*Acta Odontologica Scandinavica*, 2013; 71: 343–348

informa healthcare

ORIGINAL ARTICLE

# Impact of remineralizing agents on enamel microhardness recovery after in-office tooth bleaching therapies

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**Abstract**

***Objective.*** It has been shown that bleaching with 35/38% hydrogen peroxides may alter both enamel morphology and mineral content. This study aimed to analyze the morphology and microhardness of enamel bleached with in-office hydrogen peroxides and exposed toremineralizing agents. ***Materials and methods.*** After recording initial enamel morphology and microhardness, 60 bovine incisors were bleached using either a calcium-containing 35% hydrogen peroxide (Whiteness HP Blue) or a calcium-free 35% hydrogen peroxide (Whitegold Office) ($n$ = 30). Then, the teeth were subjected to one of three post-bleaching remineralizing treatments ($n$ = 10): storage in artificial saliva only, application of a sodium fluoride gel or application of a nanohydroxyapatite-based agent (Nano-P). After 24 h and 14 days of post-bleaching treatments, the enamel morphology and microhardness were re-evaluated. The microhardness data were analyzed by means of two-way ANOVA with repeated measurements and Tukey tests ($p < 0.05$), while the enamel morphology was analyzed descriptively. ***Results.*** Samples exposed to Nano-P presented statistically the highest microhardness 24 h after its application in comparison with other remineralizing agents. The microhardness recovery did not occur in any of the groups 14 days after treatment. The morphology of all samples 14 days after the application of all remineralizing agents presented a higher number of irregularities. ***Conclusion.*** Although some remineralizing products provided microhardness recovery and a positive effect on enamel morphology at 24 h post-bleaching, none of them were able to maintain microhardness and enamel morphology at 14 days post-bleaching.

**Key Words:** *demineralization, enamel, hydrogen peroxide, microhardness, tooth bleaching*

## Introduction

The search for a more esthetic smile has grown exponentially during the last few decades, so that tooth color is currently believed to be one of the biggest concerns for patients [1]. With careful diagnosis and appropriate attention to technique, bleaching may represent a conservative and safe means to lightening discolored teeth [2]. In such cases, in-office bleaching is a popular option for patients desiring whiter teeth. An in-office bleaching procedure is a practical alternative to in-home bleaching treatments, especially with severe discoloration, poor patient compliance or the need for rapid results [3]. This method of tooth-whitening has been around for many years and remains popular because results can be seen after one appointment [4].

While in-office bleaching has proven effective for whitening discolored teeth, whether the highly concentrated hydrogen peroxides used in the technique might soften dental hard tissues is still debated. It has been shown that bleaching with 35/38% hydrogen peroxide may alter enamel morphology and mineral content [3,5,6]. It has also been demonstrated that the use of at-home bleaching agents enhanced with calcium/fluoride can reduce enamel mineral loss after bleaching [7], although the benefits of using calcium-added, in-office peroxide are still unclear in the

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*(Received 12 December 2011; revised 8 February 2012; accepted 26 February 2012)*

ISSN 0001-6357 print/ISSN 1502-3850 online 
DOI: 10.3109/00016357.2012.681119

Table I. Materials used in this study.

| Commercial name (manufacturer) | Basic composition |
|---|---|
| Whitenees HP Blue (FGM, Joinville, SC, Brazil) | 35% hydrogen peroxide, thickener, inert violet pigment, neutralizing agent, calcium gluconate, glycol and deionized water |
| Whitegold Office (Dentsply, Petrópolis, RJ, Brazil) | 35% hydrogen peroxide, thickening agent. |
| Flúor Gel (MaquiraDental Products, Maringá, PR, Brazil) | 2% sodium fluoride, sodium saccharin, propyleneglycol, methylparaben, flavoring, excipientand purified water |
| Nano-P (FGM, Joinville, SC, Brazil) | Nanostructured calcium phosphate (nanohydroxyapatite), sodium fluoride, potassium nitrate, distilled water, thickener |
| Artificial Saliva (PhormulaAtiva, Recife, PE, Brazil) | Sodium carboxymethylcellulose, xylitol, potassium chloride, sodium chloride, sodium fluoride, magnesium chloride, calcium chloride, potassium phosphate, potassium thiocyanate |

literature. Moreover, to overcome the side-effects of using hydrogen peroxide for in-office bleaching, supplementary therapies such as the application of fluoride have been strongly recommended after bleaching [8,9]. Recently, a nanohydroxyapatite-based remineralizing agent (Nano-P, FGM, Joinville, Brazil) was introduced in the market, but few studies have evaluated whether its application after bleaching promotes enamel mineral recovery. Concerning the effects of whitening agents on mineral loss in dental hard tissues, studies that investigate external bleaching therapies often test for microhardness, since this is related to the mineral content of the tooth [10].

Thus, this study aimed to analyze the morphology and microhardness of enamel bleached with in-office hydrogen peroxides enhanced or not with calcium and following the application of fluoride or nanohydroxyapatite-based remineralizing agents. The null hypotheses investigated were: the bleaching agent has no effect on enamel microhardness; remineralizing agents applied after the bleaching procedures have no effect on enamel microhardness.

## Materials and methods

### *Sample preparation*

Sixty recently extracted permanent bovine central incisors were selected and stored under refrigeration in a saturated thymol solution until testing commenced. Teeth with any visible cracks or hypoplastic defects were excluded. The roots were removed 2 mm apically to the cementoenamel junction using double-faced diamond disks (KG Sorensen, Barueri, Brazil) and were discarded. In this study, the entire crown was used [11]. The teeth were positioned in a plastic mold and embedded using a self-curing polystyrene resin (Piraglass, Piracicaba, SP, Brazil). The enamel surfaces of the teeth were ground flat using SiC paper (80-grit) and polished using 600-, 1200- and 2400-grit aluminum oxide abrasive papers and a 0.4-μm alumina polishing suspension on a polishing machine (APL-4, Arotec, São Paulo, SP, Brazil), exposing enamel in a circular area 10 mm in diameter.

### *Bleaching procedures*

The bleaching treatment was performed over 7 days using either a calcium-containing 35% hydrogen peroxide (Whiteness HP Blue, FGM, Joinville, SC, Brazil) or a calcium-free 35% hydrogen peroxide (Whitegold Office, Dentsply, Petrópolis, RJ, Brazil) ($n$ = 30). Table I shows the chemical composition of the materials used in this study.

The samples were completely covered with 0.05 ml of bleaching material, forming a layer ~ 1 mm thick. Bleaching peroxides were left undisturbed for 40 min. Afterwards, the gel was rinsed off the enamel surface by running distilled water for 30 s. Two bleaching sessions were carried out separately on the 1st and the 7th day. When not in contact with the bleaching agents, the specimens were stored in daily replenished artificial saliva at 37°C.

Immediately after the second bleaching session, the teeth were dried and subjected to one of three post-bleaching treatments ($n$ = 10): storage in artificial saliva only (control – no product was applied), application of a 2% sodium fluoride gel (Maquira Dental Products, Maringá, PR, Brazil) or application of a nanohydroxyapatite-based agent (Nano-P, FGM, Joinvile, SC, Brazil). Next, 0.5 ml of the NaF gel was applied on the enamel surface and left undisturbed for 4 min, as recommended in the manufacturer's guideline. Excess was removed with a dry cotton pellet. Then, 0.5 ml of Nano-P was applied directly on the enamel and scrubbed for 10 s using a felt disk (Diamond Flex, FGM, Joinville, SC, Brazil) mounted in a low-speed hand-piece. The material was then undisturbed for 5 min and excess was removed with a dry cotton pellet. After the application of the remineralizing agents, samples were stored in artificial saliva.

### *Microhardness evaluation*

The enamel microhardness determination was performed with a microhardness tester (Shimadzu HMV/2000, Shimadzu Corporation, Kyoto, Japan) fitted with a 50 kgf load, which was used to make indentations on the enamel surface. The loaded

Table II. Hardness means (standard-deviations) for samples subjected to each bleaching peroxide and remineralizing agent at different time-points.

| PB | RA | Time-points | | |
|---|---|---|---|---|
| | | Baseline | 24 h | 14 days |
| Whitegold Office | Nano-P | 309.1 (59.49)$^{Aa}$ | 333.2 (91.02)$^{Aa}$ | 189.1 (63.82)$^{Ba}$ |
| | F | 306.8 (75.44)$^{Aa}$ | 251.3 (33.36)$^{ABb}$ | 196.8 (70.58)$^{Ba}$ |
| | Saliva | 304.5 (36.6)$^{Aa}$ | 311.6 (92.33)$^{Aab}$ | 165.2 (45.79)$^{Ba}$ |
| Whiteness HP Blue | Nano-P | 349.6 (37.78)$^{Aa}$ | 394.4 (86.64)$^{Aa}$ | 225.8 (76.77)$^{Ba}$ |
| | F | 319.7 (82.15)$^{Aa}$ | 385.4 (85.48)$^{Aa}$ | 172.4 (42.39)$^{Bb}$ |
| | Saliva | 355.7 (51.19)$^{Aa}$ | 206.0 (53.24)$^{Bb}$ | 198.0 (48.65)$^{Bab}$ |

PB, Peroxide bleaching; RA, Remineralizing agents; 24 h, 24 hours after exposure to remineralizing agents; 14 days, 14 days after exposure to remineralizing agents; F, 2% Sodium Fluoride.
For each peroxide bleaching, means followed by different letters (capital letters comparing periods of evaluation and lower letters comparing remineralizing products) indicate statistically significant differences ($p < 0.05$).

diamond was allowed to sink and rest on the enamel surface for 30 s and the Vickers hardness number was determined. Three indentations were performed on the center of each specimen, with the distance of 100 µm between them and they were averaged. Measurements were obtained at three time-points: baseline (before bleaching), 24 h after the application of remineralizing agents and 14 days after the application of remineralizing agents.

### *Scanning electron microscopy (SEM) analysis*

Five samples were analyzed by means of SEM in an attempt to characterize the enamel morphology at baseline, after the application of bleaching peroxides and 24 h and 14 days after the application of the remineralizing agents. High-precision impressions were taken using polyvinyl siloxane-based materials (Adsil Putty Soft and Adsil Regular Body, Vigodent, Rio de Janeiro, RJ, Brazil) and the double-impression technique. An initial putty impression was recorded and allowed to fully set. Then, a regular body material was carefully applied both into the first impression (the first impression was used as a customized tray) and on the sample in order to obtain a very precise final impression. Replicas were prepared by pouring the impressions with an epoxy resin (Epoxicure, Lake Bluff, IL). Then, epoxy replicas were sputter-coated with gold (MED 010; Baltec, Balzers, Liechtenstein) and observed using a scanning electron microscope (XL30, Philips, Eindhoven, The Netherlands). Representative images of each specimen were taken by two independent observers with a blind study design.

### *Statistical analysis*

Microhardness data were analyzed by means of two-way ANOVA with repeated measurements and Tukey tests. The Assistat 7.6 Beta Software (Federal University of Campina Grande, Campina Grande, PB, Brazil) was utilized to perform statistical tests at a pre-set significance level of 5%. Microhardness means of bleaching peroxides were compared separately. SEM images were characterized descriptively.

## Results

### *Microhardness evaluation*

*Whitegold office.* There were no statistically significant differences among remineralizing agents ($p > 0.05$). However, there were statistically significant differences among the time-points ($p < 0.05$). Comparisons among groups are listed in Table II. Before bleaching, samples of all groups were statistically similar. Samples exposed to Nano-P presented statistically the highest microhardness 24 h after its application in comparison with other remineralizing agents. Fourteen days after exposure to remineralizing agents, all three materials provided statistically similar microhardness means. Only fluoride did not recover microhardness means at 24 h, while none of the materials rehardened enamel 14 days after exposure to them.

*Whiteness HP blue.* There were statistically significant differences among remineralizing agents ($p < 0.05$), among time-points ($p < 0.05$) and in the interaction of remineralizing agents vs time points ($p < 0.05$). Comparisons among groups are listed in Table II. Before bleaching, samples of all the groups were statistically similar. Samples exposed to Nano-P and fluoride presented statistically the highest microhardness 24 h after their applications in comparison with other remineralizing agents. Fourteen days after exposure to remineralizing agents, samples on which Nano-P was applied after bleaching showed the highest microhardness means. Only saliva did not recover microhardness means at 24 h, while none of the materials rehardened enamel 14 days after exposure to them.

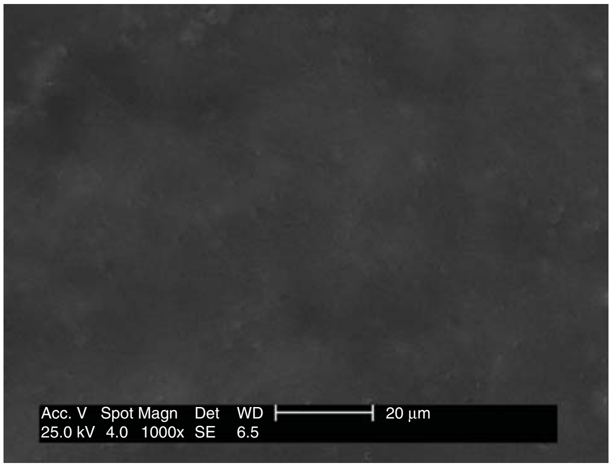


Figure 1. Baseline images showed flat and polished enamel.

*SEM evaluation*

Baseline images showed flat and polished enamel (Figure 1). After the application of HP Blue, calcium deposits could be observed (Figure 2). Conversely, after the application of Whitegold Office, no calcium deposition was observed and some irregularities such as depressions could be perceived (Figure 3). Twenty-four hours after the application of Nano-P, samples previously bleached with both Whiteness HP Blue and Whitegold Office showed a large number of nanohydroxyapatite deposits (Figure 4). For samples previously bleached with Whiteness HP Blue, 24 h after the application of saliva, slightly porous enamel with some depressions could be detected. A similar morphology was observed for samples previously bleached with Whitegold Office 24 h after the application of sodium fluoride (Figure 5). On the other hand, the morphology of samples previously bleached with Whitegold Office 24 h after the application of saliva presented characteristics similar to those from the baseline. Also, a similar morphology was observed for samples previously bleached with Whiteness HP Blue 24 h after the application of fluoride (Figure 6). The morphology of all samples 14 days after the application of all remineralizing agents presented a higher number of irregularities and depressions (Figure 7).

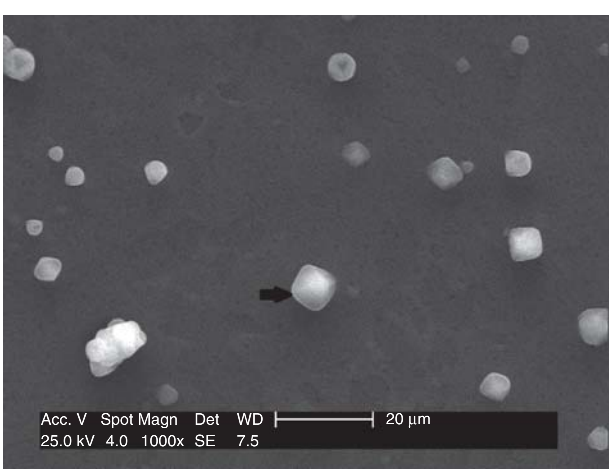


Figure 2. Calcium deposits could be observed after the application of Whiteness HP Blue.

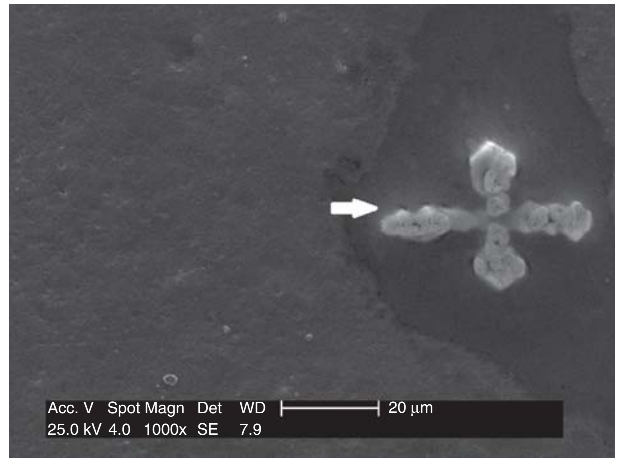


Figure 3. Some depressions could be perceived (black arrow) after the application of Whitegold Office. The white arrow shows an indentation.

## Discussion

Although 24 h after the application some remineralizing agents could recover initial microhardness values, none of the remineralizing agents was capable of maintaining microhardness values 14 days after its application. Moreover, the application of Nano-P could provide a great mineral deposition in samples only 24 h after its application. Thus, the null hypothesis that there will be no differences for enamel morphology and microhardness among tested conditions was rejected.

For the in-office bleaching of vital teeth, a high percentage of hydrogen peroxide (25–38%) is

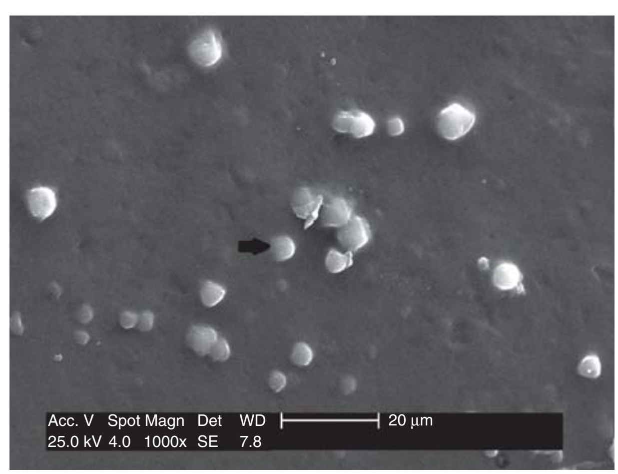


Figure 4. Samples previously bleached with both Whiteness HP Blue and Whitegold Office showed a high number of nanohydroxyapatite deposits 24 h after the application of Nano-P.

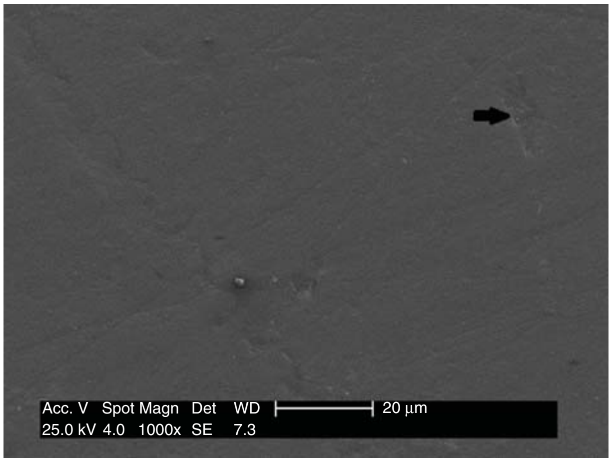


Figure 5. Slightly porous enamel with some depressions could be detected 24 h after the application of saliva for samples previously bleached with Whiteness HP Blue. A similar morphology was observed 24 h after the application of 2% sodium fluoride for samples bleached with Whitegold Office.

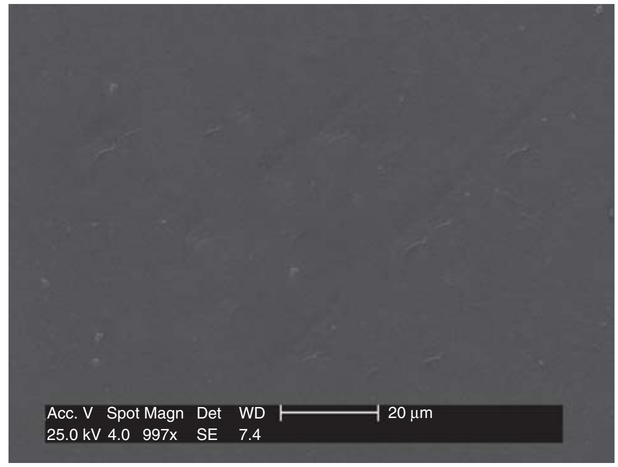


Figure 6. The morphology of samples previously bleached with Whitegold Office 24 h after storage in saliva was similar to that seen at baseline. This was also observed for samples previously bleached with Whiteness HP Blue 24 h after the application of 2% sodium fluoride.

frequently used [12,13]. It is known that the oxide-reduction reaction of the bleaching agent could lead to the dissolution of the organic and inorganic dental matrix until only carbon dioxide and water remain [14]. Additionally, it has been shown that bleaching with 35/38% hydrogen peroxides may alter enamel morphology and alter mineral content [3,6]. Thus, efforts have been made to achieve a protocol capable of remineralizing bleached enamel, recovering micro-hardness loss and surface alterations.

The finding that the application of a nanohydroxyapatite-based paste (Nano-P) provided a high mineral deposition on the bleached enamel, regardless of the type of bleaching agent used, is of great concern. In fact, it has been shown that bleaching can reduce the amount of calcium and phosphorous, in addition to modifying the morphology of a large quantity of crystals in the superficial layer, when compared with non-treated enamel [15,16]. Thus, nanohydroxyapatite crystals could have been immediately incorporated to the superficial enamel after bleaching the teeth with both of the hydrogen peroxides, favoring mineral uptake and hardness recovery.

On the other hand, 2% sodium fluoride and saliva only promoted different microhardness values and surface morphology 24 h after their application, depending on the hydrogen peroxide utilized. The finding that only 2% sodium fluoride was not able to recover microhardness and to avoid enamel injuries in samples bleached with Whitegold Office 24 h after its application might be attributed to the absence of fluoride incorporation into the bleached enamel. It is likely that a single application of 3 min was not sufficient to provide sufficient fluoride uptake. However, the calcium deposits present on enamel bleached with Whiteness HP Blue might have favored fluoride binding, recovering microhardness and promoting enamel integrity. Likewise, the application of saliva 24 h after its application promoted enamel recovery and integrity only for samples bleached with Whitegold Office. It might be assumed that the calcium and phosphorous loss after bleaching [15,16] in the absence of calcium on the surface of bleached enamel could have favored ion uptake from saliva.

Nevertheless, although some remineralizing agents in combination with peroxides could favor microhardness recovery and enamel integrity/mineral deposition 24 h after their application, they were not capable of maintaining microhardness and enamel integrity 14 days after their application. These findings might be justified by the short-term contact of the remineralizing agents with the enamel. It should be assumed that a single application is not sufficient to supply the sufficient amount of minerals to maintain

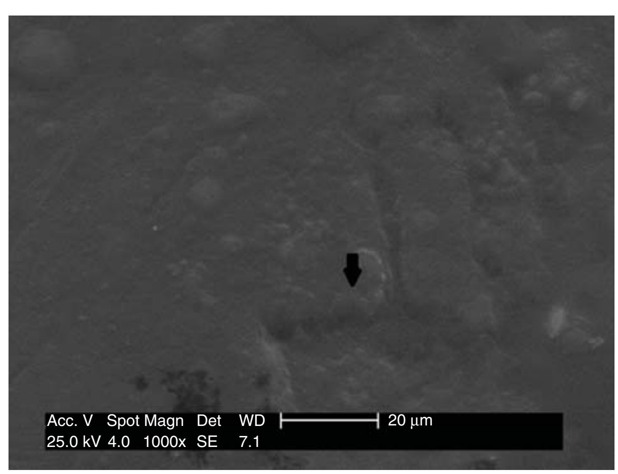


Figure 7. A high number of irregularities and depressions was observed 14 days after the application of all remineralizing agents, regardless of the bleaching peroxide utilized previously.

microhardness and enamel integrity recovered 24 h after their application. Moreover, it has been demonstrated that products released from bleaching gels remain in the enamel and dentine for up to 2 weeks [17]. Thus, a higher number of applications of remineralizing agents on bleached enamel might be necessary to maintain the benefits achieved 24 h after their application. Moreover, the incorporation of nanohydroxyapatite into high-concentration hydrogen peroxides might be another alternative to increase ion uptake, since previous studies have found some advantages to bleaching enamel with enhanced gels [7,11]. It should be noted that this is an *in vitro* study. Thus, further clinical studies should to be performed to analyze the effects of remineralizing substances on sensitivity.

According to this *in vitro* study, 14 days after the bleaching treatment, the two bleaching agents decreased the microhardness of enamel and the remineralizing gels used in this study were not able to keep the values of initial microhardness.

## Acknowledgments

The authors thank Vigodent S/A Indústria e Comércio for providing the polyvinyl siloxane based materials used in this work. The authors also show gratitude to NEPGN (Petroleum and Natural Gas Research Center) and to CTPETRO-INFRA I and FINEP/LIEM projects for SEM assistance.

***Declaration of interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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