Biodegradable Drug-Eluting Scleral Buckle Implant

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Abstract—Scleral Buckle (SB) remains a commonly used surgical method for the treatment of Rhegmatogenous Retinal Detachment (RRD). In this paper, the concept of creating a new SB implant is proposed. Biodegradable poly(lactic acid)-copoly(glycolic acid) PLA-PGA co-polymer—PLGA was processed via electrospinning to form nanofiber mat, onto which moxifloxacin and dexamethasone were immobilized. Two PLGA materials were tested, with different PLA:PGA ratio— 50:50 and 75:25. Chemical composition and implant morphology were analyzed via FTIR spectroscopy and scanning electron microscopy, respectively. Moreover, drug release from polymeric implant and its degradability were tested.

In 7 days 21.9% and 19.8% of moxifloxacin and dexamethasone were released from PLGA 50:50 and PLGA 75:25 implant samples, respectively. Both PLGA implants degraded in 20 weeks—PLGA 50:50 and PLGA 75:25 with drugs added decomposed. When materials were subjected to stretching, their decomposition was accelerated, allowing the implants to degrade in 14–16 weeks.

Formation via electrospinning of biodegradable polymeric implant, with ability to stretch, release of immobilized chemicals and decomposition over time after scleral buckle healing is a potential alternative for commonly used silicone bands during retinal detachment surgery.

Keywords—electrospinning, scleral buckle, retinal detachment, biodegradable implant, poly(lactide-co-glycolide)

I. INTRODUCTION

Rhegmatogenous Retinal Detachment (RRD) is one of the most common vitreoretinal emergencies and typically occurs when the vitreous liquefies and separates from the retina, leading to a retinal tear through which fluid flows in and leads to detachment of the neurosensory retina from the underlying retinal pigment epithelium. Scleral Buckle (SB) remains a commonly used surgical method for the treatment of RRD. This procedure involves the use of a silicone strip that is completely or partially wrapped around the eye, which exerts pressure on the eye wall and approximating the retinal tear to the underlying tissue, preventing the fluid from penetrating into the subretinal space. This method, although effective, is associated with potential implant-related complications and may result in side effects including a myopic shift, postoperative astigmatism, strabismus, scleral abscess, extraocular infections, and even endophthalmitis. These problems are partly caused by the permanent silicone SB implant after surgery.

We used electrospinning to create an innovative, drugreleasing, biodegradable SB implant for surgical treatment of RRD. The proposed new implant could potentially replace the conventionally used silicone implant in the future. Ultimately, this self-degrading new material is intended to improve the quality of life of patients undergoing retinal detachment repair, which would make it unnecessary to remove the implant in subsequent surgical procedures and would avoid the occurrence of secondary adverse events including bacterial infection and inflammation due to the constant release of biocidal and anti-inflammatory agents from the material.

The use of an improved treatment method via a drugeluting biodegradable implant could be an innovative alternative that may be of great practical importance to minimize potential side effects after SB surgery. The new method proposed in this paper could be the key to finding the "gold standard" of treatment with new biodegradable implants.

II. LITERATURE REVIEW

Rhegmatogenous Retinal Detachment (RRD) is the most commonly reported vitreoretinal emergency [1], with an estimated worldwide incidence between 5–10/100,000 [2]. RRD typically occurs as the vitreous liquefies and separates from the retina, causing a retinal break through which fluid enters and leads to the detachment of the neurosensory retina from its underlying Retinal Pigmented Epithelium (RPE) [3]. If left untreated, photoreceptors devoid of oxygen and nutrients undergo apoptosis, leading to irreversible vision loss. Scleral Buckle (SB) remains a commonly used surgical approach in the management of RRD. Multiple studies have demonstrated >85% surgical success rates of retinal reattachment with SB [4–7].

Since it was coined by Charles Schepens in 1957, the SB procedure has remained relatively unchanged [8]. Following peritomy and reflection of the conjunctiva, retinal breaks are identified and treated with transscleral cryopexy or laser photocoagulation. A buckle implant pre-treated with antibiotic solution is then sutured over (or within) intact sclera as a complete 360° or partial encirclement, or radially, depending on the location and size of the retinal breaks. The SB implant places an inward indentation force on the sclera which transduces pressure inside the eye to displace fluid away from the retinal tear site [9]. As the anatomic space between the detached neurosensory retina and underlying membrane is collapsed, the RPE cells ultimately resume their physiological ability to pump out subretinal fluid which facilitates retinal re-apposition [10]. The common SB implants are manufactured using silicone-based sponge, rubber, and/or semi-plastic elements [11-13]. Following SB placement, patients use multiple topical medications, up to a month or longer, to prevent postoperative inflammation or infection, since the soaking of the buckle prior to use is unlikely to provide sufficiently long enough antimicrobial coverage after surgery.

Despite retinal apposition being achieved shortly after the surgery, the silicone-based SB implant remains in the orbit permanently in most cases [14]. The most reported postoperative complication is a change in eye's refractive error as pressure from the SB changes the shape of the eye, in particular by increasing the globe's axial length and causing a myopic shift. Most studies report a myopic shift in eyes treated with SB for RRD [15], with every 1 mm increase in axial length corresponding to approximately 2.75 diopters of induced myopia [16]. This remains a major detriment to the quality of life of patients after SB surgery. Comparatively fewer studies have also reported postoperative astigmatism [17-19]. Strabismus and diplopia have also been reported after surgery [20], due to mechanical restriction of an extraocular muscle by the size or location of the buckle [15].

Having a permanent ocular implant also increases the lifetime risk of serious permanent vision loss and organ threatening complications. Extrusion is the most common cause of SB removal procedures [15], accounting for up to 57% of all removal surgeries [21]. Buckle extrusion typically occurs at the level of the sclera, but has been less commonly reported to extend outside the conjunctiva as well as the skin [21, 22]. Studies have similarly estimated that buckle intrusion (subretinal, into the eye) occurs in approximately 4-18% of all eyes, on average about seven years after SB surgery [23]. Given that the SB is a permanently fixated structure, it also exists as a nidus for infection [13, 20, 24-26]. Most infections after SB placement occur at a mean of 2-8 months after surgery, with scleral abscesses occurring in less than 1% of cases and extraocular infections occurring in approximately between 0.5% to 6% of cases [13, 24-27]. Endophthalmitis, a rare complication which often carries a poor visual prognosis, has also been reported after SB [28, 29]. These delayed complications are indications for SB removal surgery [23, 30], and SB removal itself is associated with a number of risks including infection [31].

Although topical ophthalmic drugs are widely used in the short term to prevent postoperative complications after SB implantation, their delivery is greatly limited by ocular barriers [32, 33]. Perhaps the most critical barrier for such delivery scheme is the inability of patients to apply medications or follow a prescribed timing regimen. Topical formulations may cause surface irritation and allergic reactions, resulting in patient noncompliance [34]. Polymeric implants are alternative drug delivery platforms used for the sustained delivery of ocular therapeutics. However, nonbiodegradable polymeric implants require surgery to be removed from the eye once the drug is depleted. Further, most of the commercially available polymeric implants do not provide the flexibility of sequential, and/or simultaneous release of multiple therapeutics [32, 35-38]. An ideal drug delivery implant would have the capacity to release therapeutic contents with a pre-determined timeframe, at proper relative drug ratios, and in a programmed manner that could be either sequential and/or simultaneous release [37].

One of the methods for creating nanofibrous materials capable to drug immobilization for further release is electrospinning [39–42]. To form nanofibers from polymers electrostatic forces are involved in the process. As a result of applying high voltage and appropriate polymer flow through

the injector, the solvent evaporates from polymer solution and an appropriately designed structure of nanofibers or nanocapsules is formed (stretched). These nanofibers are then collected on the metal collector within an electric field. In the case of electrospinning, nanofibers located in the electric field while "flying" to the collector are subject to centrifugal force, which causes the spin movement of the resulting nanostructures to be noticed (hence the name electrospinning) [43]. Materials obtained through electrospinning are characterized by a high surface-to-weight ratio, excellent mechanical properties, high porosity and flexibility [43-45]. Electrospinning is easy to use, inexpensive, and allows obtaining fibrous structures or fibers with micro or nanometer diameters with small beads, crystals or particles immobilized onto the fibers.

The typical material for SB implants is silicone but because of many disadvantages of using it, other materials have been taken for consideration. One of the most interesting and promising materials are biodegradable polymers, like gelatin, surgical gut or fibrin. Also there are reports of testing Poly(Lactic Acid) (PLA) and Poly(Glycolic Acid) (PGA) for SB implant construction material [46, 47]. These materials can be promising in ophthalmic surgery, mainly due to non-toxicity, easy degradation and absorption in 3–4 months, and removal from the body via urinary tract. In this work biodegradable implant made from PLA-PGA copolymer—PLGA was formed via electrospinning. Moxifloxacin and dexamethasone were immobilized onto PLGA nanofiber, allowing these agents to be released directly into the surgical area to facilitate a more effective and rapid healing process.

III. MATERIALS AND METHODS

A. Materials

Poly(Lactide-co-Glycolide) (PLGA) in two different Llactide:glycolide ratio (50:50 and 75:25) were purchased from Bonding Chemical (USA). Tetrahydrofuran (THF), Dimethylformamide (DMF), moxifloxacin, dexamethasone and Phosphate Buffer Solution (PBS) were purchased from Merck Chemicals (Germany) and used as received.

B. Preparation of Polymer Solutions

Both types (50:50 and 75:25) of PLGA polymer were dissolved in THF-DMF solvent mixture (THF and DMF in 4:1 ratio, respectively), by stirring the solution at 20 °C at around 800–1000 rmp. The final concentration of PLGA (50:50 or 75:25) in THF-DMF was 20% (w/v). PLGA-moxifloxacin-dexamethasone solutions (with usage of 50:50 or 75:25 PLGA polymer) was prepared by dissolving polymer and suspending/dissolving moxifloxacin and dexamethasone in THF-DMF while stirring the mixture at 20 °C at around 800–1000 rmp. Final concentrations of PLGA and dexamethasone and moxifloxacin in the solution were 20% (w/v) and 2% (w/v), and 1%, respectively.

C. Implant Preparation via Electrospinning

The electrospinning apparatus, equipped with a variable high-voltage 0-30kV power supply, was assembled in-house. The anode was connected to a stainless-steel needle (\emptyset 0.9 mm) connected directly to one 10 mL plastic syringe. The disc-shaped copper ground electrode was connected to a

stainless-steel rotating tube (a collector) with \emptyset 60 mm, where all fiber mats were collected. Collector rotation tube was fixed on 1 rmp. The experimental setup was housed in a laminar flow safety cabinet. All experiments were performed under room temperature and atmospheric pressure conditions. Polymer solutions physical properties, like dynamic viscosity, conductivity and density were determined and presented in Table 1, where electrospinning process parameters were also shown.

Table 1. Physical properties and electro-hydrodynamic processing parameters of PLGA in different PLA-PGA ratios (PLGA 50:50 and PLGA 75:25) without and with addition of moxifloxacin and dexamethasone

(PLGA 50:50+M+D and PLGA 75:25+M+D) polymeric solutions					
Sample	Dynamic viscosity (Pa·s)	Conductivity (µS)	Distance injector- collector (cm)	Voltag e (kV)	Flow rate (cm ³ /h)
PLGA 50:50	0.23±0.03	0.480 ± 0.015	15	10	1
PLGA 75:25	0.21±0.05	0.450±0.026	15	13	1
PLGA 50:50+M+D	0.20±0.08	0.725±0.031	15	11	1
PLGA 75:25+M+D	0.18±0.04	0.631±0.017	15	13	1

D. Scanning Electron Microscopy

SEM was conducted on a Helios NanoLab 650 microscope (Thermo Fisher Scientific, USA) with an accelerating voltage of 10 kV and a working distance of 40 mm. Analyzed samples were not sputtered with any conductive coating.

E. Infrared Spectroscopy

Fourier Transform Infrared Spectra (FTIR) were collected by using the Attenuated Total Reflection (ATR) attachment on Invenio S FT-IR (Bruker, USA) spectrometer. One single spectrum was averaged over 24 scans at 4 cm⁻¹ resolution in the wavelength range from 400 to 4000 cm⁻¹. All analyses were performed in duplicate under room conditions.

F. Drug Release Analysis

To perform analysis of drug (moxifloxacin and dexamethasone) release from polymeric implant samples, PLGA 50:50 and PLGA 75:25 was cut into small pieces, with a weight of around 0.02 g. Then, the material was placed into 0.5 mL of fresh, sterile PBS buffer and incubated at 37 °C up to one week. After the designated incubation time, polymeric implant pieces were dried, weighed and analyzed via FTIR spectroscopy. The PBS solutions were analyzed using UV-1800 UV-vis spectrophotometer (Shimadzu, Japan) to determine dexamethasone and moxifloxacin concentration changes. All experiments were performed in triplication.

G. Implant Biodegradation Analysis

The procedure was carried under sterile conditions, to avoid possible buffer contamination. Implant samples were cut into small pieces with a weight of 0.02–0.03 g approximately and then each piece was weighed and placed into 1 mL of sterile PBS solution. After that tested samples were placed for incubation at 37 °C to map the eye's environment to the maximum. The biodegradability of each sample was analyzed weekly, by washing one piece of each implant sample with distilled water, drying and weighing. With this analysis a weight loss of all pieces of implants in time was determined. Dried samples after incubation in PBS were also tested via FTIR, to analyze implant chemical composition changes over time. Moreover, the impact of stretching on implant samples and their biodegradability was tested. Polymeric bands made from PLGA were stretched on stretching cone lengthening them by 5 and 10%. Stretched samples were suspended in PBS solution and incubated at 37 °C. Biodegradability of stretched samples was tested in the same way as non stretched material by analysis of weight loss of the samples.

H. Statistical Analysis

Data analysis was carried out using SPSS by IBM Corporation (USA). One-Way Analysis of Variance (ANOVA) was completed to determine the significant differences between sample means, at a significant level of p<0.05. Mean comparisons were performed by the Tukey test.

IV. RESULT AND DISCUSSION

A. Implant Preparation

PLGA in different PLA:PGA ratios—50:50 and 75:25 solutions were prepared, and their physical properties were analyzed. Those analysis were conducted for polymeric solutions with and without moxifloxacin and dexamethasone addition. Table 1 shows the physical properties of polymer solutions including dynamic viscosity and conductivity. In addition, electrospinning process parameters under which scleral buckle implants were obtained are presented.

Physical properties of PLGA solutions were similar to other solutions used for fiber production via electrospinning. The process of materials production was effective and run in similar conditions [48, 49]. Addition of moxifloxacin and dexamethasone did not affect solution's dynamic viscosity, but conductivity increased significantly. This is probably an effect of addition conductive material. Electrospinning was conducted for several hours with usage of rotating collector, until all materials reached required and even thickness of 1.25±0.1 mm with maximum of homogeneity. All polymeric mats were stored in 4 °C and 0% of RH until they were used for further analysis. Fig. 1(A) presents scheme of electrohydrodynamic processing with drum collector. Polymeric solution inside a syringe is pumped through needle (injector) by infusion pump. High voltage is connected to injector and drum collector. Polymer when reaching collector is forming an implant. Fig. 1(B) shows the photograph made during formation of real degradable implant.



Fig. 1. (A): Scheme of electrospinning processing, (B): photograph took during implant production process.

B. Characterization of Implant Morphology and Chemical Composition

The morphology of fiber mats was determined via Scanning Electron Microscopy (SEM). The electron micrographs in Fig. 1 present nanofibrous structure of PLGA at different PLA-PGA ratios (50:50 and 75:25, Fig. 1(A) and (B), respectively), with and without moxifloxacin and dexamethasone particles immobilized onto it. The fibers made from pure PLGA polymers (Fig. 2(A) and (B)) were full of additional structures or forms. Fibers were interwoven with beads and particles. When moxifloxacin and dexamethasone were mixed with PLGA solution, the morphology of obtained via electrospinning mat changed. Fibers were much more homogenous and no similar like on samples where polymeric solution was free from drugs (Fig. 2(A') and (B')). When adding conductive material to the solution that later was processed via electrospinning obtained material is more homogenous [50].



Fig. 2. SEM micrographs of PLGA made from material with different PLA-PGA rations (50:50—A, 75:25—B) nanofibers. PLGA 50:50 fibers with dexamethasone and moxifloxacin mechanically immobilized onto them (A'), PLGA 75:26 fibers with dexamethasone and moxifloxacin mechanically immobilized onto them (B').

Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze chemical composition of biodegradable implants made in the project. On the Fig. 3 is presented spectra of dexamethasone, moxifloxacin, PLGA 50:50, PLGA 50:50 with drugs incorporated on the polymer fibers, PLGA 75:25 and PLGA 75:25 with immobilized drugs on the fibers. With red arrows are highlighted characteristic peaks for dexamethasone and moxifloxacin that are present on spectra of the drugs and implant samples, where dexamethasone and moxifloxacin were incorporated into polymeric fibers during electrohydrodynamic processing.

The most characteristic for dexamethasone and moxifloxacin peaks are corresponding to the wavenumbers 1670 cm⁻¹ and 1310 cm⁻¹, respectively. These peaks were also detectable on spectra of implant samples, where the drugs were incorporated onto polymeric fiber structure. By FTIR analysis it was possible to prove that the drugs used in

the project were successfully incorporated on implant samples made from both PLGA polymers.



Fig. 3. FTIR spectra of dexamethasone, moxifloxacin, PLGA 50:50 and PLGA 75:25 pure or with incorporated into polymer fibers dexamethasone and moxifloxacin.

C. Drug Release from the Implant

To perform analysis of drug (moxifloxacin and dexamethasone) release from polymeric implant samples, PLGA 50:50 and PLGA 75:25 with drugs woven into polymeric fibers, were tested. To perform the analysis, a calibration curve was created and used to determine drug concentration in PBS solution (Fig. 4). Dexamethasone and moxifloxacin were diluted in the PBS solution in different concentrations and analyzed using UV-vis

spectrophotometry. Absorbance changes on a wavelength 400 nm was detected and chosen to implant drug release analysis.



Fig. 4. Drug release calibration curve used to determine dexamethasone and moxifloxacin concentration in PBS after implant incubation in time. Absorbance measurement was taken at a wavelength of 400 nm.



Fig. 5. Dexamethasone and moxifloxacin release from PLGA implant samples into PBS solution in time.

Drug release from PLGA 50:50+M+D and PLGA 75:25+M+D implant samples containing dexamethasone and moxifloxacin immobilized onto PLGA fibers is shown on Fig. 5. From the first moment of the analysis dexamethasone and moxifloxacin were releasing from the implant. In 7 days of analysis 19.8% and 21.9% of dexamethasone and moxifloxacin were released to PBS solution from PLGA 50:50+M+D and PLGA 75:25+M+D implant samples, respectively. In case of polymer made from PLGA 50:50+M+D drug release was slower and lower than samples made from PLGA 75:25+M+D. The drug release was low in PBS solution for first 4 days of the analysis, where only 3-4% of drugs have released from the implant. After that time, in both cases, drugs released much faster, with around 20% of dexamethasone and moxifloxacin were released after 7 days of the analysis.

D. Degradation Analysis of PLGA Implant over Time

The biodegradability of each sample was analyzed, by washing one piece of each implant sample with distilled water, drying and weighing. With this analysis a weight loss of all pieces of implants in time was determined. At the same time FTIR analysis of tested samples were performed, where a peak at wavenumber of 1750 cm^{-1} was evolving. On the Figs. 6 and 7 are presented obtained biodegradability results for implants made from PLGA with different PLA:PGA ratio (50:50 and 75:25) and with moxifloxacin and dexamethasone

woven into the polymeric fibers. Moreover, implant biodegradability was tested at normal conditions, where not any force was used to stretch the material and when adopted force stretched implant samples to extend the length of the implant up to 5 and 10%. By this analysis mechanical properties of the implant samples were tested and impact of biodegradation when material was exposed to stretching.



Fig. 6. Top graph: FTIR spectra changes over time of PLGA 50:50 with added dexamethasone and moxifloxacin measured at wavenumber range of $1650-1800 \text{ cm}^{-1}$. Bottom graph: Mass loss changes over time of PLGA 50:50 with dexamethasone and moxifloxacin.

For PLGA 50:50+M+D, samples behaved similarly for the first 5–6 weeks of analysis, By this time, material degradation (mass loss) reached values of around 10–15%. After that time not stretched PLGA 50:50+M+D (Fig. 6, bottom, solid black line) started to degrade faster and in 15 weeks around 95% of the material decomposed. Practically full decomposition of not stretched PLGA 50:50+M+D was observed after 20 weeks of PBS storage. For PLGA 50:50+M+D stretched to extend the length up to 5% and 10% (Fig. 6, bottom, dotted and dashed black lines, respectively), materials degradability was slower, implant weight loss in time showed a linear trend. When material was stretched it decomposed over time and at week 15 and 18 material broke.

In case of PLGA 75:25+M+D degradation was similar for all samples—stretched or not stretched. In 20 weeks of analysis, not stretched implant degraded in more than 95% (Fig. 7, bottom, solid black line). When the samples were stretched, they decomposed and broke in week 16 and 14, for PLGA 50:50+M+D and PLGA 75:25+M+D, respectively, not allowing to stretch and continue the experiment.

Polymer degradation analysis was also tested with FTIR

technique. After 5, 10, 15 and 20 weeks of analysis, all implant samples were dried and analyzed with ATR spectroscopy to determine chemical composition changes of polymers.



Fig. 7. Top graph: FTIR spectra changes over time of PLGA 75:25 with added dexamethasone and moxifloxacin measured at wavenumber range of 1650–1800 cm⁻¹. Bottom graph: Mass loss changes over time of PLGA 75:25 with dexamethasone and moxifloxacin.

Implant samples spectra on wavenumber 1650–1800 cm⁻¹ range are presented on a Figs 6 and 7, on the top of each Figure. Peak at wavenumber around 1750 cm⁻¹, corresponding to presence of ester groups in the polymers was presented on spectra of all analyzed implant samples at the beginning of the analysis (week 0). During degradation time of analyzed material, this peak disappeared and new one was detected in week 5 of the analysis - peak at wavenumber 1745 cm⁻¹, which corresponds to C=O stretching and appearance of ketone groups in chemical structure of the polymers. From week 15 of the analysis, when most of all tested samples degraded, a final peak appeared at wavenumber 1720 cm⁻¹, which correspond to aldehyde and carboxylic acid groups detected in the samples. With this analysis it was possible to observe how analyzed samples degrade and how bonds between ester groups break and form new structures, like aldehydes or carboxylic acids-the process of polymer degradation in time was observed. Results obtained with FTIR analysis are similar to previous ones, when degradation of analyzed implants was detected with sample weight loss. In both methods it was possible to observe that implants made from PLGA 75:25+M+D degrade slower than materials made from PLGA 50:50+M+D, when no additional force is added to stretch the material.

V. CONCLUSION

Electrospinning is a proper technique to produce biodegradable drug-eluting scleral buckle implants. Obtained materials were built from fibers and beads which was confirmed by scanning electron microscopy. FTIR spectroscopy was used to determine chemical composition of produced PLGA implants and confirm the presence of dexamethasone and moxifloxacin inside their fiber structure.

Two main implant features were creation of degradable implant and an ability of drug release during degradation time. PLGA polymer as an implant scaffold was able to degrade. Polymeric implant samples produced via electrospinning degraded within 15 to 20 weeks, when PLGA 50:50 and PLGA 75:25 was used, respectively. This was confirmed using two different analysis—FTIR spectroscopy and weight loss analysis of the implant samples over time. After 20 weeks of analysis both created implant samples degraded close to 97%. Application of stretching force on both PLGA implant bands allowed the use of the implant for 14 weeks.

Drug release experiments confirmed that moxifloxacin and dexamethasone successfully eluted from tested materials, and in the first week of applying the implant in the environment that imitates human eye conditions around 20% of drugs were released to the environment. To improve drug elution from degradable scleral buckle implant, higher concentration of dexamethasone and moxifloxacin could be used. Furthermore, it could be feasible to create core-shell PLGA material to make a functional implant. The core could be made from pure PLGA polymer, and the shell would contain high drug concentration woven into PLGA fiber structure. This would improve drug elution from the implant in a first week of eye-healing process, when it is the most desirable and needed. Another option that could be considered in future studies is to find a different polymer/s to create an implant with better drug eluting properties, same or better degradation features and is still approved for use in ophthalmology.

We believe that this research has the potential to make a significant impact on enriching the state of knowledge and the possibility of using electrospinning technique and new materials in the field of ophthalmic surgery and materials engineering. Further research is needed to optimize the design and engineering of biodegradable drug-eluting surgical implants which can replace the conventional implants made of synthetic materials and improve the surgical care of patients with retinal detachment in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

SW conducted the research, analyzed data and wrote a paper; CB analyzed data and corrected a paper; all authors had approved the final version.

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