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Antiviral zinc oxide nanoparticles mediated by hesperidin and in silico comparison study between antiviral phenolics as anti-SARS-CoV-2

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which caused the coronavirus (COVID-19), is the virus responsible for over 69,613,607 million infections and over 1,582,966 deaths worldwide. All treatment measures and protocols were considered to be supportive only and not curative. During this current coronavirus pandemic, searching for pharmaceutical or traditional complementary and integrative medicine to assist with prevention, treatment, and recovery has been advantageous. These phytopharmaceuticals and nutraceuticals can be more economic, available, safe and lower side effects. This is in silico comparison study of ten phenolic antiviral agents against SARS-CoV-2, as well as isolation of the most active metabolite from natural sources. Zinc oxide nanoparticles (ZnO NPs) were also then prepared using these metabolite as a reducing agent. All tested compounds showed predicted anti-SARS-CoV-2 activity. Hesperidin showed the highest docking score, this leads us to isolate it from the orange peels and we confirmed its structure by conventional spectroscopic analysis. In addition, synthesis of hesperidin zinc oxide nanoparticles was characterized by UV, IR, XRD and TEM. In vitro antiviral activity of hesperidin and ZnO NPs was evaluated against hepatitis A virus as an example of RNA viruses. However, ZnO NPs and hesperidin showed antiviral activity against HAV but ZnO NPs showed higher activity than hesperidin. Thus, hesperidin and its mediated ZnO nanoparticles are willing antiviral agents and further studies against SARS-CoV-2 are required to be used as a potential treatment.

1. Introduction

The current COVID-19 pandemic is responsible for over 69 million infections and over 1.5 million deaths worldwide to date, 2.3 million infections and more than 55,000 deaths in Africa, and over 119,000 infections and more than 6000 deaths in Egypt [1]. In most individuals infected with SARS-CoV-2 symptoms include mild to moderate respiratory disease that might recover without physician consultation. However, individuals with chronic diseases such as cardiovascular disease, diabetes, chronic respiratory disease or cancer have a greater risk of developing acute respiratory symptoms. COVID-19 transmission is prevented by maintaining social distance, washing hands or using alcohol sterilization, and not touching the face. Where it transmit through droplets of saliva from the person nose when an infected individual coughs or sneezes [1]. All measures and protocols were considered to be only supportive but not curative. However, there are many current clinical trials evaluating potential drugs [1]. Since claiming COVID-19 as a global pandemic there was a necessity to search for pharmaceutical or traditional, complementary and integrative medicine to assist with prevention, treatment and recovery [2].

Natural products are untapped sources for compounds that can be used in the prevention and treatment of different diseases. In addition,
natural compounds have often served as cheaper and safer drug candidates against several diseases, many nutraceutical compounds were reported to have antiviral activities [3].

In the last decade, the number of new antiviral drugs extracted from natural sources has risen significantly. Natural products assist in the discovery of antiviral medicines, either directly or indirectly [4].

Therefore, screening of natural compound libraries by computational screening methods as molecular docking can save both time and money in drug development [3].

On the other hand, Zn containing compounds have shown antiviral properties toward a number of viruses according to such money in drug development [3]. discovery of antiviral medicines, either directly or indirectly [4].

Physical processes such as attachment of virus, virus infection, and natural sources has risen significantly. Natural products assist in the discovery of antiviral medicines, either directly or indirectly [4].

In this work, a number of phytopharmaceutical phenolic compounds such as rutin, coirilagin, diosmin, myricetin-3-O-xylosyl-(1→2)-rhamnoside, epigallocatechin-3-O-gallate, hesperidin, lyoniresinol, myricetin, naringenin and quercetin 3-O-glucuronide versus the crystal structure of SARS-CoV-2 M\(^{\text{pro}}\) (6lu7) [8] which were used with a resolution of 2.1 Å. It was used for in silico molecular docking. This protein is composed of two polypeptides known as proteomes A and B. This dimer has a crystallographic symmetry along two fold axis. It contains three domains; domain I (residues 8–101), domain II (residues 102–184) with an antiparallel β-sheet structure and domain III (residues 201–303) which was been arranged in an α helix, as well as cleft region between domain II and III (residues 185–200) [8].

Fig. 1. Main protease (6lu7) was been classified according to their domain regions; domain I (residues 8–101), domain II (residues 102–184) with an antiparallel β-sheet structure and domain III (residues 201–303) has five α-helices arranged as antiparallel spherical cluster. Where domain III connected to domain II by a long loop region (residues 185–200). SARS-CoV-2, M\(^{\text{pro}}\), has a Cys-His coupled catalytic activity, where the substrate-binding site is located in a cleft between domain I and domain II [8] as in Fig. 1. In the current study, molecular docking procedures were executed by the Swiss Dock service [9]. Using EADock DSS, which perform blind docking and its CHARMM energies were assessed on a grid where the most favorable energies were estimated with FACTS and clustered. These clusters can be visualized and interpreted using Chimera as reported in literature [10].

2. Materials and methods

2.1. Molecular docking

The main protease (M\(^{\text{pro}}\)) is an important enzyme encoded by the coronavirus genome, with an essential role in virus replication and transcription process, making it a suitable drug target for SARS-CoV-2. To reveal the inhibition mechanism of selected polyphenolic compounds such as rutin, coirilagin, diosmin, myricetin-3-O-xylosyl-(1→2)-rhamnoside, epigallocatechin-3-O-gallate, hesperidin, lyoniresinol, myricetin, naringenin and quercetin 3-O-glucuronide versus the crystal structure of SARS-CoV-2 M\(^{\text{pro}}\) (6lu7) [8] which were used with a resolution of 2.1 Å. It was used for in silico molecular docking. This protein is composed of two polypeptides known as proteomes A and B. This dimer has a crystallographic symmetry along two fold axis. It contains three domains; domain I (residues 8–101), domain II (residues 102–184) with an antiparallel β-sheet structure and domain III (residues 201–303) which was been arranged in an α helix, as well as cleft region between domain II and III (residues 185–200) [8].

2.2. Plant materials, extraction and isolation

Orange peels (500 g) were purchased from a local juice factory. Orange peels (200 g) were extracted with 2 L of hot distilled water till exhaustion. The extract was filtered and evaporated to become more concentrated under vacuum. The water extract was partitioned by CH\(_2\)Cl\(_2\) followed by butanol. The butanol subfraction was applied to Whatman filter paper sheets 3 MM using BAW as eluent [12,11] to yield a major dark purple band under ultraviolet (UV) light. The dark purple band was isolated and macerated in methanol, then evaporated under vacuum using rotatory evaporator. The dried material applied to a Sephadex LH -20 column using EtOH/H\(_2\)O (1:1) as eluent to yield 120 mg of hesperidin.

2.3. Nuclear magnetic resonance (NMR)

\(^1\)H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker advantage III, 400 MHz NMR spectrometer and 100 MHz for \(^{13}\)C-NMR. \(^1\)H chemical shifts (δ) were measured in ppm, relative to TMS and \(^{13}\)C-NMR chemical shifts to dimethyl sulfoxide-\(d_6\) and converted to the TMS scale by adding 39.5.

2.4. Synthesis of zinc oxide nanoparticles (ZnO NPs)

ZnO NPs were synthesized using by a method described by Attia et al. [13], in which 50 mg of hesperidin dissolved in 5 mL of DMSO mixed
with 500 mg of zinc acetate dissolved in 50 mL bi-distilled water and heated in boiling water bath for 20 min. Five drops of ammonium hydroxide was then added to the reaction to raise the pH to 12, where a ZnO NPs precipitate is formed. The mixture was left for half an hour for complete reduction of zinc acetate to ZnO NPs. The formed precipitate was centrifuged at 8000 rpm, followed by washing two times by bi-distilled water, and two washes with ethanol to yield white pellets of ZnO NPs upon freeze drying.

2.5. Characterization of metal nanoparticles

2.5.1. UV–vis spectral analysis

ZnO NPs preparations were examined using a UV spectrophotometer, Shimadzu, UV-1601 (Shimadzu Corporation, Japan). The UV spectra recorded between 200–400 nm.

2.5.2. FT-IR analysis

The functional groups of the synthesized zinc oxide nanoparticles and hesperidin compound characterized using a FTIR 6100 spectrometer (Jasco, Japan) in the range of 4000–200 cm⁻¹.

2.5.3. Transmission electron microscopy (TEM)

The morphology and the particle size of ZnO NPs mediated by hesperidin were determined by TEM (JEOL-JEM-1011, Japan). Drops of the nanoparticles suspension were placed on carbon coated copper grid and the solvent was evaporated at the room temperature prior to recording the TEM image.

2.5.4. X-ray diffraction (XRD)

The ZnO NPs X-ray diffraction pattern recorded with PANLYTICAL X-ray diffractometer using Cu Kα radiation of wavelength 1d706; −0.1541 nm in the scan range 21d703; = 20–70°.

2.6. Evaluation of the antiviral activity

The antiviral screening was performed using plaque inhibition assay at the Regional Center for Mycology and Biotechnology (RCMB, Al-Azhar University, Cairo, Egypt).

2.6.1. Mammalian cell line

Vero cells derived from African green monkey kidneys obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

The Vero cells were propagated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), HEPES buffer, 1% L-glutamine and 50 μg/mL gentamycin. All cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂ and were sub cultured two times for 7 days [14].

2.6.2. Cytotoxicity evaluation

Cytotoxicity assays were performed using Vero cells that implanted in 96-well plates at a cell concentration of 2 × 10⁵ cells per ml in 100 μL of the growth medium. Fresh media containing different concentrations of hesperidin and ZnO NPs were added after 24 h of implantation. Serial two-fold dilutions of the tested samples (started from 3000 μg/mL to 2 μg/mL) were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, Jersey, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO₂ for a period of 2 days. Three wells were used for each concentration of the investigated samples. Control cells were incubated without test samples and with or without DMSO.

After the incubation period, the viable cell yield was determined by a MTT colorimetric assay [15]. The relation between surviving cells and the concentration of tested samples were plotted to obtain the survival curves of the Vero cell line after treatment with the specified compounds. The 50 % cytotoxic concentration (CC₅₀), the concentration required to cause toxic effects in 50 % of intact cells, was estimated from graphic plots of the dose response curve for each concentration using GraphPad Prism software (San Diego, CA, USA). The maximum nontoxic concentration (MNTC) of each hesperidin and ZnO NPs were determined and used for further biological studies.

2.6.3. Virus propagation

The cytopathogenic HAV HM175 strain (ATCC VR-1402) of HAV was propagated and assayed in confluent Vero cells [16]. Infectious viruses were enumerated by determining the 50 % tissue culture infectious dose with eight wells per dilution and 20 μL of inoculum per well using the Spearman-Karber method [17].

3. Results and discussion

Ten nutraceuticals phenolic compounds were selected according to their availability and spreading as a pharmaceutical dosage forms derived from natural nutritive food sources.

These nutraceuticals were diosmin, rutin, naringenin, quercetin 3-O-glucuronide, myricetin 3-O-xylosyl-(1→2)-rhamnose, myricetin, epigallocatechin-3-O-gallate, corilagin and lyoniresinol. Hesperidin, diosmin and rutin are widely available in pharmaceutical stores under various trade names and can be derived from various natural nutritive foods reported to have antiviral properties for example, hesperidin from citrus peels has been reported to have antiviral activity [18]. On the basis of in silico screening hesperidin was also predicted to target the interaction site between SARS-CoV-2 Spike and ACE2 receptors, thus blocking the entry of the virus into the human lung cells. Therefore, hesperidin could be a promising prophylactic drug against COVID-19 [19].

In addition, naringenin from citrus peels were reported as antiviral agent against Zika virus [20], rutin [21] showed a significant results as antiviral [19]. Green tea’s epigallocatechin-3-O-gallate has antiviral effects against a number of DNA and RNA viruses [22]. Corilagin prevents liver damage by inhibiting HCV replication and modulating oxidative stress [23]. Anti-HIV activity has been reported for myricetin and its glycosylated derivatives [24]. In addition, Several lignans have been reported to have antiviral properties [25].
The binding mode, type of interaction and binding energy of phenolic compounds.

| Compound       | Binding mode | Type of interaction | Binding Energy (Kcal/mol) |
|----------------|--------------|---------------------|--------------------------|
|               |              |                     |                          |
| Hesperidin with M^pro | THR 24, MET 49 | Conv-HB | -8.84 |
|                | TYR 118     | Pi-alkyl            |                          |
|                | THR 26, ASN 142 | C-HB       |                          |
|                | LYS146, LEU168, GLY194, GLY195 | Van der Waals |                          |
|                |              |                     |                          |
| Hesperidin with HAV | SER197 | Conv-HB | -6.91 |
|                | PRO169 | C-HB | Amide-Pi Stacked |
|                | ASN196 | Stacked |                          |
|                | HIS41, CYS44, THR45, MET49, TYR118, ASN142, GLY143 | Van der Waals |                          |
|                | THR 24, THR 25 | Conv-HB | C-HB |
|                | SER46 | C-HB | Stacked |
|                | THR25, THR 26, THR 25, MET 49, ASN 119, LEU141, ASN 142, GLY143, GLN189 | Van der Waals |                          |
|                | THR 24, THR 24 | Conv-HB | Stacked |
|                | TYR 118 | Stacked |                          |
|                | THR26, LEU 27, HIS41, MET 49, ASN 142, GLY 143 | Van der Waals |                          |
|                | THR 24, THR 25 | Conv-HB | Stacked |
|                | THR 24 | Stacked |                          |
| Rutin          | THR 25, THR 26, LEU 27, HIS 41, MET 49, TYR 118, ASN 142, GLY 143, CYS 145 | Van der Waals | -8.82 |
|                | THR 24, SER 46 | Conv-HB |                          |
| Quercetin-3-O-syringyl-(1→2)-rhamnoside | THR26, LEU 27, CYS 44, SER46 | Van der | -8.01 |
|                | THR 24, THR 25, THR 25, ASN 42, GLY 143 | Van der Waals | Pi-Donor HB |
|                | LEU27, HIS41, THR 45, ASN 142, GLY 189 | Van der Waals | Carbon HB |
|                | MET 49 | Conv-HB | Stacked |
|                | THR 25, SER46, GLY 143 | Van der | Carbon HB |
|                | THR 24, THR 26, ASN 142, CYS 145 | Van der | Carbon HB |
|                | THR 24, THR 25, LEU 27, SER 46, GLY 143, CYS 145 | Van der Waals | Carbon HB |
|                | THR26 | Conv-HB | Stacked |
|                | LEU 4, THR 24, THR 25, THR 26, LEU 27, HIS 41, ASN 119, ASN 142, CYS 145, GLY 143 | Van der | Carbon HB |
|                | THR 24, THR 45, SER 46, ASN 119, ASN 142, GLY 143, CYS 145 | Van der | Carbon HB |
|                | THR 25, THR 26 | Van der | Carbon HB |
|                | LEU 27, HIS 41, MET 49 | Van der | Carbon HB |

3.1. Molecular docking

In the current research, an attempt was made to interpret and support the experimental findings regarding the inhibition mechanism of the main protease COVID-19. Ten polyphenolic compounds were docked using Swiss Dock Server, where receptor based approaches were applied to these polyphenols. The crystal structure of Covid-19 protease 6lu7, which adopts a heart shape, with two dimers of identical subunits and two active sites. A broad-spectrum inhibitor should target the active site His 41 and Cys 148 of mother bat coronavirus, as in PDB entry 4yol. This study elucidated the binding mode of the phenolic compounds to the crystal structure 6lu7 by means of molecular docking (Fig. 2). The docking study confirmed that hesperidin can form electrostatic interactions with THR 25, LEU 27, HIS 41 and GLY143 residues while form pi-stacking interaction with TYR 118 as listed in Table 1.

Most of the polyphenolic compounds showed inhibition activity in the cleft region between domain II, III (residues 185–200) and In domain I as seen in Fig. 2 while in Table 1, the polyphenolic compounds listed according to their binding energies. The relationship of RMSD to the native binding mode (the full fitness (blue, left X-axis)) and energy (the simple fitness (red, right y-axis)) was shown in Fig. A (suppl.file). Docking mode for hesperidin was shown in (Figs.B for SARS-CoV-2 M^pro and Fig. C for HAV 3C Protease [26], 2HAL crystal structure), myricetin (Fig. D.1,2), diosmin(Fig. E 1,2), epigallocatechin-3-O-gallate (Figs F1,2), rutin (Figs G1,2), quercetin 3-O-glucouronide (Figs H 1,2), coirlagin (Figs I1,2), myricitin (Figs J1,2), narigenin (Figs K1,2) and lonyrinisol (Figs M1,2), in the supplementary file.

3.2. Isolation and identification of hesperidin

As hesperidin showed the highest docking score, herein, we isolated it from a food waste (orange peels) and its antiviral activity against HAV (one of the RNA viruses) was evaluated.

The butanol fraction from the aqueous extract of the orange peels was applied to Whatman filter paper sheets using BAW as an eluent, with the standard ZnO absorption pattern, in agreement with reports ZnO NPs [28]. ZnO on a nano scale has shorter wavelengths compared to these polyphenols. The crystal structure of Covid-19 protease (Fig. 2). The docking study confirmed that hesperidin can form electrostatic interactions with THR 25, LEU 27, HIS 41 and GLY143 residues while form pi-stacking interaction with TYR 118 as listed in Table 1.

The addition of zinc acetate solution to hesperidin solution in water bath heated up to 80 °C was applied to Whatman filter paper sheets using BAW as an eluent, whereby, a major dark purple band of flavonoidal nature on the pre was applied to Whatman filter paper sheets using BAW as an eluent, (one of the RNA viruses) was evaluated.
3.3.2. FT-IR analysis of ZnO NPs and hesperidin

FT-IR spectra (Fig. 3b) of the prepared ZnO NPs and hesperidin were collected in the spectral width range from 400 to 4000 cm\(^{-1}\). Where showed peaks for O–H groups stretching of water around 3546.04, 3478.29 and 3421.80 cm\(^{-1}\) represent O–H groups of hesperidin and around 3382.99 cm\(^{-1}\) for ZnO NPs. Aromatic moieties were confirmed by carbon double bond stretching at 2918.50 cm\(^{-1}\). Bending vibration of the alcoholic —CO—H confirmed by the presence of peak at 1096.12 cm\(^{-1}\). The characteristic bands of the ZnO NPs stretching modes are assigned at 542.9 and 891.2 cm\(^{-1}\) \([13,30]\).

3.3.3. TEM analysis

TEM analysis was performed on low resolution and high resolution transmission electron microscopes and showed the formation the hexagonal ZnO NPs with particle size ranging from 20 to 30 nm, Fig. 4.
3.3.4. XRD analysis

The occurrence of ZnO NPs and investigation of their structural features were confirmed by X-ray diffraction (XRD), Fig. 5. ZnO NPs mediated by the flavonoid hesperidin showed peaks with 2θ values identified at 31.618°, 34.334°, 36.161°, 47.504°, 56.440°, 62.727°, 66.245°, 67.864°, and 68.925° corresponding to (100), (002), (101), (102), (110), (103), (200), (112) and (201). These peaks were matched with those of data card (00-003-0888).

The crystal size of ZnO NPs was calculated to be 27.73 nm. This data is very similar to TEM measurements. To calculate the crystal size we apply Scherrer’s equation

\[ \text{Crystal Size} = \frac{0.9 x \lambda}{\beta \cos \theta} \]

\[ \beta = 2\theta/2, \quad d = \text{the full width at half maximum intensity of the peak (in Rad),} \quad \lambda = 0.154060 \text{ nm} \]

3.4. Antiviral activity

3.4.1. Evaluation of cytotoxicity against Vero cells

The two samples of hesperidin and ZnO NPs showed 50 % cell cytotoxic concentration (CC50) = 620.8 ± 34.6 and 243.7 ± 12.7 µg/mL, respectively. As shown if Figs. K. &L, suppl. file and the values were listed in tables C & D, respectively. Then, these compounds were tested for antiviral effects against HAV at the maximum non-toxic concentration (MNTC) using plaque assay method illustrated in Fig. M. (supplementary file).

3.4.2. Anti-HAV activity

Flavonoids have been reported to have antiviral activities both in vivo and in vitro [31]. It was reported that the mechanism of action of some flavonoids against HIV (also an RNA virus) is due to the inhibition of phosphorylation of proteins mediated by cytokines. It should also be noted that the phosphorylation signaling mediated by cytokines is increased in COVID-19 infection, leading to the phosphorylation of numerous cytoskeletal proteins. Therefore, flavonoids, which include hesperidin, can inhibit protein phosphorylation, which in turn leads to potent antiviral activity and infection prevention of other cells [32].

ZnO NPs have also been reported to have antiviral activity [32,33].

Zn-containing compounds reported to affect diverse stages of viral replication cycles such as virus inactivation, inhibition of viral uncoating, viral genome transcription, viral protein translation and polyprotein processing. It should be noted also that the activity dependent on the type of the virus and the concentration of zinc containing compounds [34].

In our study, the confluent monolayers of vero cells were infected with fixed dilutions of HAV virus. After incubation, inoculum was removed and replaced with serum-free MEM containing 1.5 % carboxymethyl cellulose. The cells were then incubated allowing the virus to form the plaques. The cells were fixed for 2 h at 25 °C with formaldehyde that added directly to the medium till concentration of 5%. Fixed cells were extensively washed with water before being stained with a solution containing 1% crystal violet and 10 % ethanol for 30 min. After rinsing with water, the number of plaques was counted, and virus titers (counts) were calculated. The antiviral effects of the two tested samples, hesperidin and ZnO NPs against HAV at maximum noncytotoxic concentration (MNCC) are shown in the Table B (supplementary file). The zinc oxide nanoparticles showed higher activity than hesperidin at relatively low concentration due to the high surface area and small particle size, which increased the activity of zinc oxide nanoparticles. From the Table B, Fig. T, (supplementary file). Both hesperidin and ZnO NPs showed antiviral activity against Hepatitis A virus (HAV) with percentages of 44.75 and 58.83 % at maximum noncytotoxic concentration and EC50 equal to 72.4 and 176.3 µg/mL, respectively as shown in the Tables A and B (supplementary file).

4. Conclusions

The current pandemic disease COVID-19 is responsible for a lot of morbidity and mortality worldwide. Nutraceuticals and phytopharmaceuticals phenolic compounds under our study exhibit good docking scores and it could be an ideal targets for treatment of SARS-CoV-2. Hesperidin which is isolated from food waste orange peels is exhibited the highest docking score (-8.84 Kcal/mol) against SARS-CoV-2 main protease among the other tested compounds. Hesperidin is capable of reduction of zinc ions to prepare ZnO NPs with hexagonal shape and particles size about 25 nm. Hesperidin mediated ZnO NPs exhibit antiviral activity than hesperidin itself with percentages of 44.75 and 58.83 % at maximum noncytotoxic concentration. More studies should be done for hesperidin, other nutraceutical, phytopharmaceutical compounds and their ZnO NPs for finding a safe, cheap and potential treatments for SARS-CoV-2 pandemic disease.

Authors contributions

Gouda H. Attia, Yasmin S. Moemen, Mahmoud Youns, Ammar M. Ibrahim, Randa Abdou, Mohamed A. El Raey

Gouda H. Attia and Mohamed A. El Raey design the manuscript.

Gouda H. Attia, Ammar M. Ibrahim and Mohamed A. El Raey conducted the chromatographic separation and performed the structure elucidation of the pure isolated Hesperidin

Gouda H. Attia, Randa Abdou and Mohamed A. El Raey performed green synthesis of ZnO NPs and describe its features.

Yasmin S. Moemen performed all the molecular docking study and Mohamed A. El-Raey suggest the protein.

Mahmoud Youns and Randa Abdou performed the antiviral and cytotoxicity assays and their data analysis.

All authors were responsible for drafting and writing the final version of the manuscript and approved the final manuscript.

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Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

IRB approval was obtained (required for studies and series of 3 or more cases).

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfb.2021.111724.
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