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Single-walled carbon nanotubes-based RNA protection and extraction improves RT-qPCR sensitivity for SARS-CoV-2 detection

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HIGHLIGHTS
• Single-walled carbon nanotubes (SCNTs) protect RNA from degradation at room temperature.
• SCNT simultaneously immobilizes RNA and RNase to reduce their contact.
• A new method was established to enable protection and simple extraction of RNA.
• Magnetism-modified SCNTs used for SARS-CoV-2 RNA extraction improved RT-qPCR sensitivity.

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ABSTRACT
The false-negative result of nucleic acid testing is an important cause of continued spread of COVID-19, while SARS-CoV-2 RNA degradation during transportation and nucleic acid extraction can lead to false-negative results. Here, we investigated that single-walled carbon nanotubes (SCNTs) could protect RNA from degradation for at least 4 days at room temperature. By constructing magnetism-functionlized SCNTs (MSCNTs), we developed a method that enabled protection and simple extraction of SARS-CoV-2 RNA, and the RNA-bound MSCNTs can be directly used for reverse transcription polymerase chain reaction (RT-qPCR) detection. The experimental results showed that 1 μg of MSCNTs adsorbed up to 24 ng of RNA. Notably, the MSCNTs-based method for extracting SARS-CoV-2 RNA from simulated nasopharyngeal swabs and saliva samples with mean recovery rates of 103% and 106% improved the sensitivity of RT-qPCR detection by 8–32 fold in comparison to current common methods. This improvement was largely attributable to the protection of RNA, enabling increased RNA load for downstream assays.

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has continued to spread globally, causing about 610 million infections as of September 2022 [1]. Although coronavirus disease 2019 (COVID-19) vaccines are available, precise and sensitive detection of SARS-CoV-2 remains critical to reduce its transmission. Nucleic acid amplification tests are currently regarded as the gold standard, mainly using real-time reverse transcription polymerase chain reaction (RT-qPCR) assay [2]. These tests are highly specific, but require protean nucleic acid extraction from patient specimens prior to detection. Such a process is complicated and time-consuming, involving many handling steps such as pathogen lysis, nucleic acid binding, washing, and elution [3]. Additionally, repeated pipetting operations and chemically toxic agents are needed. Therefore, viral nucleic acid extraction has become a major bottleneck in current SARS-CoV-2 diagnosis [4]. A simple and rapid extraction method could improve the efficiency of molecular testing, which is beneficial to the prevention and control of the epidemic.

Several modified protocols have been developed for extraction of SARS-CoV-2 RNA, such as solid-phase extraction techniques based on nucleic acid-adsorbing matrix like silica-coated beads and nitrocellulose membrane [5,6]. Although these methods showed high extraction efficiency and were feasible for RT-qPCR testing, false-negative results on at least one occasion for SARS-CoV-2-infected patients were observed, especially in individuals with low virus levels. For instance, Fang et al. [7] reported that RT-qPCR only determined 36/51 (71%) of SARS-CoV-2 infected patients within 0–6 days of initial onset. Cao et al. [8] evaluated two groups of patients with COVID-19, of which 66.7% were negative by RT-qPCR on nasopharyngeal swabs. Meanwhile, Ingrid et al. [9] performed a systematic review and critical appraisal about the rate of RT-qPCR false-negatives, and concluded that up to 54% of COVID-19 patients had initial false-negative RT-qPCR. There are many factors that may cause such high false-negative results, including the detection timing of patients with suspected infection, the quality of nucleic acid extraction, professional operation deviation, and the type of samples collected [10]. Besides, the ongoing degradation of SARS-CoV-2 RNA during specimen collection, transportation and processing would be an important cause of false-negative results [11]. Clinically, SARS-CoV-2 samples usually require “cold chain” transportation to prevent the loss of genetic material [12], because RNA is vulnerable to prolonged high temperature (heat inactivating the virus) and ubiquitous ribonuclease (RNase) digestion [13]. The addition of protein-based RNase inhibitors to viral lysates can protect RNA, but increases assay costs and may compromise subsequent amplification reactions, particularly for extraction-free RT-qPCR which was primitive developed to address the substantial supply chain shortages of RNA isolation/purification kits [14,15]. Given the high sensitivity of RT-qPCR, which can detect 4–8 virus copies per reaction, it is difficult to further improve RT-qPCR amplification [16]. Therefore, a strategy that not only prevents the loss or degradation of virus RNA over time, but also does not affect the RT-qPCR reactions, may shed light on the confidence of downstream testing.

Single-walled carbon nanotubes (SCNTs), a member of carbon-based nanomaterials, are cylindrical structures with high surface area and large surface-to-volume ratio [17]. They are broadly applied in biomedical technologies due to their potential to interact with biomolecules such as peptides, nucleic acids, and proteins for site-directed drug delivery [18]. Of these, single-stranded DNA (ssDNA) can be rapidly adsorbed onto SCNTs via π–π stacking interactions, and ssDNA-associated SCNTs were used to purify and disperse hydrophobic SCNTs in solution [19,20]. Of note, SCNTs have been shown to protect RNA from enzymatic cleavage during cellular delivery [21]. The effect may be attributed to the interaction of SCNTs with RNase, which reduced its activity by changing the enzyme conformation [22]. However, the protection of SCNTs on RNA at distinct temperature and its timeliness have not been investigated. Moreover, the potential application of SCNTs in the field of viral RNA extraction and detection has attracted our great interest.

Thus, we demonstrated that SCNTs protected RNA in a highly efficient manner, even when RNA was exposed at room temperature for 4 days. The protection mechanism was ascribed to SCNTs immobilizing both RNA and RNase, thereby blocking their contact. Taking advantage of the protective effect of SCNTs on RNA during sample storage, we developed a simple and high-performance RNA extraction method by constructing magnetism-functionalized SCNTs (MSCNTs) (Scheme 1). The RNA-MSCNTs complexes can be directly incorporated into commercial RT-qPCR testing workflows and improved their sensitivity to detect SARS-CoV-2 in saliva or nasopharyngeal swab samples. Furthermore, the MSCNTs-based RNA protection and extraction approach could achieve accurate detection of viruses by (a) reducing false-negative results caused by RNA degradation, (b) saving redundant operation time, and (c) decreasing the cost of nucleic acid extraction reagents.

2. Materials and methods

2.1. Materials and reagents

- Single-walled carbon nanotubes (SCNTs, 15 mg/mL), multi-walled carbon nanotubes (MCNTs, 1 mg/mL), and graphene oxide (GO, 1 mg/mL) were purchased from XianFeng Nanotechnology Co., Ltd. (Nanjing, China). Magnetic beads (MBs; BeaverBeads™ NH2, 500 nm, 10 mg/mL) were obtained from Beaver Co., Ltd. (Suzhou, China). RNase A (10 mg/mL) was purchased from TianGen biotech Co., Ltd. (Beijing, China). Murine RNase inhibitor was obtained from Vazyme Biotech Co., Ltd. (Nanjing, China). SARS-CoV-2-ab II EMN pseudovirions (1.25 × 10^10 copies/mL), influenza A H1N1-M1 pseudovirions (1.7 × 10^10 copies/mL), and influenza B NS1 pseudovirions (7.1 × 10^9 copies/mL) were all purchased from FluBio Co., Ltd. (Suzhou, China). Transcript™ One-Step RT-qPCR SuperMix kit was obtained from TransGen Biotech Co., Ltd. (Beijing, China), and viral transport media was supplied by Navid Biotech Co., Ltd. (Qingdao, China). All oligonucleotides used in this study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). All other chemicals were analytical grade.

2.2. Total RNA extraction

Total RNA was isolated from Escherichia coli using a commercial TransZol Up Plus RNA kit from TransGen Biotech Co., Ltd. (Beijing, China). The RNA isolation process was performed according to the manufacturer’s instructions as previously described [23], and the purity and concentration of the obtained RNA were determined using a QuickDrop Micro-Volume Spectrophotometer (Molecular Devices, LLC, USA).

2.3. RNA protection assay

Freshly extracted RNA (about 600 ng) was mixed with 1 μg of SCNT, GO and MCNT, respectively. The mixture was vortexed for 30 s and allowed to stand for 15 min to ensure complete adsorption, followed by adding 10 ng RNase A to the mixture. Then, 2% agarose gel electrophoresis was performed to evaluate the integrity of 23S and 16S RNA. For experimental optimization, the protective effect of SCNTs on RNA at different concentrations ranging from 2.5 to 40 ng/μL was investigated. Furthermore, the protection of RNA by SCNTs at different temperature and time frame were also estimated.

2.4. Characterization of SCNT-RNA complexes

SCNTs were mixed with RNA or RNase for 15 min. A drop of the mixture was placed on silicon and dried at room temperature before measurement. Scanning electron microscopy (SEM) imaging was made
using a Regulus 8100 (Hitachi, Japan) with an accelerating voltage of 2 kV. Surface zeta potentials of the nanomaterials were measured using a Zetasizer Nano ZS90 device (Malvern, England).

2.5. Preparation of magnetism-functionalized SCNTs

Magnetism-functionalized SCNTs (MSCNTs) were prepared by the formation of amide bonds between the amino groups of MBs and the carboxyl groups of SCNTs. In details, one mL of amino-coated MBs (1 mg/mL) were washed three times with PBS (50 mM, pH = 7.4), followed by adding 100 µL of 15% glutaraldehyde solution. After vortexing, the MBs were suspended and incubated under foil wrap for 1 h at room temperature (shake every 10 min). For SCNT carboxyl activation, 40 mM 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC-HCl) and 40 mM N-Hydroxysuccinimide (NHS) were added simultaneously to 1 mL of SCNTs (1 mg/mL) and incubated for 1 h. Next, 20 µL of activated MBs were mixed with 200 µL of the above SCNTs and incubated at room temperature for 3 h and then at 4 °C overnight. The MB-coupled SCNTs were all collected by magnetic separation. Finally, the MSCNTs were washed three times with PBS and stored in 100 µL DNase/RNase-free water until further use.

2.6. SARS-CoV-2 RNA enrichment and extraction using MSCNTs

For the extraction of viral RNA from simulated clinical samples, nasopharyngeal swab specimens were obtained by collecting secretions from the posterior nasopharynx, deep throat, tonsils, or arch of the palate of healthy individual using a mini-tip swab. Four swabs were placed in 1 mL of sterile H$_2$O. One microgram of as-prepared MSCNTs was premixed with 10 µL of sterile viral transport media (10 ×), and 2 µL of 5 M hydrochloric acid was added to facilitate protonation of MSCNTs. 1 × 10$^5$ copies of SARS-CoV-2 pseudoviruses were subjected into 25 µL of swab solution and transferred to the viral transport media to mimic real sample collection conditions (equivalent to placing one swab in 1 mL of lysis buffer). The resulting mixture in a total volume of 100 µL was kept at room temperature, during which time the virus was lysed and RNA was sufficiently adsorbed by MSCNTs. The viral RNA was isolated via magnetic separation without heat inactivation or DNase/RNase-free water until further use.

2.7. RT-qPCR protocol

For RT-qPCR, a total volume of 20 µL PCR master mix containing 0.2 µM forward and reverse primers, 1 × TransScript™ One-Step Reaction SuperMix, 150 nM probe, 5 µL of original non-extracted RNA or all amount of RNA-enriched MSCNTs. The primer and probe sequences targeting open reading frame 1a (ORF1a) and nucleocapsid (N) were listed in Table S1. The amplification reactions were performed under the following conditions: reverse transcription at 45 °C for 10 min, followed by initial denaturation at 95 °C for 1 min, 45 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s using a CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA). The detection sensitivity was determined with the lowest number of detectable pseudovirus with a cycle threshold (Ct) value < 36, where the Ct cutoff was set as the criterion for evaluating positive samples [25].

3. Results and discussion

3.1. SCNTs protect RNA from degradation

First of all, we used newly extracted total RNA from Escherichia coli (E. coli) to evaluate whether carbon nanomaterials could act as effective RNA protectors. RNA degradation is mainly caused by ubiquitous RNases. Therefore, RNase was intentionally added to RNA-GO, RNA-MCNTs, and RNA-SCNTs complexes to evaluate the RNA-protective ability of these nanomaterials. The results showed that GO and SCNTs, but not MCNTs, were able to resist RNA attack by RNase, while SCNTs had stronger protective ability (Fig. S1A). By comparing the grayscale intensity of the electrophoretic bands, SCNTs could protect 73% of 23S RNA and 83% of 16S RNA from RNase digestion, while GO could only protect 22% of 23S RNA and 57% of 16S RNA (Fig. S1B).

We further investigated the RNA protective capability of SCNTs. Different amounts of SCNTs were subjected to 10 ng/µL RNA solution. In the absence of SCNTs, the total RNA was rapidly degraded by RNase, but more than 85% of both 23S and 16S RNA remained stable in the presence of 15 ng/µL SCNTs (Fig. 1A). It is well known that RNA is usually stored at extremely low temperatures (~80 °C) because RNAs maintain highly active over a wide temperature range (15–70 °C). Total RNA bound to SCNTs or not were exposed to ~20 °C, 4 °C, room temperature for 24 h, and 56 °C for 30 min, respectively. As shown in Fig. 1B, the RNA bound to SCNTs was significantly resistant to RNase digestion at ~20 °C, 4 °C, and room temperature, but not at 56 °C. However, over 80% of the RNA was degraded without SCNTs protection after 24 h exposure at room temperature. Next, the time frame for RNA
immobilized RNase by SCNTs lost most of its enzymatic activity. Fig. 2 E
3.2. The principle of SCNTs protecting RNA

Previous studies have reported an interaction between SCNTs and proteins, such as enzymes [26,27]. Thus, it is not difficult to infer that SCNTs might also immobilize RNase and prevent it from contacting with RNA. To verify this hypothesis, we took scanning electron microscopy (SEM) images of pure SCNTs, SCNTs-RNA, SCNTs-RNase, and SCNTs-RNase-RNA. The images in Fig. 2A–D clearly demonstrated the adsorption of RNA and RNase on the surface of SCNTs. Furthermore, we evaluated the enzymatic activity of SCNT-immobilized RNase. To achieve this, RNase was divided into two aliquots. One part was mixed with a large amount of SCNTs to ensure that RNase was fully adsorbed on the surface of SCNTs, and the other RNase was not treated. An equal amount of total RNA was added as the substrate. After 1 h of incubation, the stability of RNA was examined by electrophoresis analysis and quantification of recA gene levels using RT-qPCR, respectively. As shown in Fig. 2E–F, RNase completely digested 23S RNA and 16S RNA, while the immobilized RNase by SCNTs lost most of its enzymatic activity. Moreover, recA gene levels in 5 μL of RNase-SCNT dispersed solution were analyzed by RT-qPCR. The results indicated that only marginal interference of RNase-SCNTs on cycle threshold (Ct) values and curve shapes (Fig. 2G), and this result also verified the compatibility of SCNTs for RT-qPCR analysis. In contrast, we mixed RNase with excess SCNTs to generate RNA-SCNT complexes in which RNA was adequately adsorbed. RNase was added to the mixture and incubated for 1 h, and then the stability of the adsorbed RNA was assessed. In Fig. 2H–J, we obtained the electrophoresis and RT-qPCR results similar to those of RNase-SCNTs. These data demonstrated that SCNTs protected RNA from RNase cleavage by simultaneously immobilizing RNase and RNA.

3.3. RNA enrichment and extraction using MSCNTs

The potent protection and binding ability of SCNTs to RNA drove us to explore the function of SCNTs for capturing and extracting the total RNA. The continuous RNA protection ability of SCNTs ensures the long-term storage of RNA, thereby providing an intact template for subsequent use. Considering that RNA-wrapped SNCTs are well dispersed, it is difficult to be separated from solution by centrifugation [28]. To simplify the separation procedure of RNA extraction, we linked SCNTs with MBs to construct magnetism-functionalized SCNTs (MSCNTs) through the condensation reaction between the amino groups of MBs and the carboxyl groups of SNCTs. The MBs solution was sienna, and SNCTs were dark black, whereas MSNCTs were gray and fully aggregated when a magnetic field was employed (Fig. S2). The increase in particle size revealed that MSCNTs were successfully constructed (Fig. S3A). Furthermore, we determined the zeta potential of MSCNTs composite. The results showed that the surface of MSCNTs was positively charged, while the zeta potential was reversed after the addition of RNA, indicating that MSNTs had adsorbed a large amount of negatively charged RNA (Fig. S3B). In addition, Fourier transform infrared (FT-IR) spectrometry was used to characterize a certain functional groups or chemical bonds on MSCNTs. Compared with the SCNTs, the amino groups of MBs showed absorption peaks at 2924 cm−1 and 2854 cm−1, and absorption bands at 1635 cm−1, 1425 cm−1, 1219 cm−1 were attributed to the amide, while the absorption bands at 2924 cm−1 and 2854 cm−1 were attributed to the amino groups of MBs showed absorption peaks at 2924 cm−1 and 2854 cm−1, and absorption bands at 1635 cm−1, 1425 cm−1, 1219 cm−1 were attributed to the amide, which were the characteristic chemical bond on the MSCNTs (Fig. S3C). MSCNTs on the surface of MBs not only facilitate magnetic collection but also maintain the biological function of RNA adsorbed on SCNTs [29]. The protective effect of MSCNTs on RNA was also evaluated in various conditions, including the presence of RNase, different temperatures and the timeliness. The results showed that MSCNTs exhibited excellent RNA protection as similar as SCNTs (Fig. S4).

Based on prior work showing that a small amount of SCNTs had no effect on the PCR reaction [30], to investigate how much MSCNTs can be used directly for subsequent amplification, we added different amounts of MSCNTs to 20 μL of test system containing 6 pg/μL RNA and found...
that a maximum of 1 μg MSCNTs did not affect the efficacy of RT-qPCR (Fig. 3A). Afterwards, the RNA extraction capacity of 1 μg MSCNTs was investigated by taking *E. coli* total RNA as an example. The amount of extracted RNA was measured using relative quantitative RT-qPCR. As shown in Fig. 3B, RNA captured on MSCNTs increased linearly with RNA input from 62.5 pg to 32 ng, and the extraction efficiency was over 80%. However, when the amount of input RNA was 64 ng, the extraction efficiency was dramatically dropped to 38%, indicating that the extraction performance was saturated. When the captured RNA amount was 24 ng, it no longer increased with the addition of more RNA input, indicating
that this amount has reached the maximum extraction capacity of 1 μg MSCNTs, which was equivalent to 9.77 \times 10^6 copies of E. coli RNA. Therefore, this MSCNTs extraction method fully satisfied the high-sensitivity detection of trace RNA samples by molecular diagnostics.

3.4. Practical application of MSCNTs in SARS-CoV-2 detection

Next, we evaluated the practical application of MSCNTs in SARS-CoV-2 RNA detection. SARS-CoV-2 pseudoviruses (1 \times 10^4 copies/mL) were spiked into the viral transport media which served as a sample inactivation solution or virus lysing agent (termed inactivator hereafter) (Fig. 4A). This inactivator, with or without MSCNTs, was left at room temperature for 1 h, 6 h and 12 h to mimic real sample storage and shipping, during which the SARS-CoV-2 pseudoviruses were sufficiently cleaved and RNA captured on MSCNTs. All RNA-MSCNTs complexes were extracted by magnetic separation and used directly for subsequent RT-qPCR. In contrast, 5 μL of the inactivator without MSCNTs was routinely used as a control [4, 31]. We found that the Ct values of extraction-free samples were correspondingly increased, indicating that the target concentration decreased with increasing sample storage time. However, the amplification plots and Ct values of RNA extracted from our method did not alter over time (Fig. 4B), as the robust performance of RT-qPCR was largely attributable to the stability of the RNA.

According to the recommendation of the World Health Organization (WHO), in most clinical specimens, SARS-CoV-2 is usually collected using nasopharyngeal (NP) swabs [32]. Therefore, SARS-CoV-2 pseudoviruses were spiked into the inactivator along with NP swab from healthy volunteers (one NP swab per 1 mL of inactivator). We found that the addition of NP sample resulted in a \sim 4 Ct delay in the PCR amplification plots, whereas MSCNT-based RNA extraction from NP swab yielded 5 Ct lower than the NP sample without extraction (Fig. 4C). This corresponds to a \sim 32 fold increase in detection sensitivity compared to extraction-free RT-qPCR. Recently, saliva has been proposed as a suitable specimen for the diagnosis of SARS-CoV-2 infection [33]. We also investigated the MSCNTs method for RNA extraction from mock samples containing 30% saliva. Consistent with previous studies that SARS-CoV-2 RNA was stabilized in saliva over time [34, 35], our results in Fig. 4D showed that the Ct values did not differ from those of saliva samples tested 6 h after collection. However, in comparison with saliva samples without any extraction steps, the Ct values of MSCNT-based RNA extraction was decreased by 2.5–3.5, indicating that MSCNTs method improved the detection sensitivity of RT-qPCR for viruses in saliva. Furthermore, the spike recovery experiments showed the mean recovery percentage in both cases ranging from 103 to 106% with a relative standard deviation of 9.66 and 11.78 for the NP swabs and

Fig. 4. MSCNT-based RNA extraction was applied in RT-qPCR for SARS-CoV-2 detection. (A) Schematic of MSCNTs extracting SARS-CoV-2 pseudovirus RNA from artificially spike nasopharyngeal swab and 30% saliva. (B) The stability of SARS-CoV-2 RNA at 1 h, 6 h and 12 h under MSCNTs’ protection. (C) Comparison of Ct values between SARS-CoV-2 in inactivator (black), SARS-CoV-2 and NP swab in inactivator (red), and MSCNT-based RNA extraction from the inactivator with NP swab (green). (D) Comparison of Ct values between the same ways for RNA extraction from 30% saliva. (E) The detection sensitivity of RT-qPCR using MSCNT-based RNA extraction from NP swab. NP: nasopharyngeal swab; NTC: negative control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
saliva samples, respectively (Table S2), which suggested that the biological matrix had no remarkable effect on our proposed method. These observations were presumably due to the use of MSCNTs not only to enrich RNA and protect RNA from RNase digestion, but also to eliminate reaction inhibitors present in the NP swabs and saliva samples.

To more precisely depict the sensitivity improvement of MSCNT-based RNA protection and extraction to RT-qPCR assay, a series of 2-fold dilutions of SARS-CoV-2 pseudovirus were spiked into the MSCNTs-containing NP samples and detected by RT-qPCR. As shown in Fig. 4E, the dynamic detection range of SARS-CoV-2 was from 2000 to 125 copies/mL. The regression curve showed a correlation coefficient ($R^2$) of 0.9916, demonstrating a highly linear relationship between the Ct value and the logarithm of virus number. The detection limit of our method was approximately 8-fold lower than the WHO recommendation of 1000 copies/mL [36].

3.5. Detection of SARS-CoV-2 in mixture samples

As the epidemiology, clinical manifestation and care of COVID-19 and influenza share many features, there is a need for targeted differentiation and detection of the causative pathogens of both respiratory diseases [37]. To evaluate the selectivity of our proposed method, three RNA viruses ($1 \times 10^4$ copies/mL for each) were spiked into NP swab solution and examined by determining the Ct values as follows: A + B, A + S, B + S, A + B + S, where A, B, and S represented influenza A, influenza B, and SARS-CoV-2 pseudovirus, respectively (Fig. 5A). The mean Ct values from triplicate experiments were confirmed that only the SARS-CoV-2 was detected selectively among the virus mixtures when the gene ORF1a was targeted (Fig. 5B). By employing specific primers for gene N, a similar result was obtained in various viral mixtures (Fig. 5C). Furthermore, the agarose electrophoresis showing amplicon bands were well-matched with the corresponding Ct values (Fig. 5D–E).

Thus, our MSCNTs-based RNA extraction combined RT-qPCR method was sufficiently used to identify SARS-CoV-2 with high selectivity.

4. Conclusions

In summary, we demonstrated in this study that SCNTs acted an outstanding long-acting RNA protection function, which were attributed to the simultaneous immobilization of RNA and RNase. A simple RNA extraction method was realized via constructing magnetism-functionalized SCNTs. MSCNTs, as a powerful RNA stabilizer, have been successfully applied to SARS-CoV-2 detection and improved the detection sensitivity of RT-qPCR assay. It is expected to break through the “bottleneck” that SARS-CoV-2 samples must be transported in a short time under low temperature (0–4 °C) conditions. Future studies should examine whether MSCNT-based RNA protection and extraction can be applied to real COVID-19 patients or other RNA virus-infected samples.
Yong Li: designed research, performed most of the experiments and analyzed the figures, wrote the manuscript, revised this manuscript.

Xiangming Han: conducted agarose gel electrophoresis experiments.

Xiaofeng Mu: performed SEM imaging and analyzed the data.

Ye Wang: performed SEM imaging and analyzed the data.

Chao Shi: designed research, revised this manuscript.

Cuiping Ma: performed most of the experiments and analyzed the figures, designed research, revised this manuscript.

All authors have given approval to the final version of the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.340639.

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