fig. 1a. Two Ag/AgCl electrodes as anode and cathode are connected with a Keithley source meter 2400 (*Keithley* Inc.).

2.2. Extracted tooth sample preparation for tooth whitening

Freshly extracted molars (n = 8) were decontaminated and stored in tea for 7 days and then in deionized (DI) water. Each tooth was sectioned into 4 sections coronally (mesiobuccal, mesiolingual, distobuccal, distolingual) using an electric saw (*Proxxon Micro Band Saw, Germany*), and pulp tissues were removed using a blade, Fig. 1b. After storing in DI water overnight, initial shade measurement was performed using a spectrophotometer (*Olympus CrystalEye, Japan*). All shade measurements were done by one operator, repeated 3 times and values (L^*, a^*, b^*) averaged and recorded by Origin (from *OriginLab, USA*), where L^* is color along brightness axis, a^* is color along red-greenness axis and b^* is color along yellow-blueness axis [17]. Then specimens were fixed into a chamber with epoxy glue, and then flowable resin composite (3M, USA) was used to build a reservoir on the smooth surface of each sample, Fig. 1c and d. The reservoir is a circle area with a diameter

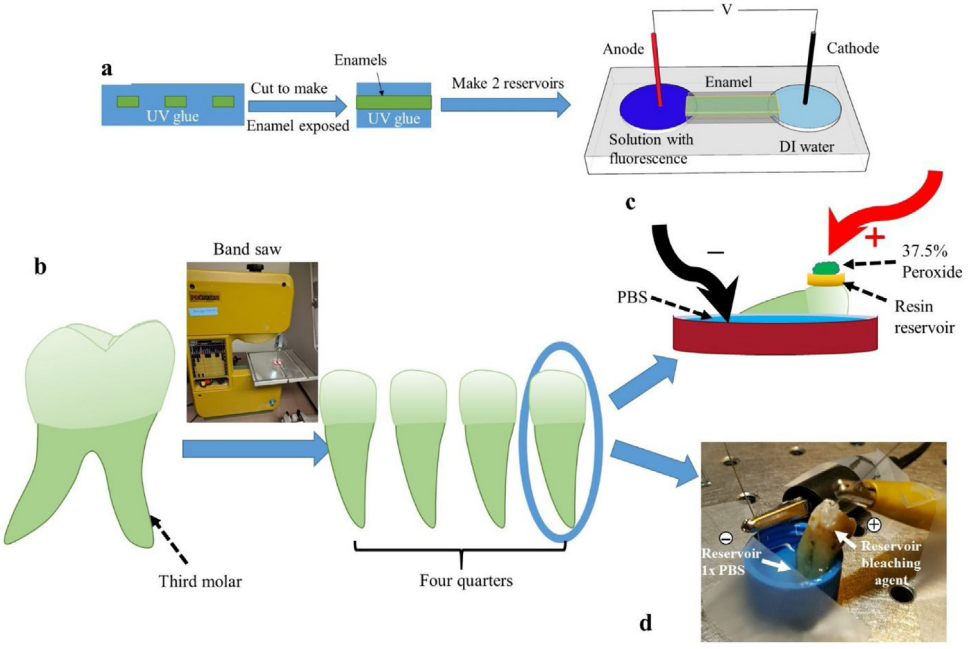

Fig. 1 – Schematic of sample prepation and experimental setup.
(a) Schematic of sample preparation and experimental setup; The gound section of enamel is embedded in UV glue, and the two enamel rod ends are exposed to two reservoirs — one filled by hydrogen peroxide solution with fluorescent probe and the other by deionized water; (b) Each tooth was sectioned into 4 quarters coronally using an electric band saw, and pulp tissues were removed using a razor blade; (c) Quarters of teeth were fixed into a chamber with epoxy glue, respectively; Then flowable resin composite (3 M, USA) was used to build a reservoir on the smooth surface of each sample; Reservoirs were filled with whitening agent; (d) Experimental chambers were filled with 1 × PBS with cathode, and the anode was placed in the reservoir on the tooth surface.

of about 3 mm. Reservoirs were filled with commercially available whitening agent (37.5% hydrogen peroxide aqueous gel; *Pola Office+; SDI, Victoria, Australia*). Chambers were filled with 1× Phosphate-buffered saline (PBS) and the AgCl cathode was put in the PBS. The stainless steel was placed in the reservoir on the tooth surface as the anode (Fig. 1d). The electrodes were connected with a Keithley source meter 2400 (*Keithley Inc.*). For each tooth, the four sections underwent varying electrokinetic flow treatment (0 V, control; 10 V; 20 V; 30 V, respectively) for 3 h. The same quarter of tooth was repeated 3 times at t = 1 h, 2 h, and 3 h respectively for shade measurements by the photospectrometer. All specimens were stored in DI water overnight prior to measurement. Samples before and after infiltration are quantitively analyzed by photospectrometer and $\Delta L^* = L^*_{post} - L^*_{pre}$, $\Delta a^* = a^*_{post} - a^*_{pre}$ and $\Delta b^* = b^*_{post} - b^*_{pre}$ are calculated respectively. The data were analyzed by statistic software (*GraphPad Prism, USA*). Differences were considered significant at $p < 0.05$.

3. Experimental results

3.1. *Infiltration of hydrogen peroxide through enamel slabs*

The enamel nanopore surface is naturally negatively charged and a thin layer of electric double layer (EDL) is formed when it is in contact with an ionic solution. When an electric field is applied, eletrokinetic flow will be induced and both positively and negatively-charged molecules will be transported by this flow [15,16]. A small direct current (DC) electric field of 5 V/mm was applied. It was shown that there was no fluorescent light at 0 s, Fig. 2a and fluorescent signal began to accumulate after 4000 s of operation, Fig. 2b, indicating that the hydrogen peroxide solution has been infiltrated through the enamel section. Fluorescence images are processed by ImageJ and the fluorescent profile along x-axis is measured in the regions of interest (ROI) shown in Fig. 2a and b. Each data point in the profile represents the average intensity across the width of the ROI. The fluorescent intensity increased dramatically when comparing before and after infiltration, Fig. 2c, which is a clear evidence that the hydrogen peroxide with fluorescent probe has been carried through the enamel section by the electrokinetic flows. As the hydrogen peroxide solution (more conductive than deionized water) is infiltrated into the enamel, the resistance inside the enamel nanopores decreases, and the measured current increases with time, Fig. 2d.

3.2. *Tooth whitening by electrokinetic flows on extracted human teeth*

In order to demonstrate the applicability of the technique in clinical application in Dentistry,

in vitro study of nanofluidic transport of hydrogen peroxide by electrokinetic flow into the whole tooth was conducted.

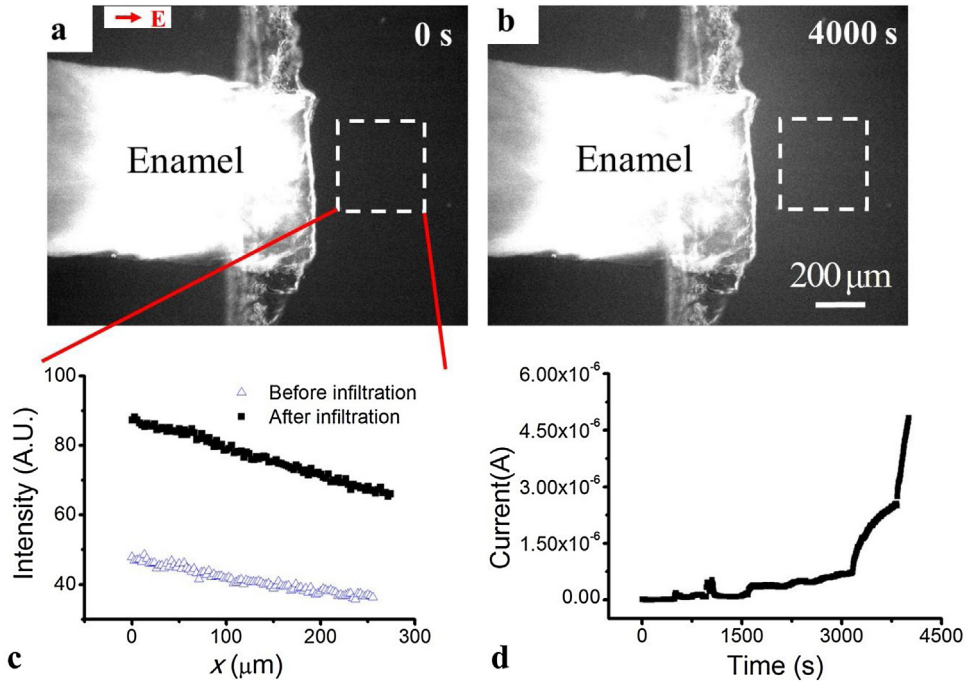

Fig. 2 – Infiltration of hydrogen peroxide solution with hydrogen peroxide fluorescence probe in the enamel by electrokinetic flows.
(a) and (b) Nanofluidic transport of hydrogen peroxide solution into the enamel slabs under electric field at 0 s and 4000 s; after 4000 s infiltration, the fluorescent signal begins accumulating in the right reservoir; direction of electric field and infiltration is from left to right; (c) Comparison of fluorescent intensity on the right reservoir before and after infiltration shows that the hydrogen peroxide solution mixed with its fluorescent probe is indeed tranported through the enamel; (d) Current increasing with time shows that resistance inside enamel nanochannels decreases with infiltration of hydrogen peroxide solution.

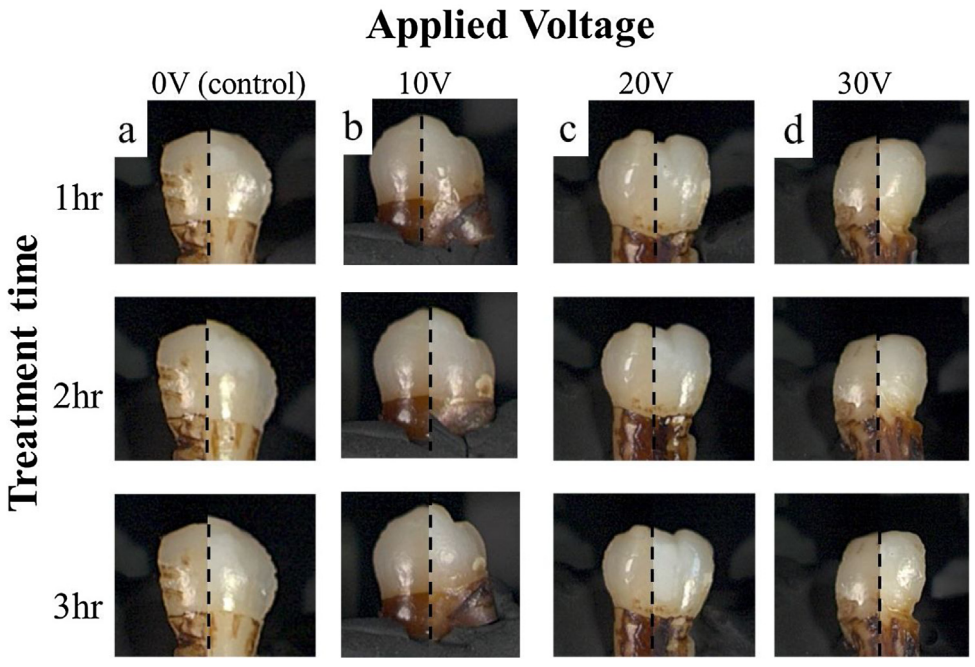

Fig. 3 – Whitening effects monitored by images taken by photospectrometer.
(a–d) Pre-treatment (left) and post-treatment (right) photos juxtaposed. Note that the white spot regions are where the reservoir locate. It shows the improving whitening effects with time and with increasing electric field. Effects are seen earlier with higher electric fields.

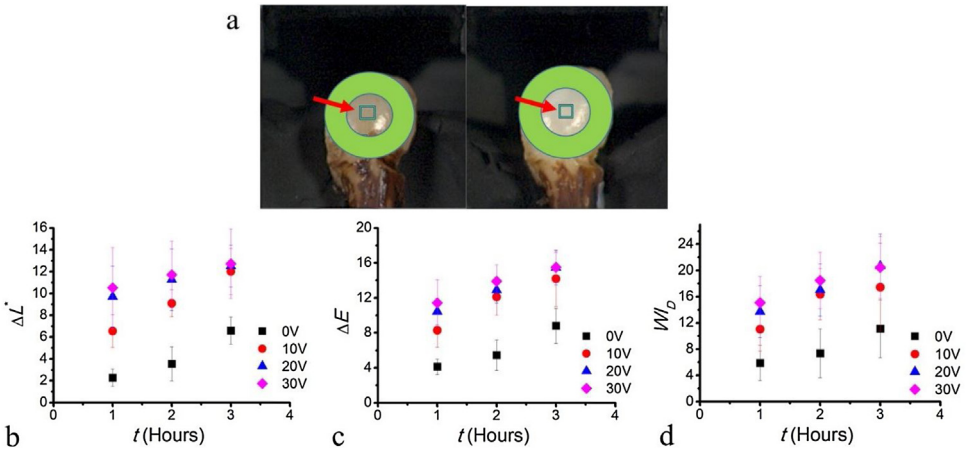

Fig. 4 – Quantitive analysis of whitening effects by photospectrometer.
(a) Red arrows indicate that the middle parts of the teeth are chosen for quantitive comparison of the whitening effects; The green circle region is covered by resin which sets the reservoir area; (b) - (d) Comparison of brightness ΔL^*, color difference ΔE and whiteness index WI_D by applying different voltages for infiltration of hydrogen peroxide into human teeth. Error bars indicate standard deviation taken for 8 teeth. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The shade measurements of the pre- and post-treatment were taken by a photospectrometer and the comparisons are shown in Fig. 3. Pre-treatment (left) and post-treatment (after) photos are juxtaposed. *x*-axis is the applied voltage (0 V, 10 V, 20 V and 30 V) and *y*-axis is the treatment time (1 h, 2 h and 3 h), in which 0 V is the control experiment without applying voltage, Fig. 3a–d. It was shown that with increasing voltage and treatment time, whitening effects were significantly greater, indicating that hydrogen peroxide has been infiltrated into the teeth enamel efficiently by using electrokinetic flows. Same or improved whitening effects were observed earlier with higher electric fields compared to the control experiment without applying voltage. Note that we used the midcrown region of unerupted third molars with completed roots. Recent data on maturation of mammalian enamel indicates that enamel maturation in the incisal/occlusal third of the tooth crown in completed when enamel secretory phase ends at the crown cervix [18]. As the length of the root is higher than the length of the crown, the fact that teeth had completed roots indicates that maturation was completed in the midcrown region used in our study. It is important to note that mineral and water volume measured from normal enamel of unerupted third molars with completed root [19] has been shown to be similar to mineral [20] and water volumes [21] measured from normal enamel of erupted permanent human teeth, suggesting that pore volume (i.e., pathways for transport of materials) in normal enamel of unerupted third molars with completed root is similar to that of erupted third molar with completed root.

These results give us qualitative understanding about how to apply different voltage with different treatment time to improve the whitening effects. However, in order to gain a better understanding of the optimal infiltration voltage with the shortest treatment time, a more quantitative analysis was conducted and will be discussed in the next section.

3.3. *Analysis of tooth whitening effects by photospectrometer*

As another positive evidence of hydrogen peroxide infiltration into the tooth enamel, samples after infiltration were quantitatively analyzed by photospectrometer (*Olympus CrystalEye, Japan*). The comparison between pre- and post-treatment is done in the middle part of the teeth, indicated by red arrows, Fig. 4a. The selected area is inside the reservoir which is directedly exposed to hydrogen peroxide. Brightness improvement ΔL^* is shown in Fig. 4b, showing that all the electrokinetic flow treatments improve the whitening effects. The whitening effect by applying different voltages was improved by 3–10 fold. Meanwhile, color difference [3] by considering the combination effects of brightness L^*, yellowness b^* and redness a^*, $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2}$ has also been significantly improved by electrokinetic flow treatments, Fig. 4c. The whiteness index [22] $WI_D = 0.511\Delta L^* - 2.324\Delta a^* - 1.1\Delta b^*$ is also calculated, Fig. 4d, which shows that whiteness of the teeth samples has been dramatically improved by applying different voltages respectively. For instance, when voltage of $V = 10$V is applied for one hour, all the values of ΔL^* ($p < 0.0001$, two sample T test), ΔE ($p < 0.0001$, two sample T test) and WI_D ($p < 0.005$, two sample T test) are significantly different from that of control group without voltage applied. The significant differences are also shown in other voltages, Fig. 4d. Please note that the effects of three-hour whitening treatment without voltage can be shortened to one-hour by using electrokinetic flow treatment. Fig. 4b–d.

3.4. *Temperature change during the whitening treatments*

In order to address any potential heating caused by the electric current in this work, a thermocouple temperature sensor (from *Grainger*, USA) is used on the surface of the tooth to mea-

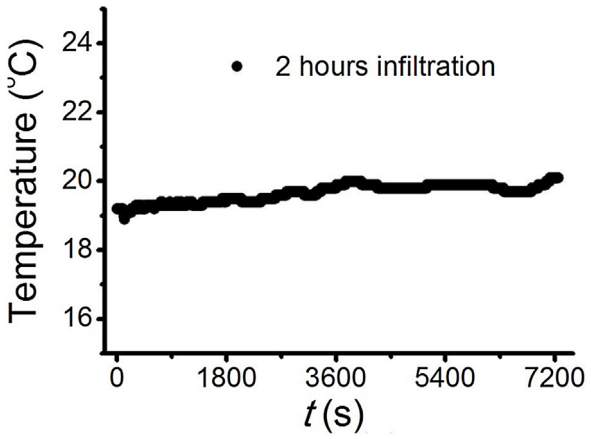

Fig. 5 – Measured temperature change during whitening treatment.
The temperature change on the tooth surface is measured by a thermal couple during the *in vitro* whitening treatment at 20 V is conducted; After 2 h operation, the operatory temperature only increases by about 0.2 °C.

sure the temperature change during the whitening treatment at the voltage of 20 V. The probe is placed inside the reservoir and after 2 h operation, the temperature only increases around 0.2 °C which is negligible in the clinical application, Fig. 5. There might be some difference regarding the temperature increase when our in vitro experimental model is compared to clinical reality. But we don't expect the difference would be noticeable because the electric current to enable the delivery of hydrogen peroxide into the teeth is considerably small («1 mA). Therefore, it is unlikely that this process will induce any drastic temperature increase, thus harm or damage to the surrounding tissue.

4. Discussions

Teeth whitening is one of the most routine dental procedures asked by patients, but current procedures rely on simple diffusion of peroxides into enamel nano channels and, therefore, requires significant contact time with peroxides which can result in post operative sensitivity and soft tissue irritation. Another main challenge of tooth whitening is the staining instability (fading) of whitened teeth, which may lead to uncontrolled multiple re-exposures of dental and periodontal hard and soft tissues to peroxo-derivatives products (whitening agents) [23]. This could contribute to exacerbate the main undesired outcomes common to all tooth whitening treatment types: tooth sensitivity, and damage to the enamel surface and to dental pulp [23]. Tooth sensitivity, which is triggered by pulp inflammation, is positively correlated with both peroxide concentration and peroxide contact time with the tooth, and is lower for at-home tooth whitening (whitening product applied by the patient at home, under supervision of a Dentist; with ~10% carbamide peroxide, corresponding to ~ 7% of hydrogen peroxide) compared to in-office tooth whitening (whitening product applied by a Dentist at a dental office; ~ 35% hydrogen peroxide) [24]. In this context, our technique could be used to decrease both peroxide concentration and peroxide contact time with teeth, resulting in an improved efficacy of tooth whitening and reduction of tooth sensitivity and other undesirable side effects. At the same time, potentially deeper penetration of peroxides may lead to longer-lasting whitening, reducing the rebound of staining and the need for repeated whitening treatments. Improvement in tooth whitening is shown here using the unique CIELAB-based, and most reliable whiteness index (WI_D) available for evaluation of tooth whitening [25].

In this work, we demonstrate the transport of hydrogen peroxide into teeth enamel by simply applying a small electric field, which, in turn, induces electrokinetic flow inside the enamel nanopores. This method is simple, cost-effective and easy to implement in the dental clinical applications. Electric current necessary to enable this delivery is small («1 mA), and, therefore, it is unlikely that this process will induce any harm or damage to the surrounding tissue. This technique could be readily translated into clinical practice since there are prior examples of applying low current («1 mA) into human tissue for clinical benefits [26–28].

5. Conclusions

In summary, we demonstrated a robust method to transport hydrogen peroxide into the enamel nanopores by nanofluidic flows driven by electrokinetics. The induced electrokinetic flows are able to carry the hydrogen peroxide through the nanopores, which efficacy is verified by monitoring the electric current during treatment and an analysis by photospectrometer. We demonstrated that the whitening effects can be significantly improved by using electrokinetic flow treatment in a short period of time compared to the diffusion-only treatment. The technique in this work is less invasive and does not require acid etching or other pretreatments. No heating or burning issue is noted during the treatment. The results of our *in vitro* study suggest that nanofluidic transport using electrokinetic flow can become a powerful tool to infiltrate various materials to greater depths in a short period of time through enamel nanochannels without any acid etching. The demonstrated level of nanofluidic transport of hydrogen peroxide into the human teeth opens opportunities in improving the whitening effect in a time period short enough to be feasible for clinical application in Dentistry. Combined with the shorter treatment time, it may also be used to infiltrate potassium nitrate (known desensitizing agent) concomitantly with the whitening agent in order to reduce tooth sensitivity. Our work may also find applications including, but not limited to, treatment of early carious lesions by transporting material to remineralize enamel, as well as the delivery of challenging biomaterials such as bones.

Author contributions

C. P. and S. P. performed the experiments. J. H., F. S., S. L. and C.P. conceived the research. C.P., F. S., H.G., S. P., S. L. and J. H. analyzed the data. W. W., S. L. and S. P. proposed the studies, discussed and provided inputs. All authors participated in discussing and writing the manuscript.

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