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ORIGINAL ARTICLE

Analysis of dentinal erosion and removing smear layer of different irrigation protocols: an *in vitro* study

ABSTRACT

Aim: *A material with an acidic pH and desiccating action (HybenX) has been generated to destroy the dental biofilm. This study aims to investigate the effect of HybenX used as an irrigating solution, estimating its efficacy in the elimination of the smear layer and rating how it may influence dentinal erosion.*
Methodology: *One hundred extracted, single-rooted, human teeth were used. Five groups were made in a random way (n=20), considering the type of irrigant used at the end of the instrumentation with ProTaper Gold sequence SX F4: Group A (NaOCl), Group B (NaOCl - EDTA 17%), Group C (NaOCl - EDTA 17% - NaOCl), Group D (NaOCl - HybenX), Group E (NaOCl - HybenX - NaOCl). The amount of the smear layer and the erosion were evaluated according to the Torabinejad method using a scanning electron microscope. A Kruskal-Wallis test was performed at each portion (i.e. apical, middle, coronal) and overall for both smear layer removal and erosion variables. A multiple comparison analysis was implemented as well within each portion and overall for both variables.*
Results: *The difference in debris removal at all three levels of the canals was statistically significant, comparing the five treatment groups (p<.0001). The statistic test showed a statistically significant greater erosion overall between group A and the other four groups (p<.0001).*
Conclusions: *Under the conditions of the present study, the use of a combination of NaOCl and HybenX, efficiently removes smear layer and produces a lower degree of erosion if compared with 17% EDTA.*

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Introduction

The instrumentation of a root canal produces smear layer, a 1 to 2 μ amorphous structure coating the canal walls, formed by an organic and an inorganic component, that gets into the dentinal tubules (smear plug) as far as 40 μ (1-3).
Its presence impedes irrigants, medications and filling materials to penetrate into the dentinal tubules and also might prevent them from touching the canal walls.
To remove the smear layer, a number of chelating substances and acid solutions have been suggested.
The most recommended combination of irrigants, to efficiently remove the smear layer from the root canal wall, has resulted being EDTA and NaOCl; a careful evaluation of this combination has been analysed, concerning application time and volumes, irrigation mode and the alternation of these two solutions (4-8).
Some authors pointed out that the above mentioned irrigation protocol can significantly modify the dentin mechanical properties (9-11).
A different irrigating solution (HybenX®, EPIEN Medical, Saint Paul, MN, USA) has been generated to destroy the dental biofilm; its composition is a mixture of hydroxybenzenesulfonic acid (37%) and hydroxymethoxybenzene acids (23%), sulphuric acid (28%), and water (12%). The product is currently marketed by the producer both for use in Periodontics (HybenX Oral Tissue Decontaminant) and for Endodontics (HybenX Root Canal Cleanser). The two forms of the product, which have the same chemical composition, differ in consistency: more viscous gel for periodontal use and more liquid gel for endodontic use.
This study aims to investigate the effect of HybenX used as an irrigating solution, estimating its efficacy in the elimination of the smear layer and rating how it may influence dentinal erosion.

Materials and Methods

In this study, 100 maxillary and mandibular, no decay, single-rooted, human teeth extracted for periodontal reasons were used. The local Ethical Committee approved the study protocol. For all patients an informed consent was obtained in order to include their teeth in the study.
Teeth with coronal restorations or endodontic treatments were excluded. To be sure that all teeth had a single canal, no complicated anatomy and apical curves and no calcifications, each one was checked with digital X-rays (Gendex, Hatfield, PA, USA). The selected teeth were mechanically cleaned from soft tissue and debris, stored in saline water and at a temperature of +4 °C.
After cutting out the crowns at the cement-enamel junction using a high-speed bur, the roots were standardized to a 15 mm length using a diamond disc at low speed. Fine barbed instruments were used to remove any residual pulp tissue.
With the use of a water-cooled diamond disc, parallel grooves were traced along the buccal and lingual surfaces, without touching the inner face.
To establish the working length (WT), an ISO size #10 K-type file (Dentsply Sirona, Ballaigues, Switzerland) was introduced into the root canal until just visible at the apical foramen. The apex was left unsealed, to guarantee a communication of air and vapour with the external surroundings.
ProTaper Universal Rotary instruments (Dentsply Sirona), up to apical size (F4), were used to prepare the root canal and a size 5 Gates Glidden drill (Dentsply Sirona), in the coronal 5 mm, to create a reservoir for the irrigant solution.
Between each file, 2 ml of 5.25% NaOCl (Ogna, Lab Srl, Muggiò, MB, Italy) were utilized to irrigate the canals, with a 30-G syringe needle (Kerr Dental, Orange, CA, USA), moved back and forward and keeping the needle 1 mm shorter than the WL.
The final irrigation, at the end of the shaping's procedures, was carried out with 5 ml of 5.25% NaOCl for 1 minute, followed by 5 ml of distilled water for 1 minute.
Five groups of 20 teeth each were made in a random way, considering the type of irrigant used at the end of the instrumentation.
• Group A: NaOCl

- Group B: NaOCl - EDTA 17% (Ogna, Lab Srl, Muggiò, MB, Italy)
- Group C: NaOCl - EDTA 17% - NaOCl
- Group D: NaOCl - HybenX
- Group E: NaOCl - HybenX - NaOCl

5 ml of each solution from groups A, B and C were applied to remove the smear layer from the surface of the root canals; the exposure time was approximately 2 minutes.

Groups D and E irrigation mode were slightly different, due to the tested solution's physical features. HybenX is indeed a thicker and more viscous liquid, so, while the exposure time remained the same (2 minutes), the product was put in the upper half of the canal and then spread to the remaining part of the canal by a fitting size sterile paper point (ProTaper F4, Dentsply Sirona), moving it up and down all the time.

A final irrigation with 10 ml of sterile distilled water, followed by the insertion of a paper point to dry the canal was the last step of the procedure for all groups.

Evaluation by Scanning Electron Microscopy

Parallel grooves were traced along the buccal and lingual surfaces of 100 roots split in two halves, along the longitudinal axis, obtaining 200 sections in total.

Each group, containing 40 sections, 20 of which had been randomly chosen and the other 20 discarded.

A scanning electron microscope (SEM) (FEI Quanta 200 © 2018 Thermo Fisher Scientific) operating at 8 kV at a magnification of 2,000 was used to observe the samples of each group.

Three random images of the apical (0-5 mm), middle (5-10 mm) and coronal (11-15 mm) portions of each sample, from the canal dentin wall surface, were acquired using a motorized specimen stage. The area to be analysed was inspected at a low magnification (200x). The magnification was then, increased (2,000x), without moving the microscope.

In total, 60 images for each experimental group were obtained.

Two trained and blinded evaluators independently rated each masked fragment. Evaluators had no prior knowledge of the cleaning/shaping procedures; the types of irrigant used at the end of the instrumentation, and were well acquainted with qualitative analysis of the SEM images. When evaluator scores disagreed, the lower score was taken.

A four-level scoring system based on the severity of smear layer retention was used to evaluate the efficacy of smear layer removal (12). This scoring system' s criteria were:

1. no smear layer. Absence of smear layer on the surface of the root canals; all tubules open and clean;
2. moderate smear layer. Absence of smear layer on the surface of root canal, but tubules contained debris;
3. heavy smear layer. Smear layer covered the root canal surface and the tubules.

Then the degree of erosion of dentinal tubules was also scored as follows:

1. no erosion. All tubules normal in size and appearance;
2. moderate erosion. The peritubular dentin was eroded;
3. severe erosion. The intertubular dentin was damaged and tubules were connected with each other.

Statistical Analysis

The analysis was produced using SAS® version 9.4 in a secure and validated environment. The procedure "NPAR1WAY" was used.

A one-way ANOVA, which for Wilcoxon score is known as the Kruskal-Wallis test, with group as fixed factor was performed at each portion (i.e. apical, middle, coronal) and overall for both smear layer removal and erosion variables.

Additionally, Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis, which is based on pairwise two-sample Wilcoxon comparisons was implemented as well within each portion and overall for both variables. A multiplicity correction for 10 simultaneous comparisons was applied within each portion and overall by using the Šidák alpha adjustment (i.e. $1-(1-\alpha)^{\frac{1}{m}}$ with m representing the number of pairwise contrasts).

Table 1

Descriptive statistics

(Median [interquartile range])

		Group A	Group B	Group C	Group D	Group E
		N=20	N=20	N=20	N=20	N=20
Erosion	Overall	1 [0]	2 [1]	3 [0]	2 [1]	2 [1]
	Coronal	1 [0]	2 [0]	3 [0]	2 [0]	2 [0.5]
	Middle	1 [0]	2 [2]	3 [1]	2 [0.5]	2 [1]
	Apical	1 [0]	1 [0]	3 [1]	1 [0]	1 [0]
Smear Layer	Overall	3 [0]	1 [1]	1 [0]	1 [1]	1 [1]
	Coronal	3 [0]	1 [0]	1 [0]	1 [0]	1 [0]
	Middle	3 [0]	1 [0]	1 [0]	1 [1]	2 [1]
	Apical	3 [0]	2 [1]	1 [1]	2 [0.5]	2 [0]

Table 2

Kruskal-Wallis test

		Chi-Square	Pr>Chi-Square
Erosion	Overall	142.0893	<.0001
	Coronal	74.505	<.0001
	Middle	46.0793	<.0001
	Apical	58.5154	<.0001
Smear Layer	Overall	159.5796	<.0001
	Coronal	92.0204	<.0001
	Middle	51.567	<.0001
	Apical	142.0893	<.0001

Results

The descriptive statistics is shown in Table 1.

The Kruskal-Wallis test resulted highly statistically significant ((p<.0001) at each portion and overall indicating a difference between groups for both Erosion and Smear Layer variables (Table 2).

In group A the root canals showed heavy smear layer along its whole length (Figure 1).

The difference in debris removing at all three levels of the canals and overall was highly statistically significant, comparing group A vs. groups B, C, D and E (p<.0001).

Additionally, at apical third the smear layer removal's is significantly higher in group C (p=0.002) and E (p=0.004) than in group B.

Considering the multiple pairwise contrasts for the erosion, groups B, C, D and E presented a significantly greater erosion than in group A overall, and at apical and middle third.

At the coronal third exactly the same situation such as above was observed (Figure 2).

The lower erosion of group A with respect to the other four treatment groups was endorsed also at the middle third (p<.0001). Group C confirmed to have significantly greater erosion than group D and E (p< 0.05) while, if compared to group B, only a trend is shown (p=0.0647) (Figure 2).

The greater erosion in group C compared to groups A, B, D and E at the apical third was also confirmed (p<.0001).

The multiple comparisons both for erosion and smear layer are fully shown in Table 3.

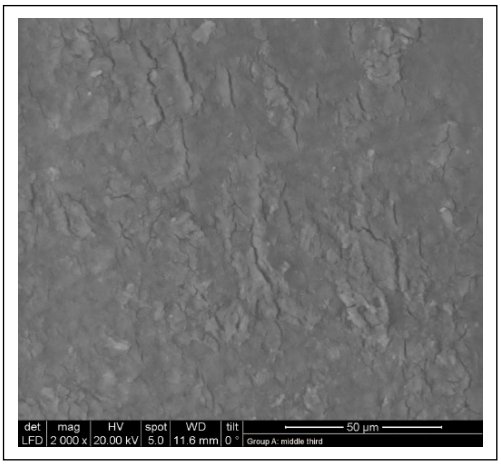

Figure 1.
Heavy smear layer in group A (middle third).

Discussion

Based on the results observed in this study, the use NaOCl only didn't remove the smear layer as efficiently as in the samples in group B, C, D and E, in which the root canal surface and the dentinal tubules appeared to be much cleaner.

This study's results confirm that substances containing EDTA in groups B and C, and HybenX, in groups D and E are fundamental to remove smear layer efficiently.

In literature, on the other hand, is well documented how the use of a chelating agent to dissolve the inorganic components of the smear layer, can lead to a different levels of erosion (13-19). In addition some

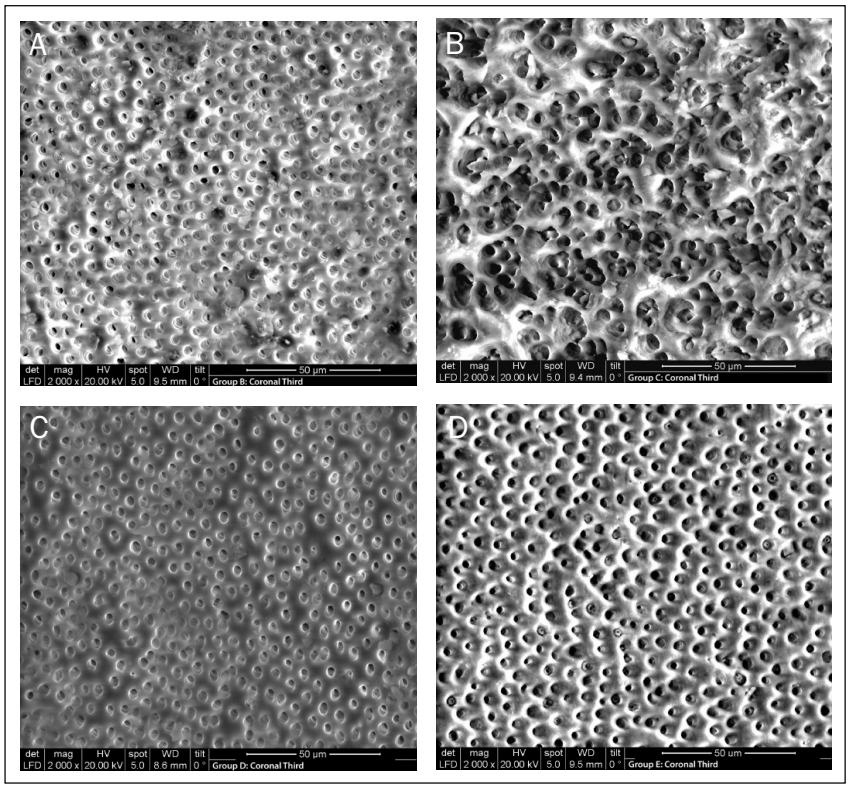

Figure 2 A-D.
Aspects of erosion in groups B (A), C (B), D (C) and E (D) respectively.

studies demonstrate that irrigants can alter the structure of collagen and mineral content of the dentin, changing the proportion of phosphate and calcium. This results in a reduction of microhardness and flexural strength of the dentin, which could be a potential risk factor for vertical root, fractures (20-23).

HybenX ability in removing the smear layer is not due to a chelating action but is likely produced by its chemical features: it is a mixture of sulphonic/sulphuric acids, so it has a strong affinity to water. Its acidic pH is active in dissolving the inorganic debris. Beyond that, the sulphate group exhibit oxygen atoms that provide it with a large negative charge; this attracts water molecules since their hydrogen atoms have a positive charge. Consequently, the determined chemical effect is dehydration and disintegration of organic biofilms.

From our observations it's clear that the higher level of erosion is shown in the irrigation sequence of group C: NaOCl - EDTA 17% - NaOCl. Especially at the coronal and middle third, the dentin is seriously eroded, the dentinal tubules are widened, their entrances look irregular and the peritubular dentin has totally disappeared. In some sites, the intratubular dentin has completely collapsed and has created pits.

In view of all these considerations it can be observed how in group B the erosion is definitively less and mainly confined to the peritubular dentin. In group D the grade of erosion is insignificant, the dentinal surface is homogeneous, smooth, with tubules free of debris. In group E the dentinal surface is smooth, with slight pits at the tubule's entrance (Figure 3). In group E, a dissolving action is shown, due to the NaOCl final rinse: this event is much lighter in group E than in group C, because HybenX's eroding action is definitively lower than EDTA's.

The results of the present study are based on laboratory SEM experiments in according to the Oxford Centre for Evidence Based Medicine are of grade 2 level of evidence (24).

Figure 3 A-C.
Aspects of the root canal walls in group E at the apical third (A), middle third (B) and coronal third (C) respectively.

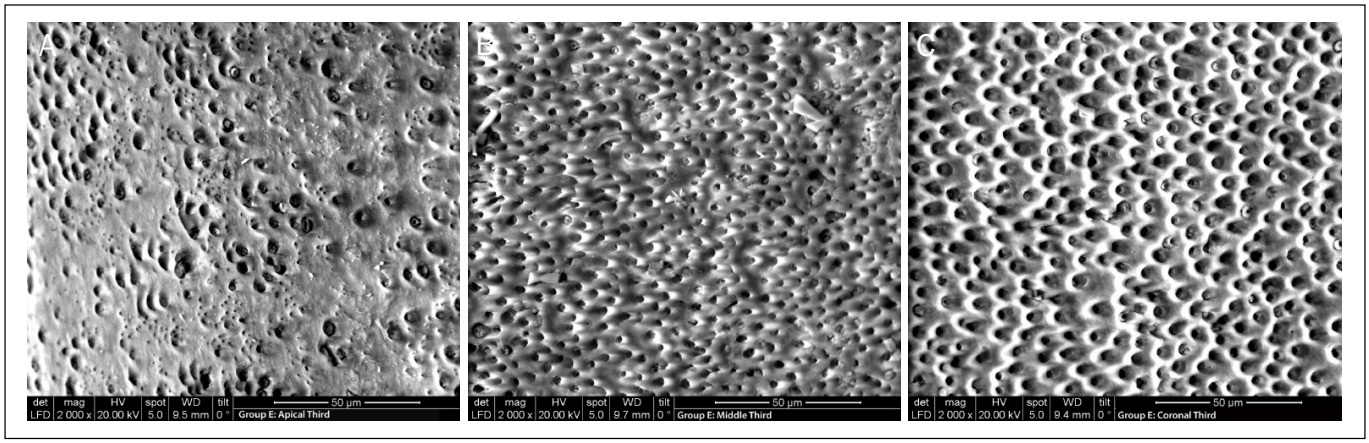

Table 3

Multiple Comparison

		Comparison	DSCF Value	Pvalue	Significance considering Sidak alpha adjustment
EROSION	Overall	Group A vs. Group B	9.40	<.0001	Yes
		Group A vs. Group C	14.43	<.0001	Yes
		Group A vs. Group D	10.21	<.0001	Yes
		Group A vs. Group E	10.55	<.0001	Yes
		Group B vs. Group C	9.91	<.0001	Yes
		Group B vs. Group D	0.28	1.00	No
		Group B vs. Group E	1.21	0.91	No
		Group C vs. Group D	10.60	<.0001	Yes
		Group C vs. Group E	9.64	<.0001	Yes
		Group D vs. Group E	1.05	0.95	No
	Coronal	Group A vs. Group B	7.79	<.0001	Yes
		Group A vs. Group C	8.73	<.0001	Yes
		Group A vs. Group D	7.91	<.0001	Yes
		Group A vs. Group E	8.44	<.0001	Yes
		Group B vs. Group C	7.02	<.0001	Yes
		Group B vs. Group D	0.99	0.96	No
		Group B vs. Group E	1.77	0.72	No
		Group C vs. Group D	7.82	<.0001	Yes
		Group C vs. Group E	6.31	<.0001	Yes
		Group D vs. Group E	3.02	0.20	No
	Middle	Group A vs. Group B	6.33	<.0001	Yes
		Group A vs. Group C	8.40	<.0001	Yes
		Group A vs. Group D	6.72	<.0001	Yes
		Group A vs. Group E	6.68	<.0001	Yes
		Group B vs. Group C	3.72	0.06	No
		Group B vs. Group D	0.89	0.97	No
		Group B vs. Group E	0.31	1.00	No
		Group C vs. Group D	5.25	0.002	Yes
		Group C vs. Group E	4.42	0.02	No
		Group D vs. Group E	0.64	0.99	No
	Apical	Group A vs. Group B	1.41	0.86	No
		Group A vs. Group C	7.75	<.0001	Yes
		Group A vs. Group D	2.94	0.23	No
		Group A vs. Group E	2.94	0.23	No
		Group B vs. Group C	7.52	<.0001	Yes
		Group B vs. Group D	2.07	0.59	No
		Group B vs. Group E	2.00	0.62	No
		Group C vs. Group D	6.25	<.0001	Yes
		Group C vs. Group E	6.94	<.0001	Yes
		Group D vs. Group E	0.22	1.00	No

		Comparison	DSCF Value	Pvalue	Significance considering Sidak alpha adjustment
SMEAR LAYER	Overall	Group A vs. Group B	12.88	<.0001	Yes
		Group A vs. Group C	14.43	<.0001	Yes
		Group A vs. Group D	13.83	<.0001	Yes
		Group A vs. Group E	14.55	<.0001	Yes
		Group B vs. Group C	4.15	0.03	No
		Group B vs. Group D	0.48	1.00	No
		Group B vs. Group E	0.24	1.00	No
		Group C vs. Group D	3.96	0.04	No
		Group C vs. Group E	4.97	0.002	Yes
		Group D vs. Group E	0.83	0.98	No
	Coronal	Group A vs. Group B	8.73	<.0001	Yes
		Group A vs. Group C	8.83	<.0001	Yes
		Group A vs. Group D	8.73	<.0001	Yes
		Group A vs. Group E	8.83	<.0001	Yes
		Group B vs. Group C	1.41	0.86	No
		Group B vs. Group D	0.00	1.00	No
		Group B vs. Group E	1.41	0.86	No
		Group C vs. Group D	1.41	0.86	No
		Group C vs. Group E	0.00	1.00	No
		Group D vs. Group E	1.41	0.86	No
	Middle	Group A vs. Group B	8.40	<.0001	Yes
		Group A vs. Group C	8.73	<.0001	Yes
		Group A vs. Group D	8.07	<.0001	Yes
		Group A vs. Group E	8.33	<.0001	Yes
		Group B vs. Group C	0.05	1.00	No
		Group B vs. Group D	2.75	0.29	No
		Group B vs. Group E	4.53	0.01	No
		Group C vs. Group D	2.93	0.23	No
		Group C vs. Group E	4.82	0.01	No
		Group D vs. Group E	1.97	0.63	No
	Apical	Group A vs. Group B	5.78	0.003	Yes
		Group A vs. Group C	7.39	<.0001	Yes
		Group A vs. Group D	7.36	<.0001	Yes
		Group A vs. Group E	8.50	<.0001	Yes
		Group B vs. Group C	5.22	0.002	Yes
		Group B vs. Group D	3.51	0.10	No
		Group B vs. Group E	4.94	0.004	Yes
		Group C vs. Group D	2.83	0.26	No
		Group C vs. Group E	2.83	0.27	No
		Group D vs. Group E	0.67	0.99	No

Some authors (25) pointed out how conventional scanning electron microscopy can produce considerable distortions: for examples they don't ~~not~~ allow the observation of wet areas since the sample-chamber operates under high vacuum.
In this study an environmental electron microscope (ESEM) that allows the visualization of fresh dentinal preparations without having to subject them to dehydration and metallization processes was used, minimizing this bias.
Regarding the quantification of the results, the subjective nature of scoring systems required preliminary training to reduce interexaminer differences; two evaluators (VG and DL) were trained to read SEM images: a calibration kit of 100 original images not associated with the study and representing a wide range dentinal aspect was used. Agreement between and within examiners was determined by using the intra-class correlation coefficient.
An ideal model for evaluating intracanal cleanliness does not yet exist (26). For that reason it was possible to detect the following limitations of the present study. The preparation of samples was standardized with a high apical diameter (size 40) which, generally, does not represent a common clinical situation; this was done to evaluate exclusively the action of the irrigating solutions in a standard experimental situations, unbound from intrinsic anatomical variables naturally present in clinical situations.
Furthermore, increased apical size and taper allowed enhanced irrigation in all areas of the root canal system and larger instruments may be employed to improve contact with canal walls, thereby producing more efficacious cleaning (27-29).
The evaluation of the erosion degree was carried out only through a surface score and therefore it is not possible, with this kind of study, to define the extent of the phenomenon along the thickness of the dentinal wall nor to determine any variations in the dentin microhardness.
Clearly, these *in vitro* preliminary data should be followed by further *in vitro* and *in vivo* investigations.

Conclusions

Our observations showed that, under these experimental conditions, the use of HybenX, was able to effectively remove the smear layer after irrigating the canal with 5% sodium hypochlorite.
Furthermore this irrigant produces a significantly lower degree of dentinal erosion than EDTA both if it was used alone and when its action was followed by irrigation with sodium hypochlorite (Figure 3).

Clinical Relevance

The present study showed a valid irrigation protocol that can successfully remove the debris on the root canal wall and, at the same time, reduce the erosion of the dentine, if compared with chelating agents, such as 17% EDTA.

Conflict of Interest

The authors deny any conflicts of interest.

Acknowledgements

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