



# Dentinal tubule blockage using nanobioglass in the presence of diode (980 nm) and Nd:YAG lasers: an in vitro study

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## Abstract

**Objectives** The main objective of this study was to enhance the blockage of dentinal tubules using nanobioglass in the presence of diode (980 nm) and Nd:YAG lasers in order to reduce permeability and dentin hypersensitivity.

**Materials and methods** Thirty-six dentinal samples were randomly divided into 6 subgroups ( $n = 6$ ): (A) control, (B) diode laser (980 nm, 3-W), (C) Nd:YAG laser (1064, 1.0-W), (D) nanobioglass, (E) nanobioglass + diode laser (980 nm), (F) nanobioglass + Nd:YAG laser. The average number of open dentinal tubules was qualitatively and quantitatively evaluated by scanning electron microscopy (SEM). Data were evaluated by SPSS software version 22, Kruskal–Wallis test, and Mann–Whitney tests with Bonferoni’s correction ( $\alpha = 0.008$ ).

**Results** Based on the results of Mann–Whitney test, there was a significant difference in the mean number of open dentinal tubules between the control group and the other groups ( $p < 0.008$ ). However, the difference among the other groups was not statistically significant ( $p > 0.008$ ).

**Conclusions** Findings of this study showed that high-power laser radiation, such as Nd:YAG and diode (980 nm) alone or with nanobioglass, has a significant effect on the blockage of dentinal tubules.

**Clinical relevance** Introduction of non-invasive methods with long-term and lasting effect on reducing pain and discomfort caused by dentin hypersensitivity

**Keywords** Dentin hypersensitivity · Nanobioglass · Nd:YAG · Diode laser (980 nm)

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## Introduction

One of the most common issues in dentistry is that patients have to deal with dentin hypersensitivity (DH). The importance of this issue is evident as around 8–57% of adults suffer from increased dentin hypersensitivity [1]. Also, the prevalence of DH in patients with periodontal disease or those receiving basic therapies (such as scaling and root planning), as well as periodontal surgeries, is much higher (60–98%) [2]. Therefore, DH can be considered as one of the most painful chronic conditions with the least predictive value in terms of success in dental sciences. This is a painful response due to a variety of chemical, thermal, or osmotic stimuli on the uncovered dentin [1]. The success of various therapeutic methods and agents, such as fluoride and nitrate toothpastes, topical desensitizers (e.g., fluoride salts, potassium nitrate (KNO<sub>3</sub>), strontium chloride, and arginine), and resins, has been evaluated in the past studies. Also, the use of laser technology for treating tooth sensitivity has received attention [1].

More than 90% of the areas involved in DH are dentinal surfaces adjacent to the gingiva [2]. Recent studies have shown that healthy and non-sensitive dentin surfaces often have tubules with closed openings, while sensitive tissues have a large cumulative volume of open tubules [3–7]. The most important causes of uncovered root surfaces and exposure of dentinal tubules can be due to erosion, attrition, abrasion, or gingival recession following scaling and root planning [5].

The primary goal of DH treatment is changes in diameter and tubular content, and to this end, various desensitizer compounds and non-pharmacological treatments have been introduced. Generally, desensitizer agents can reduce dentinal sensitivity in two ways. The first method is to reduce the flow of tubular fluid, and the second one involves reducing the neuronal function through ion interactions affecting the transmission of neural signals. Consequently, compounds such as arginine 8% with calcium phosphate or ammonium hexafluorosilicate are substances that are primarily involved in the first method, while steronium chloride, potassium nitrate, and varnish fluoride are the compounds used in the second mechanism for the treatment of dentin hypersensitivity [5, 8, 9].

Laser technology is one of the most important non-pharmacological treatments that have been widely employed for treating DH. In this regard, various types of lasers, such as CO<sub>2</sub>, Nd:YAG, Er:YAG, and He-Ne, have been used [2, 5]. The effect of laser radiation on reducing dentinal hypersensitivity can be due to tubular obstruction or a change in the irritability threshold of pulpal neurons. However, several studies have been conducted on

the ability of different lasers to obstruct tubules and to compare their function with desensitizer compounds [2, 10–12].

Another method involves using nanoparticles of various compounds to improve the quality of tubular obstruction by reducing the size and facilitating the penetration of particles into dentinal tubules. Bioglass with the ability to stimulate crystallization and to create a new mineral layer can be one of the most important agents in this field [13].

Although previous studies have introduced various therapeutic and non-drug therapies and some of them were somewhat successful, treating dentin hypersensitivity with long-term success is still considered as a major problem. Hence, the purpose of this study was to treat tooth sensitivity by enhancing the blockage of dentinal tubules using nanobioglass in the presence of diode (980nm) and Nd:YAG lasers.

## Materials and methods

In this study, 36 dentinal samples were selected from third molar teeth without decay or restoration. Periodontal tissues were removed and placed in 0.1% thymol for 1 month and then kept in distilled water at 4 °C [14]. Then, the enamel of the occlusal surface was removed and dentin disks with dimensions of 4 \* 4 \* 2 mm were prepared by the microtome machine (Leica SP1600, Nussloch, Germany). Following this, the dentin disks were immersed in acrylic polymethylmethacrylate (Acropars, Iran). Leveling and polishing the dentin surface was carried out using silicone waterproof sandpaper (APC, Iran) with sequences of 150, 240, 400, and 600 grits [15]. Each sample with cracks or structural defects was excluded from the study. In order to open the dentinal tubules in a more effective way, we put 14% ethylenediaminetetraacetic acid (EDTA pH 7.4) on the dentin slabs for 2 min and then rinsed thoroughly with water spray [16].

## Synthesis of nanobioglass

The nanobioglass was synthesized using the sol-gel method [17]. Briefly, in a solution of water/ethanol (2:1), tetraethyl orthosilicate (TEOS 98%, Merck, Germany) was mixed with calcium nitrate tetrahydrate (Merck, Germany). By adding citric acid (1 M, Merck, Germany), the pH of solution was adjusted to 2.0. The process was followed by stirring until a clear solution was obtained (solution A). A solution of 2% polyethylene glycol (MW: 2000, Merck, Germany) and diammonium hydrogen orthophosphate (Sigma-Aldrich Canada Ltd.) was prepared and through adding ammonia (25% Merck, Germany), its pH was fixed to 10 (solution B). Both solutions A and B were mixed under the stirring for 10 h to obtain a homogenous gel. After being washed with deionized water and filtering, the obtained white gel was

dried and lyophilized and finally it was calcined at 650 °C for 10 h with a heating rate of 5 °C/min.

## Characterization of nanobioglass

### XRD examination

X-Ray diffraction pattern (XRD) was used to investigate the crystal structure of nanobioglass. Panalytical Xpert PRO X Ray Diffractometer (Panalytical, Netherlands) model Xpert Pro MPD with wavelength 1.5405 Å and power 40 kV/30 mA was used to study the structure and crystal phase of the nanobioglass by scanning it in the range of 20 to 80°.

### SEM examination

The surface morphology, shape, and size of synthesized nanobioglasses were assessed by scanning electron microscope (SEM) model XL30 (Philips, Netherlands).

Subsequently, the prepared samples were randomly divided into 6 subgroups ( $n = 6$ ):

- A. **Control:** No use of nanobioglass or laser radiation.
- B. **Diode laser (980 nm):** With a specific radiation characteristics (980-nm wavelength, 3-W power, SSP mode, 5-mm non-contact), scanning in the occluso-apical and mesio-distal directions and vice versa, 1 min/cm<sup>2</sup> total radiation time [18].
- C. **Nd:YAG laser:** With specific radiation characteristics (wavelength 1064 nm, perpendicular to the surface, contact, 1.0-W power, 10-Hz frequency, 85 J/cm<sup>2</sup> per pulse energy density), scanning in the occluso-apical and mesio-distal directions and vice versa, 1 min/cm<sup>2</sup> total radiation time [16].
- D. **Nanobioglass:** A 50% solution of bioglass nanoparticles and distilled water was prepared. Then, ultrasonic probe with the characteristics of 20-kHz was used for 20 s for 20 cycles in order to homogenize the solution. Subsequently, the solution was applied using an applicator brush for 20 s and was applied to the surfaces in two steps.
- E. **Nanobioglass + diode laser radiation (980 nm):** According to the group B method.
- F. **Nanobioglass + Nd:YAG laser radiation:** According to the group C method.

## Evaluation of the interaction pattern between dentin and nanobioglass with SEM

Scanning electron microscopy (SEM) was used to study the morphology and surface topology of dentin, and also the interaction pattern between dentin and nanobioglass. The

surface of the dentin disks was covered with a 100 A0 gold layer using the Bal-Tec (Swiss) instrument, and then the prepared specimens were carefully examined with the XL30 SEM (Philips, Netherlands) microscope at 250, 500, 1000, and 2000 magnifications. The average number of open dentinal tubules at 2000 magnification was counted twice by a skillful operator.

## Statistical analysis

Then, the average number of open dentinal tubules in different groups was statistically evaluated by SPSS software version 22, Kruskal-Wallis test, and Mann-Whitney tests with Bonferoni's correction ( $\alpha = 0.008$ ).

## Results

### Nanobioglass SEM examination

The SEM image was used to analysis the surface topology and morphology of nanobioglass. The results of this study show the presence of silica in the glassy phase of a spherical-shaped structure with the average size of 50 nm (Figure 1).

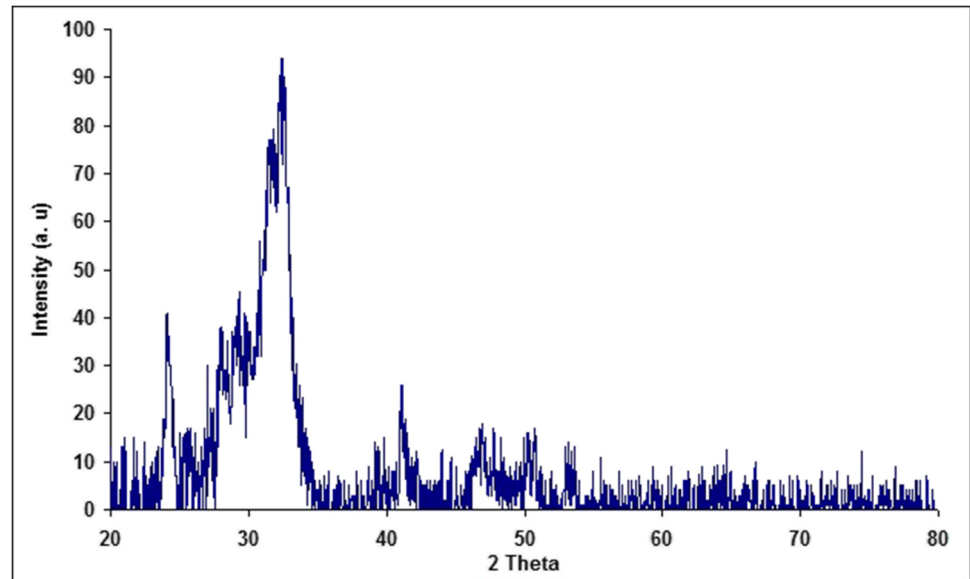
### Nanobioglass XRD examination

XRD pattern shows a clear apatite phase (Figure 2). Both SEM and XRD data indicate the deposition of sodium and calcium ions over the crystal structure from the silica matrix which is due to the calcinations process. The XRD pattern of synthesized nanobioglass is consistent with the Larnite crystalline mode corresponding to Ca<sub>2</sub>SiO<sub>4</sub> (JCPDS # 33-0302) [19]. A sharp peak shown in Figure 2 at  $2\theta=32.16$  is indexed as (300).



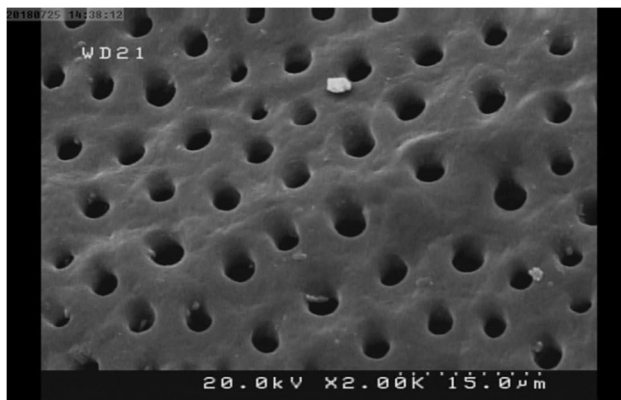
Fig. 1 A 6000<sub>x</sub> view of the nanoparticle size of bioglass

**Fig. 2** XRD pattern of nano-bioglass



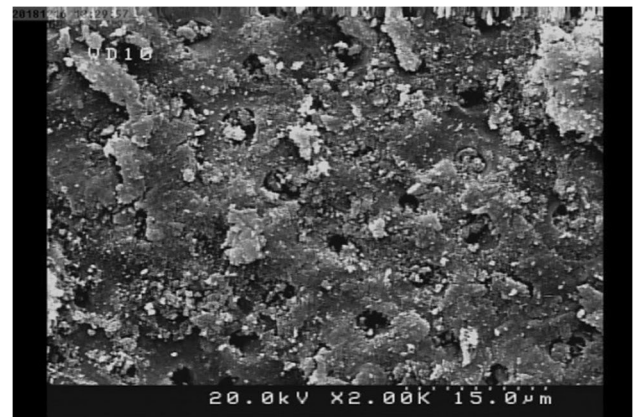
**Table 1** Frequency distribution of average number of open dentinal tubules

Groups	N	Mean	Std. deviation	Minimum	Maximum
Control	6	62.16	11.30	42.00	75.00
Diode	5	26.00	6.40	17.00	33.00
Nanobioglass-diode	6	14.16	5.56	6.00	20.00
Nd:YAG	5	12.80	8.04	.00	19.00
Nanobioglass-Nd:YAG	6	8.00	7.21	.00	17.00
Nanobioglass	5	19.00	11.33	5.00	33.00

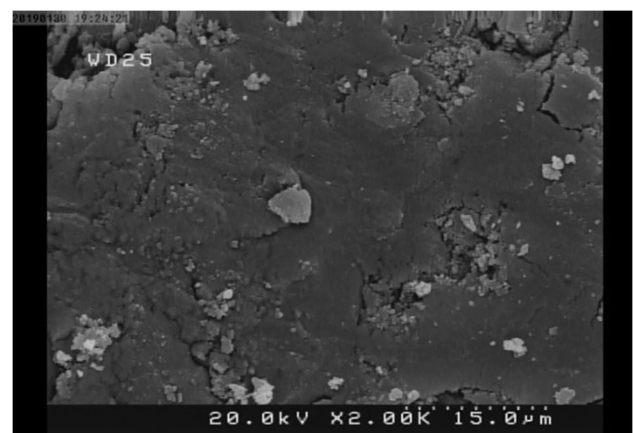


**Fig. 3** A 2000<sub>x</sub> view of the dentinal tubules openings

Based on the data presented in Table 1, frequency distribution of the averages of the open dentinal tubules in all 6 groups of control (Figure 3), nanobioglass (Figure 4), diode, nanobioglass-diode (Figure 5), Nd:YAG, and



**Fig. 4** A 2000<sub>x</sub> view of the nanobioglass penetration to the dentinal tubule openings



**Fig. 5** A 2000<sub>x</sub> view of the diode (980 nm) laser site and nanobioglass

nanobioglass-Nd:YAG (Figure 6) is 62.16, 19.00, 26.00, 14.16, 12.80, and 8.00, respectively.

According to the results of Mann-Whitney test (Table 2), there is a significant difference in the mean number of open dentinal tubules between the control group and the other groups ( $p < 0.008$ ). However, the difference among the other groups is not statistically significant ( $p > 0.008$ ).

In the following, SEM images represent the dentinal tubule obstruction and the surface coverage of control and study groups.

### Discussion

Dentinal hypersensitivity (DH) can be considered as a type of pain caused by thermal, chemical, or osmotic stimulations on the uncovered dentin that does not match the causes and origins of other dental pains. However, it should be noted that the presence of uncovered dentin cannot be simply a reason for the painful feelings resulting from the stimuli. Since, according to several studies, the number and diameter of dentinal tubules play an important role in the emergence of DH, many therapeutic approaches focus on reducing the number of open dentinal tubules and diameter of their openings [1, 20, 21]. Thus, this study aims to reduce the permeability of dentin surfaces using nanotechnology to improve penetration of particles through tubular openings and employing high-power laser radiation to melt minerals (Figure 7).

In this study, nonparametric tests (Kruskal-Wallis and Mann-Whitney) were used due to the lack of normal distribution of data and the impossibility of using parametric methods. According to the results of this study, all the surface treatments, such as diode (980nm) and Nd:YAG lasers with specific radiation characteristics as well as the use of nanobioglass with or without laser radiation, significantly reduce the number of open dentinal tubules compared with

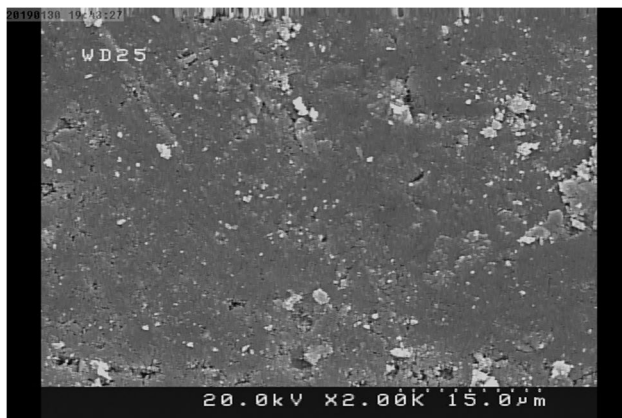


Fig. 6 A 2000\_x view of the Nd:YAG laser site and nanobioglass

Table 2 The results of Mann-Whitney tests for comparing the groups with each other

Groups		Sig. dif*
Control	Diode	0.006
	Nanobioglass-diode	0.004
	Nd:YAG	0.006
	Nanobioglass-Nd:YAG	0.004
	Nanobioglass	0.006
Diode	Nanobioglass-diode	0.018
	Nd:YAG	0.028
	Nanobioglass-Nd:YAG	0.008
	Nanobioglass	0.295
Nanobioglass-diode	Nd:YAG	0.926
	Nanobioglass-Nd:YAG	0.147
	Nanobioglass	0.784
Nd:YAG	Nanobioglass-Nd:YAG	0.196
	Nanobioglass	0.600
Nanobioglass-Nd:YAG	Nanobioglass	0.169

\*  $\alpha = 0.008$

the control group ( $p < 0.008$ ). On the other hand, the highest reduction in open dentinal tubules is observed in the nanobioglass-Nd:YAG laser group.

The use of laser Nd:YAG is the best type of laser radiation in the treatment of dentinal hypersensitivity because it is able to obstruct the tubules by melting and resolidifying the dentin while there is no palpation and cracks. In addition, the formation of an obstructive layer with a thickness of about 4 µm following the laser radiation of Nd:YAG has a

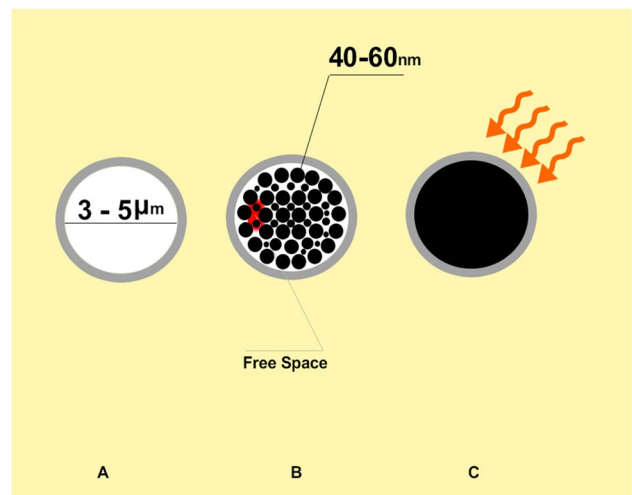


Fig. 7 A Completely open dentinal tubule with an average diameter of 3–5 µm. B Theory of using nanoparticles with a diameter of about 40–60 nm for the relative occlusion of the dentinal tubule. C High power laser radiation with the aim of melting mineral compounds and creating complete occlusion of the dentinal tubule

significant role in the immediate reduction of dentinal hypersensitivity [22, 23]. According to a clinical study by Suri et al., laser diode (980nm) with 2-W power, and also with desensitizer compounds such as 5% NaF varnish, had a significant effect on dental sensitivity reduction. As the authors argued, the combination of laser irradiation and desensitizer agents has synergistic effect on DH reduction [24]. In this study, the inefficiency of laser radiation in creating uniform tubular obstruction was one of the important reasons why we decided to use laser treatment in combination with nanoparticles. The results of SEM images indicate that the quality of blockage of dentinal tubules in nanobioglass-diode (980 nm) and nanobioglass-Nd:YAG groups is much better than that of laser radiation alone.

As mentioned above, nanobioglass was another method used in this study. The composition of bioglass has been studied extensively due to their high ability for reconstructing and remineralizing hard tissues by creating a layer of carbonate apatite [25–28].

One of the most crucial challenges in treating DH has been finding a long-lasting treatment. Therefore, one of the most important properties of bioactive glass is the continuous remineralization as well as its contribution to the deposition of mineral particles. This can help to improve the obstruction of dentinal tubules and reduce DH over time. This theory was evaluated and confirmed by Mitchell et al. [13]. According to the findings of their laboratory study, Abbassy et al. believe that a mixture of bioglass powder (45S5) and 50% phosphoric acid can release calcium, phosphate, and sodium and the penetration of these compounds into the outer layer of the enamel cause remineralization of white spot lesions [28]. Also, according to the study of Lopez et al., the proximity of bioglass compounds with stem cells derived from deciduous teeth (SHEDs) can play an important role in cellular differentiation, thereby helping to form tertiary dentin and to increase the mineral matrix deposition [29].

The results of the present study show that a radiation of 980-nm diode with 3-W power significantly decreases the number of open dentinal tubules, which is consistent with the clinical findings of Tabibzadeh et al. In addition, according to the other clinical results of Tabibzadeh et al., no pulpal damage was observed due to thermal changes of diode (980nm, 3-W) laser radiation [18].

Also, in this study, Nd:YAG laser radiation with 1-W power was used. According to the findings of the study by Zapletalová et al., Nd:YAG laser radiation with a power of more than 1.5-W can cause microcracks and carbonization and also increase intrapulpal temperature, resulting in irreversible damage [30]. Although in the clinical study by Lopes et al. the use of Nd:YAG laser with 1-W led to significant reduction in DH pain, some patients still had degrees of pain after treatment. Lopes et al. considered the cause of this phenomenon to be an uncompleted blockade of dentinal tubules [22]. Our findings

in this study confirm their hypothesis. The study of Gholami et al. was another study that has consistent results with our findings. They also found significant differences in reducing the diameter of dentinal tubules after Nd:YAG laser radiation at 1-W [5]. The White et al. study was another investigation that examined the appropriate radiation characteristics with the aim of preventing intrapulpal damages due to temperature changes of Nd:YAG pulsed laser. They believe that Nd:YAG laser radiation with 0.3 to 3.0-W and frequencies of 10 and 20 Hz cannot cause excessive temperature rise in the pulpal tissue [31]. Also, based on clinical findings, Birang et al. stated that the thermal changes caused by the 1-W Nd:YAG laser irradiation have no effect on the pulp [32]. Moritz et al. used a 1.5-W Nd:YAG laser to minimize thermal damage to periodontal tissues [33]. The radiant power used in the study was 1-W, which is the peak power, and in pulsed radiation, this power will be less than 1-W. On the other hand, the radiation method was scanning and the laser radiation was not concentrated in one point. Both factors, together with blood circulation in the vital pulp, reduce thermal damages.

Although laboratory findings indicated that the therapeutic methods used in this study may have a potential effect on reducing DH, the need for long-term clinical studies for investigating the durability of DH therapy is necessary. Given that many existing therapies meet the immediate needs of patients, the main problem of these methods is that their effects are unstable. Furthermore, laser irradiation has biological properties that can only be examined under in vivo conditions.

## Conclusions

According to the results of this study, the use of high-power laser radiation, such as Nd:YAG and diode (980nm) alone or with nanobioglass, has a significant effect on tubular obstruction. However, high-power Nd:YAG laser radiation accompanied by nanobioglass seems to have a higher ability to obstruct and reduce dentinal permeability compared to their separate application.

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## Declarations

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** For this type of study, formal consent is not required.

**Conflict of interest** The authors declare no competing interests.

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