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SYNTHESIS, CHARACTERIZATION OF CuO NPs USING GREEN SYNTHESIS AND ITS APPLICATION AS A SELECTIVE NON-ENZYMATIC GLUCOSE BIOSENSOR

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ABSTRACT

Biosensors development has become very important by using NPs showing enzyme mimic properties. In this study, it was aimed to synthesize CuO nanoparticles (NPs) by green synthesis method and to use to determine the amount of glucose in some food samples. CuO nanoparticles were synthesized by using Fig (*Ficus carica*) fruits by green synthesis method and it was used to determine the glucose amount in some food samples. The resulting CuO NPs were characterized by FT-IR, XRD and SEM analyzes. From the results, it was determined that the CuO NPs were symmetrical, spherical and in the range of 20-66 nm. Then, CuO NPs were used to detect glucose levels in different types of foods samples as non-enzymatically. The high accuracy results were observed with CuO NPs having enzyme mimic properties. In the new targeted method, the glucose oxidase (GOx) enzyme with CuO NPs mimicking peroxidase was used to develop a spectrophotometric method for the determination of highly sensitive and stable - enzymatic glucose in different foods. The developed method was found to be highly linear in the 0.25-2 g/L glucose range. The optimum operating parameters of the biosensor were, respectively, temperature, (40°C), pH (4.0) and H_2O_2 concentration (12.5 mM). It was observed that this method showed a high selectivity to glucose.

Keywords: CuO nanoparticles; green synthesis; fig (*Ficus carica*); nano-biosensor; glucose amount; food samples.

INTRODUCTION

Glucose biosensors are the first synthesized biosensors and are widely used in clinical chemistry, food industry and environment [1,2]. In general, the glucose oxidase (GOD) enzyme in glucose biosensors is the most commonly used enzyme in the determination of the amount of glucose. Although enzymes have high specificity and sensitivity, they are limited to unstable complex immobilization process and high enzyme costs [3]. Non-enzymatic glucose biosensors are being developed to overcome these disadvantages. There is a need for a non-enzymatic glucose biosensor generated without the use of enzymes, a material capable of catalyzing redox reactions and efficiently transferring electrons to the electrodes [4]. Nanoparticles are promising and suitable sensing materials, because the combination of components can provide an expanded interface in which load and energy transfer are significantly improved [5].

Copper oxide (CuO) NPs is a potential material due to its unique properties such as being chemical inert, biocompatible, non-toxic in forming glucose biosensors without enzymes [6]. CuO NPs can greatly stimulate electron transfer in the biosensor mechanism. In this way, this material is a very suitable electron conductor for enzyme mimic biosensors [7].

Diabetes is a chronic disease and affects millions of people worldwide [8]. Following the amount of glucose is a very important routine analysis that should be done to prevent the health problems of diabetes patients [9, 10]. Therefore, the rapid and accurate determination of glucose has become a field of research in both clinical diagnosis and Nano biotechnology research [11]. Currently, glucose in biological fluids, such as blood or serum, is assessed using techniques that rely on the electrochemical oxidation of glucose using glucose oxidase enzymes due to excellent selectivity, accuracy and sensitivity [12]. The potential of the enzyme-immobilized biosensors is limited by the complexity of electrode manufacturing and poor enzyme stability to harsh environmental influences. Furthermore, the responses of the enzyme-based biosensors are affected by the humidity in the environment during use or during storage. Therefore, in both in vivo and in vitro, a simple, cost-effective protocol for measuring glucose concentrations with high accuracy and precision is very desirable [13,14]. We aimed to determine the glucose in different samples (food, blood and serum) by combining the peroxidase-like activities of the CuO NPs. Since finding a suitable detection material is the primary task in a research that focuses on non-enzymatic biosensors, CuO NPs were prepared and used as a non-enzymatic glucose biosensor in a green synthesis [14,15].

MATERIALS AND METHODS

Materials

Fig (*Ficus carica*) fruits was obtained from Sakarya (Turkey) province in September, 2017. $Cu(SO)_4$ Sodium phosphate (Na_2HPO_4), Sodium carbonate (Na_2CO_3), Sodium hydroxide (NaOH), Hydrochloric acid (HCl) were purchased from Sigma-Aldrich GmbH, (Sternhe I Germany). The other chemicals were obtained from Merck. Distilled water was used in all experiments.

Preparation of Plant Extract

Fig (*Ficus carica*) fruits were washed with distilled water several times for cleaning dust and soil on plants. Small pieces (25 g) were thoroughly shattered to form a homogeneous mixture in blander using 250 mL, 10 mM sodium phosphate buffer (pH: 6.0). Then, it was centrifuged at 5.000 *xg* for 10 min and the supernatant was used for green synthesis [16].

Green Synthesis of CuO NPs

Interaction time; For this purpose, the reaction medium prepared with $Cu(SO)_4$ was taken with a 3-minute interval and the spectrophotometer was monitored for 240 minutes by measuring the absorbance (240 nm) against the blunt solution, and it was determined that the time of formation of the CuO NPs was highest.

Optimum pH; for the synthesis of CuO NPs, it was investigated which pH was more efficient by using different buffers solutions.

For this purpose, the reaction medium was formed using phosphate buffer for pH:

2-3, acetate buffer for pH: 4-6, phosphate buffer for pH: 7-8 and carbonate buffer for pH: 9-11, and the changes in absorbance at 240 nm were measured by UV-Vis spectrophotometer.

Optimum Temperature; In order to determine the temperature at which the CuO NPs are synthesized more effectively, reactions were made at 10-90°C to determine the optimum temperature by analyzing the solutions taken from the reaction medium against the blind solutions [17].

Characterisation of CuO NPs

CuO NPs were firstly synthesized using Fig (*Ficus carica*) fruit extract and they were characterized with scanning at range of 200-1000 nm by using UV–vis spectro-photometer (Epoch Nanodrop UV–vis spectrophotometer). Second determina-tion of surface topography CuO NPs was performed by SEM. Then, XRD analysis was determined in determination of crystallinity of CuO NPs. The surface morphologies of the CuO-NPs were examined using a Metek, Apollo prime, active area 10 mm^2, Microscope inspect S50, SE detector R580 SEM operated at 20 kV on samples with energy-dispersive X-ray (EDX) analysis attached to scanning electron microscope (SEM). X-ray diffraction (XRD) patterns were performed on Panalytical empyrean equipped with Ni-filtered Cu Kα radiation (λ = 0.1542 nm) in the range of 10°–80° at a scanning rate of 4° min^{-1}. FTIR analysis of CuO NPs was recorded using Vertex 80 Model FTIR Frontier spectrophotometer with attenuated total reflection (ATR) technique in the 4000-400 cm^{-1} region.

Investigation of Peroxidase-like Activity of CuO Nanoparticles

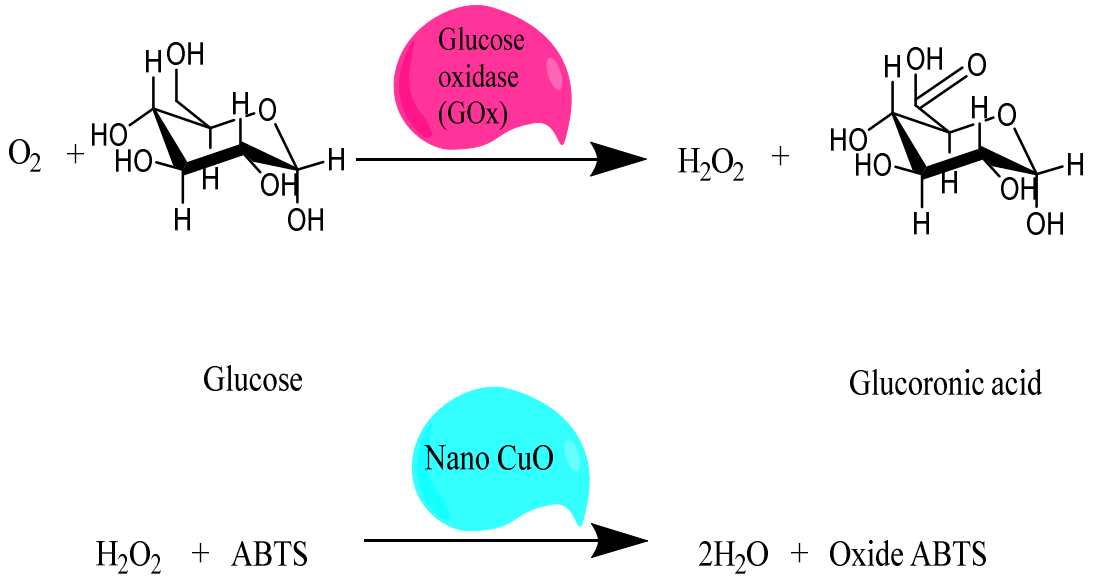
The CuO NPs obtained were measured using the substrate of the enzyme to determine if it had peroxidase activity. For this purpose; 24 µL of the ABTS, 60 mM was mixed with CuO NPs and 24 µL of the H_2O_2 were added and the enzyme activity was determined by measuring UV-Vis spectrophotometer (412 nm) and calculated. One EU of peroxidase enzyme was defined that oxidize 1.0 µmole of ABTS per minute [18]. After the method was optimized by using Nano-CuO peroxidase enzyme, the amount of glucose was decreased according to the following principle (Schema 1).

Measured Glucose Amount Using CuO NPs

The amount of glucose in some fruit juices (such as Cherry juice, Orange juice, peach juice, molasses, honey, milk, energy drink, coke and bread samples) was determined using CuO NPs using spectrophotometric method [19] .

Preparation of glucose standard concentrations: Glucose analysis was performed using CuO NPs at different glucose concentrations between 0.25 g/L and 1 g/L. Using UV-Vis spectrophotometry, changes in the absorbance values of all samples were measured at a wavelength of 240 nm.

Fruit juice, milk and similar samples: All samples must be clear and homogeneous in order to perform glucose analysis. For this purpose, fruit juices were filtered using Watman filter paper to obtain a clear image. Glucose analysis was performed after 1/2 dilution of all samples.



Schema 1. Analysis principle of glucose using CuO NPs

Samples of carbonated beverages and the like: The carbonic acid in the samples was stirred at 35°C for 3 minutes to separate from the sample. Glucose analysis was performed after ½ ratio (v / v) dilution of all samples.

Honey samples: After homogenizing the samples such as honey, 5 g of the sample was completed with 20 mL of pure water. The solution was then homogenized by heating with a heater and then passed through a Watman filter paper. Glucose analysis was performed after dilution of the sample to ¼ (v / v).

A similar spectroscopic and colorimetric based commercial kit was purchased to compare the method. The working principle of the kit was similar to ours, but was based on the use of different substrates and two separate enzymes. Glucose analysis set and also the results of the studies were performed by HPLC method and the results were compared.

RESULTS AND DISCUSSION

Characterization of the Synthesized Cupper Nanoparticles

The absorbance values of synthesized cupper nanoparticles were measured against distilled water using UV-VIS spectrophotometer and the sharpest peak of these NPs was observed at 240 nm (Fig. 1).

This is attributed to the formation of the CuO nanoparticles [20]. Even after prolonged storage, the UV-visible spectrum of CuO NPs has not changed. It is clear from the spectrum that the CuO nanoparticles obtained by the green synthesis give light to the conclusion thatthey are symmetrical and spherical [20, 21].

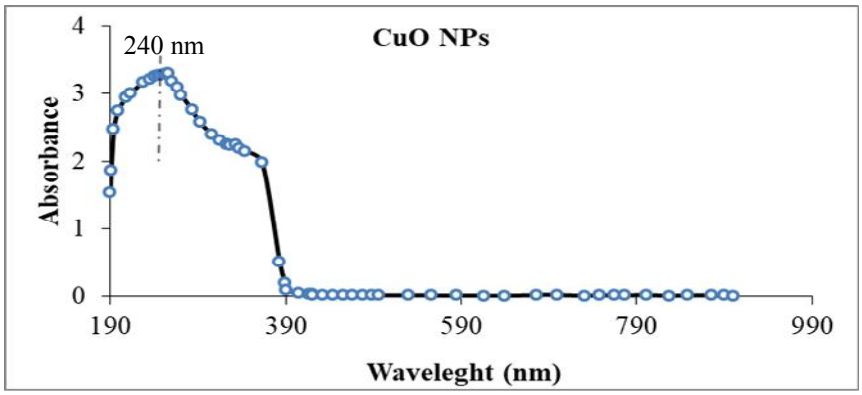


Fig. 1. Scanning of CuO NPs wavelength

Chemical Properties of CuO NPs Synthesis

The effect of pH on the electrode response was investigated by using different buffer systems (50.0 mM) between pH 3.0 and 11.0 with an increment of 1.0. As can be seen from Fig. 2A, the current value of the enzyme electrode increases significantly from pH 3.0 to 5.0, and then a decrease is obtained at pH values higher than 4.0. As a result, pH 4.0 was chosen as optimum pH for further studies.

The effect of the temperature on the synthesis of CuO nanoparticles was also investigated using acetate buffer (pH: 4.0 and 50.0 mM) with an increase in the temperature between 10°C and 20°C. As can be seen from Fig. 2B, the optimum temperature value in the synthesis of CuO NPs is 40°C, followed by a reduction in temperature values greater than 40°C.

Characterization of CuO NPs and Biosensor Assays

The synthesized CuO NPs were characterized by UV-Vis spectrum, scanning electron microscope (SEM), FTIR, EDX and X-Ray diffraction. Then, copper oxide (CuO) NPs were investigated using as a new biosensor for identify glucose.

Surface Characterization of CuO NPs

Chemical and mineralogical compositions of synthesized green Copper NPs were determined by scanning electron microscopy (SEM), which was used to examine the surface of the adsorbent (Fig. 3A). The picture clearly shows that the CuO NPs sizes were in nanometers and they have a short nanorod-like structure.

XRD Studies

Copper oxide nanoparticles' XRD which was produced in its peroxidase enzyme catalyst and its crystallographic analysis are given in Fig. 3B.

Fig. 3B showed the XRD patterns of the as-prepared CuO-NPs using Fig (*Ficus carica*) fruit extract. XRD analysis revealed small distinct diffraction peaks at 32.0, 39.7 and 56.6 for the CuO NPs. The present experimental results were found to be consistent with reported diffraction patterns of CuO NPs [22].

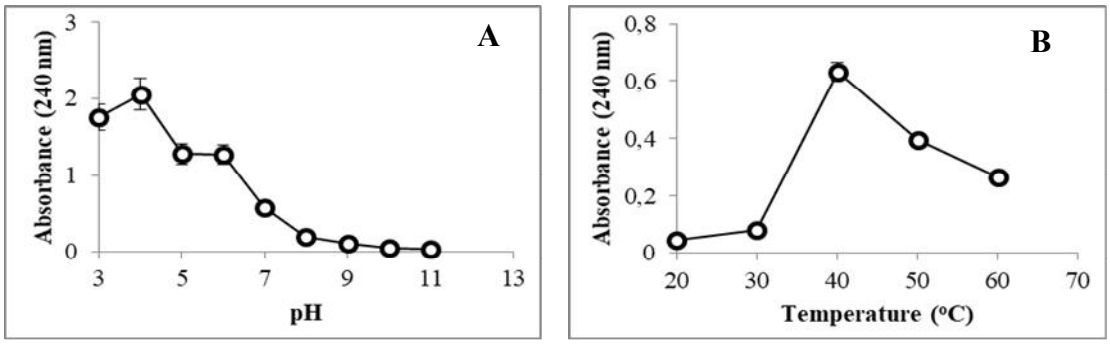


Fig. 2. The effect of pH, (A) and temperature (B) on the synthesis CuO NPs using green synthesis

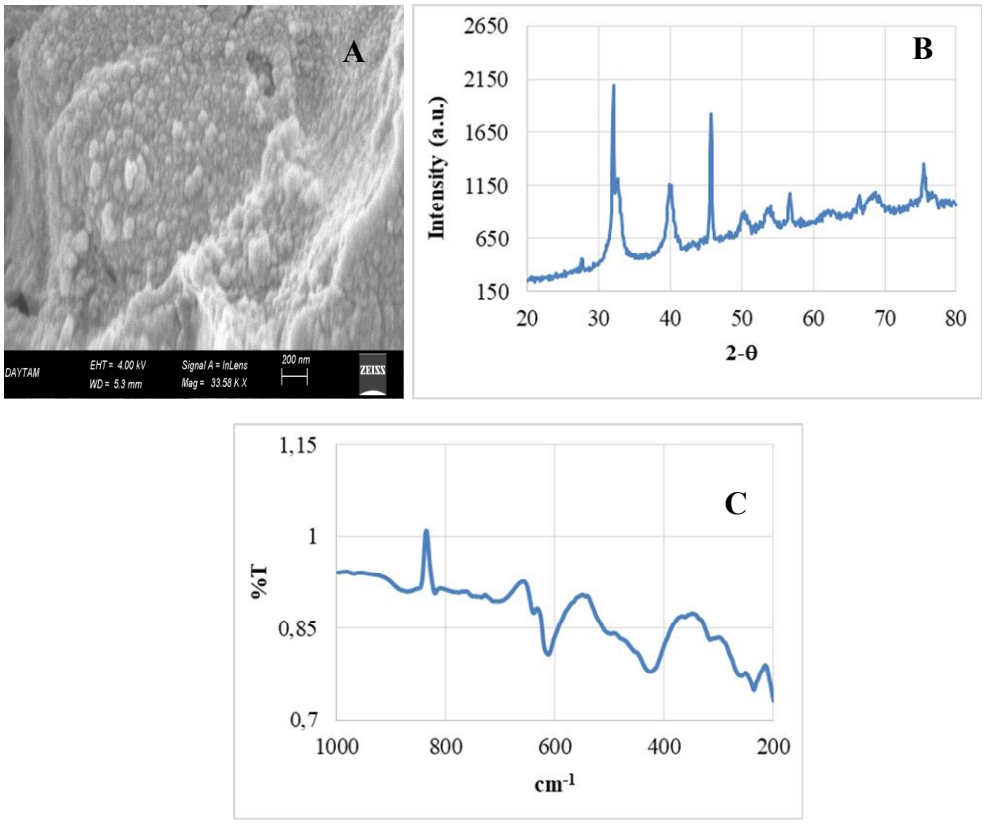


Fig. 3. XRD image of CuO NPs (A), SEM images for Cu O NPs (B), FTIR images for CuO NPs (C)

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fig. 3C showed the FTIR of the CuO NPs prepared by the solvo thermal method, in the range of 1000-200 cm^{-1}. A broad absorption band was observed at around 422 cm^{-1}. The FTIR results showed the high purity of the obtained CuO NPs (Fig. 3C).

Determination of Glucose by Using CuO NPs

In Fig. 4, linearity was observed when the concentration graph was drawn against absorbance in the range of 0.25-1 g/L glucose concentration. Each error bar 5 showed the standard deviation for the measurement.

The concentration absorbance graph was drawn by using glucose standard solutions which was prepared at different concentrations and the glucose concentrations of the samples were calculated using the observed equation from this graph.

Glucose Concentration (g / L) = (Absorbance Increase Measured for Sample / The slope of the calibration graph) (1)

After optimizing the method we developed by using CuO NPs, the glucose analysis was performed using the glucose analysis kit method to compare the accuracy of the measurement results. In the glucose assay kit, glucose oxidase and peroxidase enzymes as well as different colorimetric substrates can be used to perform glucose analysis. The comparison of the commercially available glucose analysis kit and the CuO NPs based method assay method was done by measuring the amounts of standard glucose samples in different concentrates and the results were given in Fig. 4.

The selectivity of the method based on CuO NPs was performed for glucose in the concentration range of 0.25 (g/L) – 2 (g/L), as shown in Table 1. Compared to the other method, the data obtained from CuO NPs were found to be closer to the standard for all glucose concentrations. CuO NPs synthesized by the green synthesis method and the new determination method using the GOx enzyme were tested for specificity by using different sugar samples to test the specificity of glucose determination and the results of the new method were measured.

Analysis of glucose content in food samples according to different methods and newly developed method was used for comparison. Glucose amount was determined for some food samples such as Cherry juice, Orange juice, peach juice, molasses, honey, milk, energy drink, coke and bread samples using the new method. Glucose determination assays were repeated using the glucose analysis kit for comparing to the new method results. In the same operating conditions, biosensor system response was measured by two different methods. The results are given in Table 1 in comparison with the glucose analysis kit method. In order to be a reference, the amount of glucose in the foods determined from the TURKOMP database was also used [23,24].

The recovery values of the spectrophotometric analytical signal for each of the food samples diluted with CuO NPs were calculated and shown in Table 1. From the recovery values, it can be concluded that the developed biosensor can be used for the detection of glucose even in complex matrix systems such as different food samples. When the results were examined carefully, it was observed that the results obtained with the use of CuO NPs for all foodstuffs gave

nearly 100% similarities to the commercial glucose kit. These values are within acceptable limits for the feasibility of the system developed for the detection of glucose in food samples [25,26].

As it can be seen in Table 1, it was determined that all results obtained by using CuO NPs and GOx in foods were more compatible with the database (TURKOMP 2018).

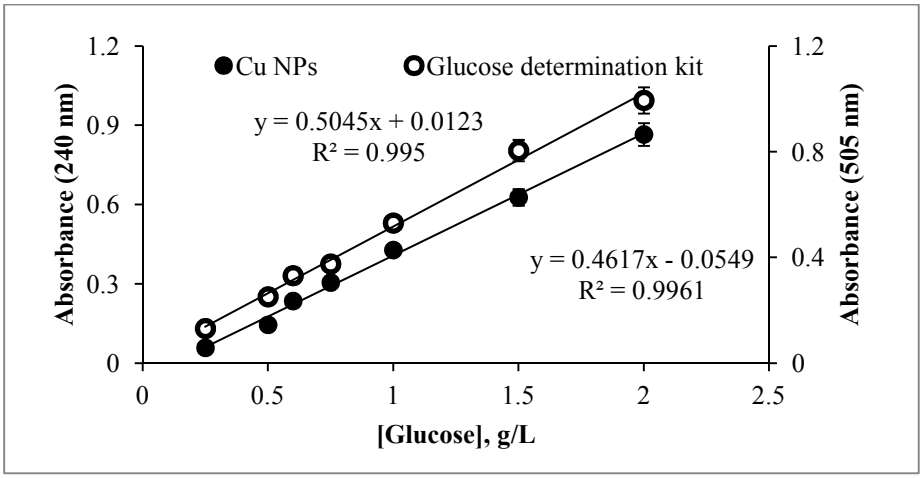


Fig. 4. CuO NPs based glucose determination method calibration chart

Table 1. The results of amount of glucose in various food samples

Food sample	CuO NPs based spectrophotometric Method (gr/L)	Glucose analysis kit with spectrophotometric method (gr/L)	The amount of glucose in foods from the literature HPLC (gr/L)
		Fruit juice	
Cherry juice	108.8 ± 1.18	103.6 ± 1.44	106.2
Orange juice	58.3 ± 1.2	52.6 ± 0.6	46.4
Peach juice	56.4± 0.17	58.8 ± 1,17	56.1
		Sweet foods	
Molasses	12.58 ± 2.65	16.6 ± 1.78	10.0
Honey	288.9 ± 3.09	278.9 ± 4.01	283.9
		Skimmed milk	
Milk	48.8 ± 0.3	42.7 ± 1.18	50.0
		Some drinks	
Energy drink	226.0± 1.5	217.0 ± 1.70	215.5
Coke	51.9 ± 1.55	54.0 ± 1.16	52.3
		Bakery food	
Bread	28.11 ± 2.16	29.85 ± 2.15	-

CONCLUSION

In this paper, CuO nanostructures were successfully synthesized by using green synthesis by using copper nitrate ($CuSO_4$) precursor. SEM, XRD and FTIR results showed that the formation of CuO nanostructures can be achieved by one step green synthesis method. We had determined that the preparation of CuO NPs for glucose detection in some food samples could be used as an easy and useful biosensor. It was determined that CuO NPs resulted in rapid, stable and sensitive responses in bioactivity, stability and glucose biosensitivity under operational conditions (pH: 4.0, 40°C). On the other hand, the data obtained from the sample application section showed better results than the commercial glucose assay kit of biosensing responses [27-29]. Compared to other glucose biosensors in the literature, this system is thought to provide practical, precise and robust glucose detection in real samples. On the other hand, the use of nanomaterial in the biosensor structure strengthened the effect of the system and as a result a more robust biosensor was obtained.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

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