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Gel properties of carboxymethyl hyaluronic acid/polyacrylic acid hydrogels prepared by electron beam irradiation

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Abstract: Semi-synthetic hydrogels made of carboxymethyl hyaluronic acid (CMHA) and polyacrylic acid (PAA) were synthesized using electron beam irradiation. CMHA with a degree of substitution of 0.87 and molecular weight of 141 kDa was mixed with linear PAA and slightly crosslinked PAA (Carbopol). Equal weight ratio of CMHA-Carbopol blends (10% CMHA 10% Carbopol) were successfully crosslinked even at low irradiation dose of 20 kGy, producing hydrogel with 60% gel fraction and 430 g/g degree of swelling. The gel properties of this formulation showed good stability when exposed in PBS (pH 7.4) at 37 °C. Furthermore, the FT-IR spectra of the 10% CMHA 10% Carbopol blends showed increase of peak intensity at 1405 cm^{-1} due to neutralization reaction between the COOH and COO^- groups of PAA and CMHA polymers. Interaction effects between the concentration of CMHA and PAA and varying irradiation doses in the gel properties in CMHA-PAA hydrogels will be explored in the future study. Radiation-crosslinking biocompatible CMHA to other synthetic polymers such as PAA provides a cleaner method of producing biomaterials with tunable properties that are ideal for pharmaceutical, medical, and cosmetic applications.

Keywords: carboxymethyl hyaluronic acid, polyacrylic acid, radiation crosslinking, hydrogel.

1. Introduction

Semi-synthetic hydrogels are widely used biomaterials which are extensively studied for use in pharmaceutical, medical, and cosmetic applications. The benefits of mixing synthetic and natural polymers allow semi-synthetic hydrogels to be tailored to specific applications[1]. Rosiak, et al. (1999) reported that one of the most efficient ways to produce semi-synthetic gels for biomedical applications is by radiation crosslinking [2]. Previously, we successfully produced radiation-crosslinked biocompatible hydrogels made from pure carboxymethyl hyaluronic acid (CMHA)[3,4]. Despite successful crosslinking of pure CMHA, it is worthy to explore semi-synthetic hydrogels using CMHA combined with other synthetic polymers.

Polyacrylic acid (PAA) and its derivatives belong to the most commonly used synthetic water-soluble polymers applied for the production of hydrogels. Because PAA hydrogels contain ionizable carboxylic moieties, their gel properties such as degree of swelling are greatly affected by the degree of neutralization, as well as the pH and ionic strength of the dispersing medium[5,6].As a biomaterial, it demonstrated good biocompatibility and bioadhesive properties [7,8]. However, using it alone has disadvantages when administered biomedically, and these issues are resolved through combination with other polymers.

In this study, CMHA will be mixed with linear and slightly crosslinked PAA to produce semi-synthetic hydrogels by using electron beam irradiation. The gel properties such as gel fraction and degree of swelling will be evaluated. The FT-IR spectra and thermal

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properties, as well as the in-vitro biodegradability of CMHA-PAA hydrogels will be evaluated.

2. Materials and Methods

2.1 Materials

Cosmetic grade and 1800 kDa HA (Stanford Chemicals Company), sodium hydroxide (99%, RCI Labscan), chloroacetic acid (99.5%, LOBA Chemie) and isopropyl alcohol (ACS reagent, J.T Baker) were all used as received for the carboxymethylation process. Analytical grade sodium chloride (AR, Univar) was used to prepare the mobile phase for the GPC analysis. Linear PAA and cosmetic grade Carbopol-940 were obtained from Merck Sigma-Adrich and Alysons' Chemical Enterprises, Inc., respectively. As with the in-vitro biodegradation experiment, Phosphate Buffer Saline (PBS) tablet (Merck Sigma-Aldrich) was used.

2.2 Carboxymethylation of HA

Carboxymethyl hyaluronic acid was prepared using the previously reported protocol with minor modification [9]. HA (20 g) was mixed with 50 ml of 45% w/v NaOH to form a paste, then mixed for 10 min. Isopropanol (2.0 L) was added and stirred for 1 h. About 60 g of solid chloroacetic acid was added and continuously stirred for 2.5 h. CMHA was collected by filtering the mixture through a non-woven fabric and then washed three times with isopropyl alcohol before being dissolved in 1.0 L deionized water. The CMHA solution was acidified until pH 7 with 12 N HCl and dialyzed using MWCO 12–14 kDa tubing for 3 days with frequent change of water. The solution was lyophilized and the purified CMHA solid was stored at 4 °C for analysis and preparation of hydrogels.

2.3 Determination of Molecular Weight and Degree of Substitution

Potentiometric back titration was used to determine the degree of substitution (DS) of purified CMHA[9]. Briefly, cationic exchange resin (Amberlite H-form) was mixed in 5 mg/mL HA and 10 mg/mL CMHA aqueous solutions. The mixtures were swirled in a water bath for 2 h at 37 °C then filtered through a stainless steel 200-mesh wire screen. The filtrates were passed through 0.45 μm and 0.22 μm syringe filters then freeze-dried. About 0.1 g of dry acidified HA or CMHA was dissolved in 10 mL of standardized 0.1 M NaOH and diluted with 15 mL deionized water. This solution was then titrated with standardized 0.05 N HCl using phenolphthalein as an indicator. Blank titrations contained no sample. The DS was calculated based on the following equations:

$$n_{COOH} = \frac{(V_b - V)_{HCl}}{1000} \text{ x } C_{HCl} \quad (1)$$

$$A = \frac{n_{COOH\,(CMHA)}}{m_{CMHA}} - \frac{n_{COOH\,(HA)}}{m_{HA}} \quad (2)$$

$$DS = \frac{A \text{ x } MW_{DSU}}{1 - (A \text{ x } MW_1)} \quad (3)$$

where n_{COOH} = mole of carboxyl groups, V_b = volume of HCl used to titrate blank, V = volume of HCl used to titrate sample, C_{HCl} = concentration of HCl, MW_{DSU} = molecular weight of unsubstituted disaccharide unit, m = mass of dry CMHA or HA, and MW_1 = molecular weight of carboxymethyl group.

Gel permeation chromatography (Shimadzu Prominence) equipped by refractive index detector was used to determine the average molecular weight (MW) of the purified

CMHA. Chromatography columns used were Tosoh TSK gel guard column PWXL and four TSK gel serially connected analytical columns (G6000 PWXL, G4000 PWXL, G3000 PWXL and G2500 PWXL). Elution was performed using 0.2 M NaCl at a flow rate of 0.5 mL/min while setting the temperature of the detector and columns both at 40 °C. A calibration curve was constructed using polyethylene oxide (PEO) and polyethylene glycol (PEG) as standards. Molecular weights reported in this study are based on standards and are not absolute.

2.4 Preparation and irradiation of polymer blends

Polymer blends of CMHA and PAA were prepared by combining the freeze-dried CMHA with linear PAA (CMHA-PAA) or with slightly crosslinked PAA (CMHA-Carbopol). The CMHA-PAA and CMHA-Carbopol blends were thoroughly mixed with water before being kneaded in plastic PE sheets and were allowed to stand overnight to allow complete dissolution. Afterwards, samples were kneaded again to ensure homogenization of the polymer blends prior to vacuum-sealing. Electron beam irradiation was carried out using a 2.5 MeV accelerator with 12 mA current and conveyor speed of 4.3 - 8.5 m/min. The resulting radiation-crosslinked hydrogels were then freeze-dried. Formulation of the samples were listed in Table 1.

Table 1. Formulation and irradiation doses of CMHA-PAA and CMHA-Carbopol blends

Sample	CMHA Blend Concentration	Weight Ratio	Dose (kGy)
CMHA-PAA	37.5% CMHA 2.5% PAA	15:1	20, 40
	35% CMHA 5% PAA	7:1	20, 40
CMHA-Carbopol	10% CMHA 10% Carbopol	1:1	20, 60, 120
Carbopol	10% Carbopol	-	20,60,120

2.5 Determination of Gel Properties

Gel fraction and degree of swelling of the hydrogel samples were determined based on our previous study [3].Approximately 0.2 g of freeze-dried samples were immersed in 1.0 L of deionized water at room temperature for 72 h. Every 24 h, swollen samples were weighed and re-immersed in freshly-changed deionized water. Swollen samples were then dried at 50°C to a constant weight to obtain the gel fraction. The gel fraction and degree of swelling were calculated as follows:

$$\text{Gel Fraction } (\%) = \frac{W_d}{W_i} \text{ x } 100\% \quad (4)$$

$$\text{Degree of Swelling } \left(\frac{g}{g}\right) = \frac{W_s - W_d}{W_d} \quad (5)$$

where W_d = weight of dried insoluble part after immersion for 3 days, W_i = initial weight of dried sample after irradiation, and W_s = weight of swollen gel. All measurements were done in triplicates.

2.6 FT-IR and Thermogravimetric Analysis

The FT-IR spectra (600-4000 cm-1) of hydrogel samples without sol fraction were obtained by PerkinElmer Spectrum One FTIR spectrophotometer in attenuated total reflectance

(ATR) mode. TGA thermograms were collected using a Netzsch STA449 F3 Jupiter at temperatures ranging from 25 to1000 °C at a heating rate of 10 °C/min in a nitrogen atmosphere.

2.7 In-Vitro Biodegradability

About 20.0 mg of freeze-dried hydrogels were placed in a 20-mL vials and submerged in 4.0 mL PBS (pH 7.4) or in a 1: 200 ratio of dried hydrogel to PBS. Samples were allowed to swell and incubated at 37 °C and weighed periodically (1, 3, and 5 d). The pH of the buffer was adjusted to 7.4 (1.0 mM HCl and NaOH solution) and replenished as needed. The degree of swelling in PBS solution was calculated from the weight of swollen gel and the initial weight of freeze-dried sample as shown in the equation:

$$\text{Degree of Swelling in PBS } \left({}^{g}/_{g}\right) = \frac{W_{s,PBS} - W_i}{W_i} \quad (6)$$

where $W_{s,PBS}$ = weight of swollen hydrogel in PBS solution, while W_i = weight of freeze-dried hydrogel with insoluble fractions.

In-vitro degradation of the hydrogel blends was evaluated by determining the weight loss of freeze-dried hydrogel exposed in PBS (pH 7.4) at 37 °C. Submerged hydrogels in a 1: 200 ratio of dried hydrogel to PBS were collected periodically in intervals of 1, 3, and 5 d. Swollen samples were washed with PBS and then freeze-dried. The degradation was calculated as follows:

$$\text{Degradation (\%)} = \frac{W_{d,PBS}}{W_i} \text{ x } 100 \quad (7)$$

where $W_{d,PBS}$ = weight of freeze-dried CMHA submerged in PBS at day 1,3, or 5.

3. Results and Discussion

3.1 Degree of Substitution and Molecular Weight of Carboxymethyl Hyaluronic Acid

Carboxymethylation of hyaluronic acid introduces a new functional group, which offers many possible prospects for the synthesis of new products from this simple polysaccharide with interesting chemical and biological properties. In radiation processing, the chemistry of carboxymethyl groups leads to the formation of radical intermediates which are helpful in forming chemical bonds with other polymer chains, resulting in high-purity hydrogels. We previously attempted to make pure carboxymethyl hyaluronic hydrogels using high energy radiation and discovered that higher DS, MW, concentration, and irradiation dose are required to make biocompatible CMHA hydrogels [3,9]. In this particular study, the DS and MW of the CMHA produced were 0.87 and 141 kDa, respectively. This will be used to investigate milder irradiation conditions for the formation of hydrogels by combining CMHA with linear and slightly crosslinked PAA. The sections that followed discusses the characterization of the polymer blends, such as their gel properties, molecular structure, thermal stability, and in-vitro degradation using PBS (pH 7.4).

3.2 Gel Properties of CMHA-PAA and CMHA-Carbopol Blends

Hydrogels for biomedical uses undergo initial characterization through the determination of their gel content and swelling properties. These two properties can be used as a means to assess the stability of hydrogels in aqueous solutions [10]. The gel properties of CMHA-PAA are shown in Figure 1. The gel fractions substantially increased as absorbed dose becomes higher from 20 to 40 kGy (Figure 1a). Hydrogel blends of 35% CMHA 5% PAA had higher gel fractions compared to the 37.5% CMHA 2.5 % PAA blend for both doses.

These values show that higher irradiation doses and higher concentrations of PAA result to more crosslinking, hence the higher gel fractions.

Different trends for the degree of swelling were observed as dose increases (Figure 1b). The degree of swelling of the 35% CMHA 5% PAA blend decreased from 1115 to 616 g/g while formulation with 37.5% CMHA 2.5 % PAA increased from 522 to 895 g/g. From the results of the gel fraction experiment, higher degrees of swelling are expected with the hydrogels with more PAA and are irradiated at higher doses due to more crosslinking. However, the swelling ratio obtained for the 35% CMHA 5% PAA did not follow the same trend at 40 kGy possibly because the higher degree of crosslinks caused the hydrogel to be more rigid, hence restricting its ability to swell and hold water in its network.

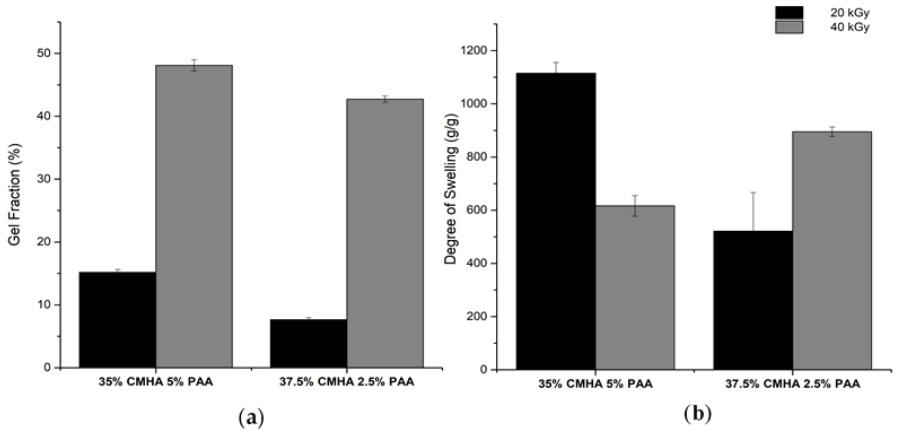

Figure 1. Gel fraction (a) and degree of swelling (b) of CMHA-PAA hydrogels

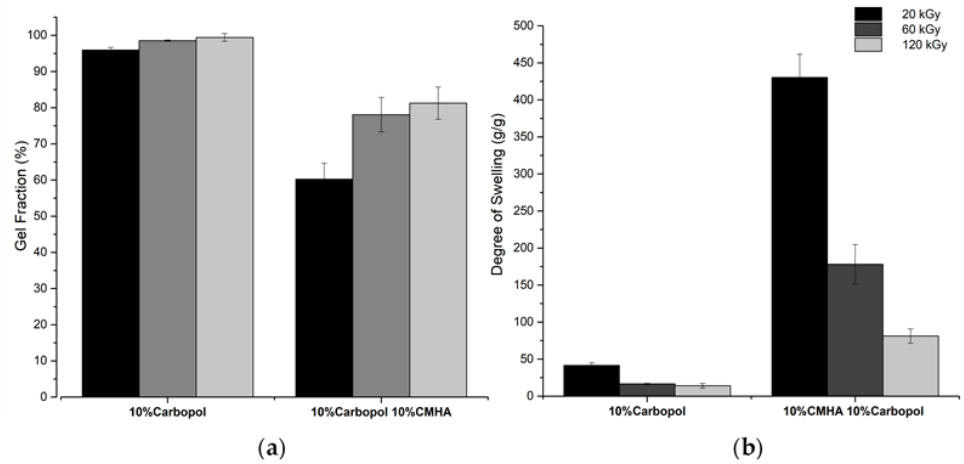

Figure 2. Gel fraction (a) and degree of swelling (b) of CMHA: Carbopol blends

For the CMHA-Carbopol hydrogel blends, it was also observed that gel fractions increased proportionally with dose for all samples (Figure 2a). However, the gel fractions decreased when CMHA was mixed to Carbopol, with the 10% pure Carbopol samples obtaining gel fractions in the range of 95-99%, while the 10% Carbopol 10% CMHA obtained 60-81%. The soluble fraction from the 10% Carbopol 10% CMHA hydrogel may have predominantly originated from the degraded CMHA chains. This could be due to the fact that some of the CMHA polymeric chains were degraded since polysaccharides, including hyaluronic acid and their derivatives, are susceptible to chain scission when exposed to high energy radiation.

As previously discussed, the degree of swelling decreases for samples with high PAA concentration at higher doses due to the more formation of crosslinks within the hydrogel (Figure 2b). Higher degrees of swelling of 10% Carbopol 10% CMHA hydrogel blends were observed compared to the pure 10% Carbopol samples which may have been caused by the presence of more flexible polymer chains having a network of lesser crosslinks.

The hydrogel blends based on CMHA and polyacrylic acid polymers produce interesting gel properties that can be controlled by modifying either the polymer concentrations or the irradiation dose or both. It is also possible to produce gels using milder irradiation conditions by considering concentrations with equal or lower weight ratio of CMHA to PAA polymers (1:1 or lower than 7:1).

3.3 FT-IR and TGA Analysis

One way to determine the successful crosslinking or blending of polymer chains within the hydrogel is by analyzing their FT-IR spectra and comparing them from their pristine raw materials[11]. The FT-IR characteristic peaks of the raw materials HA, CMHA, PAA, and Carbopol, as well as the CMHA-PAA hydrogel blends were shown in Figure 3. The 35% CMHA 5% PAA hydrogel irradiated at 40 kGy have shown more resemblance to the spectrum of its dominant polymer CMHA but with new peak around 1705 cm-1 due to the C=O carboxylic group of PAA. The FT-IR spectra of 10% CMHA 10% Carbopol irradiated at 20 kGy showed a significant deviation from its pristine material. Specifically, the C=O carboxylate group from PAA showed a strong peak at 1410 cm-1 obscuring the C=O carboxylic groups of hyaluronic acid as well as the decrease of peak intensity at 1040 cm-1 corresponding to the C-OH alcohol moiety from CMHA. It was also observed that the change of shape of the characteristic bands of -OH at 3000- 2850 cm-1 changes depending on the weight ratio of the polymers.

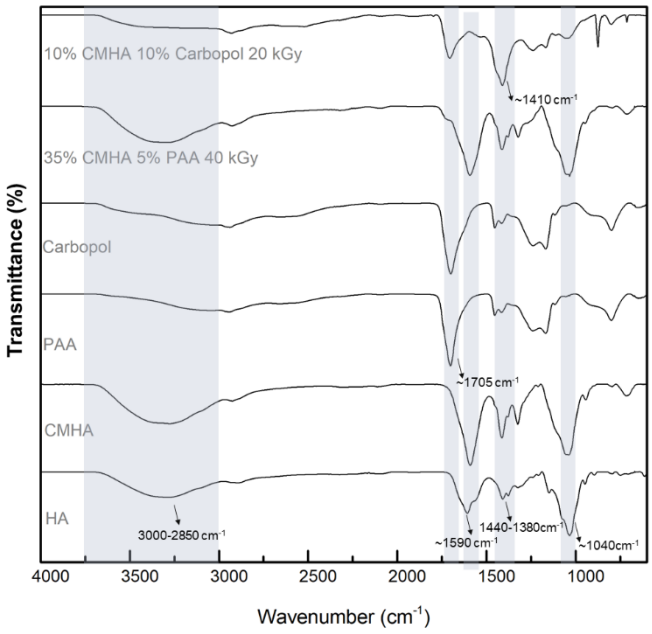

Figure 3. FT-IR spectra HA, CMHA, crosslinked CMHA, crosslinked CMHA-PAA, and crosslinked CMHA-Carbopol hydrogels

The thermograms of the CMHA:PAA and CMHA/Carbopol hydrogels without soluble fractions were shown in figure 5. The TG and DTG profiles depicted multistep decomposition of the samples. Possible dehydration of 35% CMHA 5% PAA 40 kGy was 71.7 °C that is generally lower than the 164.5 °C of 10% CMHA 10% Carbopol 20 kGy hydrogels

(Figure 5). Previous studies had also observed great difference of dehydration temperature between their PAA-based samples [12]. At 185-315 °C, decarboxylation and depolymerization of both PAA and CMHA main chains of the hydrogel blends occur [9,13]. Observed mass loss greater than 315°C were potentially from crosslinked-PAA chains with different crosslink density formed after irradiation[14].

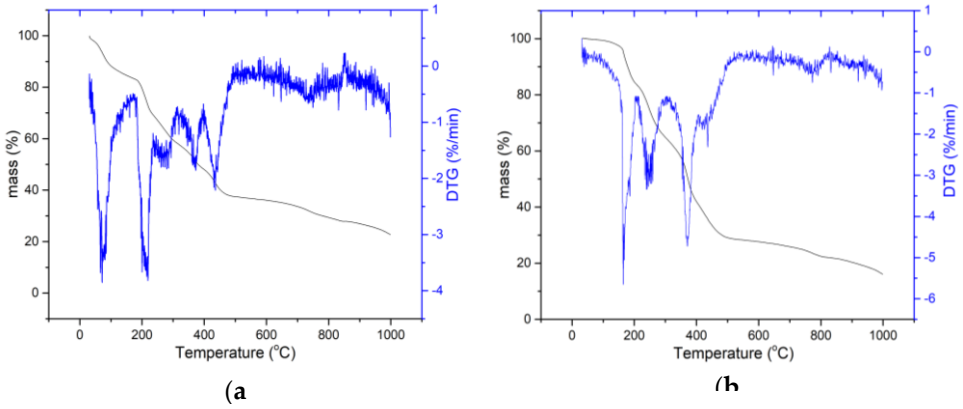

Figure 4. TGA and DTG profiles of (a) 35% CMHA 5% PAA irradiated at 40 kGy and (b) 10% CMHA 10% Carbopol irradiated at 20 kGy hydrogels.

3.4 In-Vitro Biodegradation of Hydrogels in Phosphate Buffer Solution (PBS)

By using simulated ionic and pH conditions of biological fluids such as PBS (pH=7.4) at 37 °C, the stability of the samples can be initially assessed[15]. Figure 5 evidently showed that formulation with equal weight ratio or 10% CMHA 10% Carbopol irradiated in 20 kGy have a stable crosslinking network exhibiting stable swelling property without significant increase of degradation percentage in PBS Biomedical hydrogels with stable gel properties in biological fluids can be potentially use for applications that need longer exposure when implanted in the body.

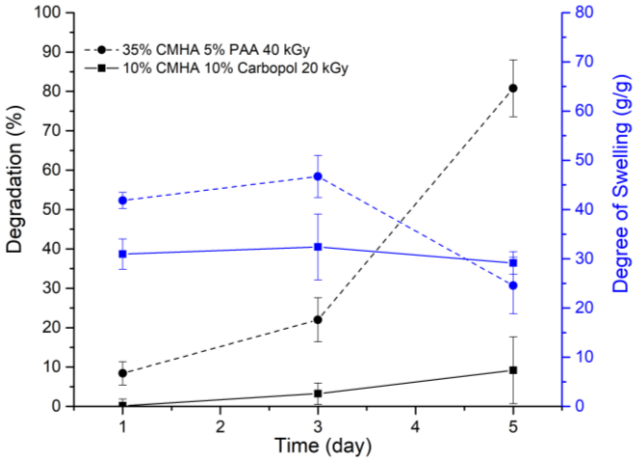

Figure 5. In-vitro degradation and swelling property of CMHA with poly(acrylic) acid hydrogels in PBS (pH 7.4) at 37°C

4. Conclusion

Semi-synthetic hydrogels based on CMHA with polyacrylic acid (CMHA-PAA) and CMHA-Carbopol) were produced using electron beam irradiation without using of toxic

initiators or crosslinkers. The gel properties of the produced gels can be easily modified by changing the polymer weight ratio and absorbed radiation dose, according to the results. Crosslinking between CMHA and PAA polymer chains was assessed by changes in the IR spectra and thermal stability of the samples. Moreover, in-vitro biodegradability study showed stability of the produced hydrogels. Since hyaluronic acid is ubiquitous to human body, CMHA-based hydrogels have the strong potential to be used for biomedical applications.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Alvin Kier R. Gallardo and Alyan P. Silos. The investigation and supervision were led by Lorna S. Relleve. The first draft of the manuscript was written by Alvin Kier R. Gallardo and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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