Development of MnO₂ hollow nanoparticles for potential drug delivery applications

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Abstract
This study reports the development of hollow nanoparticles, formed from manganese dioxide (δ-MnO₂) sheets, that are coated with polydopamine for potential immobilization of chemical agents. The biodegradability and colloidal stability of the uncoated hollow MnO₂ nanoparticles were investigated in comparison to commercially synthesized solid MnO₂ nanoparticles and graphene oxide sheets. The MnO₂ hollow nanoparticles degraded at a faster rate and seem to have a higher surface area and better colloidal dispersion than solid MnO₂ nanoparticles. Xanthan gum (as a dispersant) was proven to improve colloidal dispersion of these hollow nanoparticles and were used for further cell studies. In this study, cancer and healthy cells were treated with coated hollow nanoparticles, and the studies indicate that this novel nanoparticle can internalize cells. Particle aggregation has shown to inhibit cell growth. Further studies with this novel hollow nanoparticle may lead to a groundbreaking solution to new drug delivery systems for cancers or other applications.

Keywords: MnO₂ hollow nanoparticles, drug delivery system, biodegradability, colloidal stability, dopamine

(Some figures may appear in colour only in the online journal)

1. Introduction

Nanoparticles have been widely used in a variety of different applications due to their very unique and desirable properties. Certain carbon nanomaterial allotropes, such as carbon nanotubes, can be stronger than steel, offering mechanical properties desirable in certain applications. Nanoparticles in general have very high surface areas (the order of 10–1000 m² g⁻¹) [1] and have good adsorptive properties,
electronic properties, and even magnetic properties. These properties allow them to be widely used for energy storage, high performance and high temperature wear-resistant materials, superconductive applications and biomaterials [2, 3]. In biomedical applications, nanoparticles can be used as drug delivery carriers to deliver drugs to cells [4]. There is active research on the use of carbon allotropes, such as carbon nanotubes [4], fullerenes [3], carbon nanosheets [5], carbon nanoparticles [3], and graphene oxide (GO) nanosheets [3, 5] as drug delivery vehicles due to their mechanical stability, high surface area, and huge versatility in terms of structure, but biodegradation and cytotoxicity of these carbon allotropes have not been proven to be favorable [3, 6]. Although biodegradable polymeric nanoparticles have also been used in many applications [4], their hydrophobicity and acidic degraded products limit their widespread use in certain applications. Therefore, there is a new need to develop biocompatible, biodegradable inorganic nanoparticles for potential biomedical applications.

Manganese dioxide has been studied as a drug delivery system and as a scaffold for stem cell transplantation, and it has been proven to be biodegradable, non-toxic, and aid in stem cell differentiation [6]. As compared to many other nanomaterials, MnO$_2$ is a metal oxide, and can degrade and undergo a redox reaction [7] to break down efficiently in the body. MnO$_2$ has even been used in cancer therapies before, with the degraded Mn$^{2+}$ ions used for MRI imaging [6]. MnO$_2$ hybrid nanoscaffolds have also been developed as a biodegradable and biocompatible system to deliver therapeutics and for stem cell transplantation for treating central nervous system diseases [6]. These nanoscaffolds, comprised of laminin-coated MnO$_2$ sheets, were tested in their ability to deliver stem cells and small molecules [6]. Stem cells were successfully loaded and able to differentiate within the scaffold, and successful drug loading and release of Rhodamine B was observed [6]. The entire system showed desirable degradation properties as well [6]. MnO$_2$ nanosheets have also been used in a drug delivery system to surround silica-coated Fe$_2$O$_3$ particle cores to deliver and release Congo red as well as to monitor glutathione concentrations in cells [8, 9]. In addition, MnO$_2$-PEG-folic acid layered nanosheets have been used to deliver doxorubicin to tumor cells both in vitro and in vivo, and hyaluronic acid-modified mannan-conjugated MnO$_2$ nanoparticles have been studied as a delivery mechanism to help alleviate tumor hypoxia and enhance chemotherapy response by reacting with endogenous H$_2$O$_2$ in hypoxic environments [9]. MnO$_2$ nanosheets have also been used to deliver photosensitizer C6 to cells to aid in photodynamic therapy, and they have helped to deliver DNA enzymes for gene silencing therapies [9].

The unique physiochemical properties of MnO$_2$ and its versatility in structure [2, 6, 10] make it a great candidate for use in drug delivery applications. Once an MnO$_2$ drug delivery system is inside of a cell, the MnO$_2$ particle should degrade and release the drug. Higher concentrations of glutathione are generally present in cancer cells compared to within normal cells [11, 12], so once the hollow nanoparticles are inside, if MnO$_2$ proves to degrade well in glutathione (which this study confirms), they should degrade quickly in cancer cells but may not degrade in normal cells. In this regard, MnO$_2$ hollow nanoparticles can be a groundbreaking advancement in nanoscale drug delivery for targeting cancer treatments.

Much like carbon-based species, MnO$_2$ can be formed into many different crystal structures and macroscopic structures [2, 6, 10, 13], which is intriguing in terms of drug attachment in drug delivery systems. Nanoparticles have very high surface area, but surface area is only limited to the particle’s outer wall, and drug attachment can be very challenging depending upon the chemistry of both the drug and the drug delivery system. Therefore, there is still a need for a very high surface area, biocompatible, and biodegradable drug delivery system that can safely and efficiently deliver therapeutics for cancer treatment.

MnO$_2$ hollow nanoparticles have been successfully developed for use in high performance Zn–MnO$_2$ batteries [2]. However, hollow MnO$_2$ (hMnO$_2$) nanoparticles have not been used in tissue engineering and drug delivery systems. Their biodegradation has not yet been studied. The hollow structure can enhance the drug loading efficiency by offering a much larger surface area (internal and external walls) for drugs to attach to, allowing for higher amounts of drugs to be delivered at a time on a single substrate [14]. This may improve cost, since better loading efficiency would result in the need for less substrate. This high surface area may also result in more rapid, enhanced degradation, as the more surface area that is exposed to a degradation medium, the faster the material will degrade. The hollow structure would also result in a lower density particle [14], which may allow for improved transport throughout the body due to improved buoyancy and low likelihood that a particle will sink or get stuck in the wrong location.

In this study, we fabricated MnO$_2$ hollow nanoparticles for use in drug delivery applications. The same fabrication method used in the aforementioned Zn–MnO$_2$ battery cathode was used in this study [1]. These nanoparticles were characterized and compared to other potential drug delivery candidates, solid spherical MnO$_2$, and GO nanosheets. The solid MnO$_2$ (sMnO$_2$) nanoparticles allowed for a direct comparison of hollow to non-hollow particles, since the chemistry should be the same. The GO provided a carbon-based material, of which many viable drug delivery systems are made. This allowed for a material comparison of carbon-based particles to MnO$_2$ materials.

To overcome the hardships of drug attachment/adsorption to the surface of the drug delivery substrate, dopamine was coated and left to polymerize on the surface of the MnO$_2$ hollow nanoparticles. Dopamine is found in the body, so it is biocompatible, and it can be polymerized easily. It self-polymerizes on the surface of a substrate under high pH [15], allowing for a tight coating to form around the core particle. In the case of these hollow nanoparticles, the polydopamine coating can form on the inner and outer walls of the hollow nanoparticles. This polydopamine coating allows for drugs with amino functional groups to attach easily through the Michael addition/Schiff base reaction [16].
In this study, to prove the concept, we used a fluorescent dye, which contains NH$_2$ groups, as a drug model to investigate whether dopamine-coated MnO$_2$ nanoparticles can load the fluorescent dye. Furthermore, in this study the effect of the hollow nanoparticles on cell viability was also investigated.

2. Materials and methods

2.1. Synthesis of MnO$_2$ hollow nanoparticles

An SiO$_2$ template was used to create the hollow nanoparticles. A sol-gel method was implemented to develop the SiO$_2$ particles [2]. In a single batch, 100 ml of ethanol (Fisher Scientific, MA, USA), 20 ml of deionized (DI) water, and 2 ml of 28% ammonia were added together in a beaker and stirred under medium level aggression. 8 ml of tetrapropyl orthosilicate (Fisher Scientific) was added to the mixture as it was stirring, and the mixture was left to stir for 12–14 h. A murky white suspension formed [2].

The suspension was centrifuged at 4000 × g for 20 min, and the supernatant was discarded. DI water was added back in as a wash, and the suspension was vortexed and then centrifuged again with the same parameters. This wash was done three to four times until the supernatant was no longer murky.

After centrifuging and washing, the suspension was resuspended in 60 ml of DI water, and 1.96 g of potassium permanganate was added. The suspension was sonicated for 30 min and then put into a Teflon-lined autoclave inside an oven. The oven was ramped up to 150 °C, and the suspension was heated for 48 h.

The new purple/brown colored product was removed from the autoclave, after fully cooling down, and transferred to a beaker with 300 ml of 2 M sodium carbonate. The beaker was covered and placed in an oven for 24 h at 60 °C to etch out the SiO$_2$. After cooling, the final product was centrifuged and washed with DI water at 4000 × g for 20 min four to five times until the supernatant was clear and all excess potassium permanganate (purple coloring) was removed. The final product was then suspended in a small amount of DI water, frozen at −80 °C overnight, and freeze dried.

2.2. Characterizations of nanoparticles

2.2.1. Scanning electron microscopy (SEM).

The morphologies of the hollow nanoparticles were observed using SEM on a JEOL 6330F Field Emission SEM with EDS. Dry hMnO$_2$ nanoparticles were sonicated (Branson ultrasonic bath) in pure DI water at 0.05% concentration for a single cycle of 20 min at room temperature on full power, dropped onto a glass slide, left to dry, and the slide was coated in gold with a Pelco SC-7 sputter coater and imaged. Commercially synthesized sMnO$_2$ nanoparticles (98%, 50 nm) from US Research Nanomaterials, Inc. and GO from Graphitene were used and imaged as control samples. The GO was prepared for SEM in the same way as hMnO$_2$ was described above, and sMnO$_2$ was prepared by placing dry powder on carbon tape and coating in gold in the same manner.

2.2.2. Transmission electron microscopy (TEM).

Particle size and more detailed electron microscopy (including qualitative observations of crystallinity and sheet thickness) was observed using TEM. A few milligrams of nanoparticles including commercially synthesized sMnO$_2$ nanoparticles, GO, and synthesized hMnO$_2$ nanoparticles were dispersed in an ethanol solution, each as a separate sample. A carbon-coated Cu-grid mesh 400 was used as a TEM holder to sample the nanoparticles. A Philips CM200 TEM was used to image the ultra-microstructural morphologies of the nanoparticles at 200 kV of acceleration voltage.

2.2.3. Particle size distribution.

Quantitative particle size distribution was measured using dynamic light scattering with the Zetasizer Nano ZS90 ZEN3690 model on sMnO$_2$ nanoparticles, GO, and hMnO$_2$ nanoparticles. Dry nanoparticle samples were added to DI water at 0.05% concentration and sonicated in the Branson ultrasonic bath on full power at room temperature for a single cycle of 30 min in a 15 ml centrifuge tube before pipetting 1 ml of the sonicated sample into a quartz cuvette for DLS testing. Each sample was then sonicated for another single 30 min cycle on the same settings (shown as the 60 min sample) in order to observe the effects of sonication on aggregation. Sample measurements were taken in triplicate, and graphical representations represent averages of each sample set.

2.2.4. Fourier-transform infrared spectroscopy (FTIR).

FTIR was performed on sMnO$_2$ nanoparticles, GO, and hMnO$_2$ nanoparticles to identify the chemical groups of each sample. A Thermo Scientific Nicolet iS10 FTIR spectrometer was used with the diamond crystal ATR accessory. A small sample of dry powder was placed on the diamond crystal to fully cover the area underneath the pressure tip for testing. Spectra were collected with a range of 3000 cm$^{-1}$ to 400 cm$^{-1}$.

2.2.5. X-ray diffraction (XRD).

XRD was carried out to identify the crystal structures of sMnO$_2$ nanoparticles, GO, and hMnO$_2$ nanoparticles using a Siemens D5000 x-ray Diffractometer with monochromatic CuK$_\alpha$ radiation, 40 kV accelerating voltage, a current of 30 mA, and scanning in the 2θ range from 10° to 65° with a step size of 0.05.

2.2.6. In vitro degradation.

Qualitative degradation testing was performed on sMnO$_2$ nanoparticles, GO, and hMnO$_2$ nanoparticles to determine the degradation rate of the nanomaterials.

Three different types of media were used for the degradation testing: 1 × PBS, 20 mg ml$^{-1}$ ascorbic acid in DI water, and 20 mg ml$^{-1}$ glutathione in DI water [6]. Ascorbic acid was used, because there are fairly high intracellular concentrations of ascorbate in many cells in the body due to uptake in the small intestine from humans’ vitamin C-rich diets [7]. Glutathione is present in high concentrations in cancer cells [11, 12], so this was also used. Both of these solutes are antioxidants.
that have been known to degrade metal oxides. PBS was used as a control in which degradation was not likely to occur. Multiple samples of 2–4 mg of dry sMnO₂ nanoparticles, GO, and hMnO₂ nanoparticles were weighed out in 2 ml centrifuge tubes, and the weights were recorded. All samples were carried out in triplicate. The weights of all centrifuge tubes were recorded as well. The appropriate amount of diluent was added to each sample (three samples each of each type of dry powder in each of the three diluents) so that a weight ratio of 10:1 dry solute to nanoparticles was upheld in all samples. The samples were inverted a few times to mix and placed on a rocker plate in a 37 °C incubator for multiple time points of 1, 3, and 7 d.

After each time point, the samples were removed from the incubator, and photos were taken to show any qualitative change in color or nanoparticle dissolution. Samples were done in triplicate. The samples were centrifuged, and the supernatant was then removed from each sample and set aside in −20 °C to −80 °C for later cell testing. The centrifuged samples were washed and centrifuged three times with DI water, and all samples were freeze dried. All samples were then weighed again, and the weights were recorded and analyzed to properly observe the weight loss of each sample. The weight loss presented for each time point is the average weight loss for each sample set.

2.2.7. Settling tests and zeta potential measurements. Qualitative settling tests were completed to compare the suspension stability of synthesized MnO₂ hollow nanoparticles to the other nanoparticles under study for drug delivery applications. GO, sMnO₂ nanoparticles, and hMnO₂ nanoparticles were all tested. Samples of dry powder were added to DI water as 0.05% suspensions and sonicated in the Branson ultrasonic bath on full power at room temperature for a single 20 min cycle. DI water was used as a neutral solvent for suspension. Each sample was transferred to a small vial and left to sit for multiple timepoints, at which point photos were taken.

Additional settling tests were completed to determine the most ideal dispersion aid for hMnO₂ nanoparticles in suspension. hMnO₂ nanoparticles were suspended in human epithelial cell (HEC) media (0.05% suspension concentration) (Human Epithelial Cell Media with kit H6621 from Cell Biologics with additional 10% fetal bovine serum (FBS)). The cell culture medium with FBS protein was used as a neutral solvent to mimic the environment hMnO₂ nanoparticles will be exposed to and suspended in after they are intravenously injected into a patient. Different potential dispersion aids were added to the epithelial cell media as 0.1% solution concentrations, mixed thoroughly until fully dissolved, and then MnO₂ was added as a 0.05% concentration. The suspension was then sonicated for about 20 min. Each sample was transferred to a small vial and left to sit for multiple timepoints, at which point photos were taken. The samples consisted of a control sample with no dispersants. The dispersants were: Tween 20 surfactant, PEG 400, xanthan gum (XG), sodium alginate, and sodium pyrophosphate.

Once a dispersion agent was decided upon, additional testing was completed to further demonstrate proper dispersion. Another settling test was completed with XG as the dispersant. This test used the same human epithelial cell media as stated previously and also RPMI-1640 cell culture media for cancer culture, and XG was added to further investigate the stability of the nanoparticles in media with FBS.

The last settling test was completed in 1 × PBS, as PBS is a neutral solvent that can be used to suspend the nanoparticles. The test was completed with four samples: sMnO₂ nanoparticles with/without XG dispersant and hMnO₂ nanoparticles with/without XG dispersant. 0.05% of MnO₂ was added to 1 × PBS, sonicated for about 20 min, and then the samples that required XG were transferred to a stir plate to stir aggressively while 0.1% XG was slowly added in. The samples without XG were also stirred on a stir plate in the same manner for consistency, but nothing additional was added. Each sample was transferred to a small vial and left to sit for multiple timepoints, at which point photos were taken. The excess sample was used for later potential measurements.

Zeta potential testing was performed using the Zetasizer Nano ZS90 ZEN3690 model on multiple samples. The samples discussed in the last two settling tests (samples with/without XG dispersant in two types of cell culture media and samples with and without XG dispersant in 1 × PBS) underwent zeta potential testing. All samples were tested in triplicate.

2.3. Coating hMnO₂ nanoparticles

To investigate the potential of loading any drug or therapeutic agents onto the hMnO₂ nanoparticles for drug delivery, we utilized the grafting ability of a polydopamine coating, as it can easily react with an agent with NH₂ functional groups [16]. The dopamine coating was carried out first by forming a suspension with 50 mg of dry, pre-synthesized hMnO₂ powder in 25 ml of TRIS-HCl buffer (pH 8.5) for each sample. The suspension was sonicated for 15 min. Dopamine-HCl (50 mg) was added to the sonicated mixture (2 mg ml⁻¹) and left to mix on a rocker plate for 24 h at room temperature. The sample was then centrifuged and washed with DI water 2–3 times via vortexing and centrifuging to remove any excess dopamine.

A fluorescent dye, fluoresceinamine isomer 1 (Sigma-Aldrich, MO, USA), having NH₂ groups was used as a model drug to be loaded onto the dopamine-coated hMnO₂. A stock solution of fluoresceinamine isomer 1 in dimethyl sulfoxide (DMSO) (5 mg ml⁻¹) was prepared, and 5 ml of this stock solution was added to 20 ml of TRIS-HCl buffer to obtain a final fluoresceinamine concentration of 1 mg ml⁻¹. The dopamine-coated and non-coated samples were resuspended in this 25 ml of fluoresceinamine DMSO/TRIS-HCl buffer and left to mix on a rocker plate for 24 h, wrapped in foil, in the dark. The samples were then centrifuged and washed in the same manner described previously to remove excess, unattached dye. The nanoparticles were then frozen at −80 °C and freeze-dried.

To understand how the dopamine coating affected zeta potential, MnO₂ alone and dopamine-coated MnO₂ were tested. These samples were put into human epithelial cell
media as a 0.05% solution and sonicated for 20 min before testing. Samples were tested in a quartz cuvette.

2.4. Cell testing

To properly understand the effect of MnO₂ hollow nanoparticles on both cancer and healthy cells, fluorescence imaging and MTT testing was completed. Esophageal squamous cancer cells (KYSE30) from EACCC (Sigma-Aldrich) and healthy human esophageal epithelial cells from Cell Biologics (CA, USA) were used in all cell tests.

2.4.1. Cell culture—cancer (KYSE30) and healthy epithelial cells. The following applies to both cancer and healthy epithelial cells. RPMI-1640 cell media was used to culture an esophageal squamous cell carcinoma, KYSE30. Epithelial cell culture media from Cell Biologics was used to culture healthy esophageal epithelial cells. A T75 flask was pre-coated with gelatin-based coating solution from Cell Biologics for epithelial cell culture only (cancer cell culture did not require coating). The coating solution (2.5 ml) was added to the T75 flask, tilted to mix and cover the whole surface, and left to sit for 2 min. After 2 min, the remaining solution was aspirated, and the flask was left to sit for 15 min. Cells were seeded in the flasks, and the flasks were placed into a 37 °C incubator with 5% CO₂. Cell media was replaced after the first day and every 2–3 d thereafter until about 80% confluence was reached.

2.4.2. Fluorescence testing on cells treated with nanoparticles. After the cells reached confluence, cells were trypsinized into single suspension. Then, 10⁶ cells were added to each well in a 24 well plate and incubated at 37 °C to allow the cells to attach overnight. All media was then aspirated from the wells and discarded. Cells were then treated with five groups: no addition of nanoparticles, hMnO₂, dopamine-coated hMnO₂, non-coated hMnO₂ with loading dye, and dopamine-coated hMnO₂ with loading dye. These nanoparticles were autoclaved on the dry setting and removed from the autoclave after cooling overnight. On the day of sample addition, each sample was made into a 1 mg ml⁻¹ solution in appropriate cell culture media (RPMI-1640 for cancer cells and human epithelial cell media for epithelial cells) and sonicated for 20 min. 1 ml of the appropriate MnO₂ sample was added to each appropriately labeled well, and the well plate was placed back into the 37 °C incubator for 6 h. All samples were done in triplicate.

After the 6 h incubation period, all media was removed from the wells and discarded, and each well was washed three times with 1 × PBS to remove any excess media and MnO₂. The cells were fixed with 4% paraformaldehyde (PAF) solution and left to sit for 20 min at room temperature. The PAF was then aspirated, and each well was washed three times with 1 × PBS, and DAPI was then added to each well for staining cell nuclei before the wells were washed twice with PBS. Fluorescence images were taken for each sample on an inverted fluorescent microscope (TE2000, Nikon).

2.5. Cell viability

After the appropriate number of cells were grown, KYSE30 and epithelial cells were detached using trypsin-EDTA, and about 4 × 10⁴ cells were added to each well in multiple 24 well plates individually, and the plates were put into the 37 °C incubator to allow the cells to attach overnight. Then cells were treated with the following samples: cells alone, hMnO₂ nanoparticles, dopamine-coated hMnO₂ nanoparticles, XG only, ascorbic acid supernatant from hMnO₂ degradation only, glutathione supernatant from hMnO₂ degradation only, ascorbic acid only, and glutathione only. All samples were done in triplicate.

All dry powders of MnO₂ hollow nanoparticles were autoclaved on the dry setting, along with multiple beakers and stir rods and allowed to cool overnight. On the day of testing, each of these autoclaved samples was added directly to appropriate cell culture media as a 0.5 mg ml⁻¹ solution and sonicated for 20 min. Each solution was then transferred to a stir plate under the cell culture hood, stirred aggressively, and 1 mg ml⁻¹ of XG was added slowly during stirring as a dispersant to keep the nanoparticles suspended for testing. Each sample was left to stir aggressively for 10 min.

The samples from the degradation tests of hMnO₂ were sterilized using a 0.22 μm filter, and a 1 μml⁻¹ solution of these samples in appropriate cell culture media was made to reach an overall ascorbic acid concentration of about 100 μmol⁻¹. Ascorbic acid and glutathione only samples were made using 20 mg ml⁻¹ samples in DI water. This solution was then added to appropriate cell culture media as a 1 μmol⁻¹ solution to reach an overall ascorbic acid concentration of about 100 μmol⁻¹, and the samples were sterilized using a 0.22 μm filter.

After each designated time point of day 1, 2, and 3, all media was removed from the wells and discarded, and each well was washed three times with 1 × PBS to remove any excess media and MnO₂. A fresh MTT solution in media (0.5 μg/mL) was added to each well, and the well plates were placed back in the incubator for 4 h. Afterwards, DMSO was added to each well to dissolve the purple crystals for 15 min at room temperature. About 150 μl of each sample was transferred to a 96 well plate, and the well plate was tested using a SpectraMax Microplate Reader at wavelengths of 490 nm and 570 nm as a reference. All measurements were taken in triplicate.

2.6. Statistical analysis

Zeta potential graphs were presented as mean ± standard deviation to properly represent multiple samples from each sample set, and statistical significance was assessed using Brown–Forsythe and Welch ANOVA tests as well as Dunnett’s T3 multiple comparisons tests to compare each sample. MTT assay graphs were presented as mean ± standard deviation to properly represent multiple samples from each sample set, and statistical significance was assessed using a two-way ANOVA test followed by Tukey’s multiple comparisons test.
A probability of $p < 0.05$ indicated statistical significance in all statistical analyses.

3. Results and discussion

3.1. Morphology of $\text{sMnO}_2$, $\text{hMnO}_2$ nanoparticles, and GO nanosheets

In this study, hMnO$_2$ nanoparticles were made and observed with SEM and TEM imaging and compared to sMnO$_2$ nanoparticles and GO, alternative nanoparticles being studied in the field of drug delivery [3, 5]. SEM images in figure 1 show that sMnO$_2$ nanoparticles have the 50 nm diameter and showed heavy aggregation of nanoparticles (figure 1(A)), which was to be expected due to the high surface area and surface reactivity exhibited in nanoparticles [17]. The synthesized hMnO$_2$ nanoparticles are shown be about 200 nm in diameter (figure 1(B)), indicating that the largest synthesized SiO$_2$ template nanoparticles were around this size, and all smaller SiO$_2$ particles may have been removed during centrifugation. The hollow nanoparticles also seem to have a rougher outer surface due to the formation of multiple sheets, compared to the solid nanoparticles consisting of smaller, smoother single spheres. A rough surface translates to a higher surface area, which can be desirable for drug delivery applications. The hollow nanoparticles are very indicative of $\delta$-MnO$_2$ structure [10, 13] and may be spherical aggregations of MnO$_2$, separate from what was formed around SiO$_2$ nanoparticles.

TEM imaging of GO nanosheets showed that the GO is a very thin, crystalline, continuous, sheet-like structure (figure 1(C)). As a widely studied nanomaterial, GO is used for promoting stem cell differentiation and drug delivery [18]. Here we used GO as a control. As another control nanomaterial, we used the same material (MnO$_2$) but with a different structure—solid nanoparticles were used. TEM showed that a crystalline morphology of sMnO$_2$ nanoparticles contain some sections that are more crystalline, and heavy aggregation is still observed (figure 1(D)). However, the hollow nanoparticles are hollow inside, with a very thin wall of randomly oriented crystalline sheets (figures 1(E) and (F)). There is less aggregation in hollow nanoparticles than in solid nanoparticles, as the TEM photo in figure 1(E) shows an aggregate of only four particles, compared to large aggregates seen in the solid nanoparticles.

Both SEM and TEM photos show that hollow nanoparticles maintained their spherical morphology and remained intact after sonication, indicating good mechanical stability. The discontinuous layer of sheets and incomplete sphere formation in certain particles indicates that there are certain locations where MnO$_2$ did not fully form due to particles leaning against the wall of the autoclave liner (figure 1(F)). These discontinuous spheres may be extra beneficial for drug loading, offering an easy-access entryway for drug attachment on the inner wall, aside from any pores present in the hollow nanoparticles.

Although the hollow nanoparticles are larger in diameter, generally leading to a smaller surface area per unit volume than a smaller particle, their rough surfaces and inner wall surface available for use on the inside may lead to higher overall surface area, leading to a more desirable morphology for drug delivery applications. The large availability of space on each particle can be of noteworthy use when high drug loading is necessary, and therefore less substrate material (the nanoparticles) is needed. Less substrate leads to a lower cost drug delivery mechanism and a lower concentration of substrate within the body, reducing any toxicity or likelihood of a negative response from the body. Minimal aggregation is also beneficial in terms of dispersion in media during injection or other type of administration to the body.
The success of etching out SiO$_2$ nanoparticles, as shown in both TEM and SEM, further proves that there is porosity in the fully formed nanoparticles, as the sodium carbonate was able to get inside. This porosity is the mechanism in which drug loading may occur in any fully formed particles. The few nanoparticles that may only be partially formed can still be used, as drug loading would occur both directly on each surface and through the pores.

### 3.2. Aggregate size distribution of sMnO$_2$, hMnO$_2$ nanoparticles, and GO

The synthesized hMnO$_2$ nanoparticles have the most narrow and consistent particle size distribution when compared to solid nanoparticles and GO as shown in figure 2(A). It is important to note that these are representative of aggregate size, not primary (individual) particle size. Aggregates are fused individual particles. sMnO$_2$ nanoparticles have a very wide distribution with aggregates ranging from 50 nm to around 6000 nm in diameter (figure 2(B)). Two cycles of sonication were tried here to investigate how additional sonication may impact aggregation. The smaller aggregates are seen after both 30 and 60 min of sonication, and larger aggregates are seen mostly in samples that have only been sonicated for 30 min. Aggregates much larger than 3000 nm are not seen in samples that were sonicated for more time. GO has two somewhat distinct distributions, with most aggregates being either 1000 nm or 5000 nm in diameter (figure 2(C)). The larger aggregates are mainly seen in samples sonicated for a longer amount of time (60 min). Hollow nanoparticles have a single narrow distribution with most aggregates around 750 nm in diameter (figure 2(A)). This is consistent with the aggregate size seen in the hollow nanoparticle TEM photos in figures 1(E) and (F), and it indicates aggregates of around four primary particles wide. All samples sonicated for 30 min formed a narrower distribution than samples sonicated for 60 min.

The average polydispersity index (PDI) of the hMnO$_2$ nanoparticles was 0.19 after 30 min of sonication and 0.05 after 60 min. The average PDI of sMnO$_2$ was 0.43 after 30 min of sonication and 0.35 after 60 min, and the average PDI of GO was 0.40 after 30 min and 0.44 after 60 min. This also indicates that hMnO$_2$ is a much more heterogenous sample than the other samples and that the aggregates are likely all of very similar size.

The difference in distributions of samples sonicated for 30 and 60 min indicates that additional sonication causes more aggregation in GO and hollow nanoparticles. Solid nanoparticles do not have a consistent aggregate size, but the opposite trend can be seen for them. Additional sonication may help to break up aggregates. This may be because GO and hMnO$_2$ nanoparticles consist of thin sheets. Excess sonication (above a certain threshold somewhere between 30 and 60 min) may cause the sheets to exfoliate, producing more surface area with higher surface activity, resulting in additional aggregation. Individual primary particles in solid nanoparticles cannot be broken down further, so sonication may cause aggregates to break up and particles to simply bounce around forming smaller and larger aggregates at the same time, which would explain the inconsistent aggregate size shown in figure 2(B). If any trend can be seen for solid nanoparticles, it is expected that excess sonication would reduce aggregate size, since additional energy put into the system should lead to de-aggregation. Aggregation can cause blockages and can reduce flow of nanoparticles after administration to a patient. Due to high surface areas, aggregation of nanoparticles is inevitable. In addition, aggregation consists of fused individual particles, and these contact points between particles result in less exposed surface area for drug attachment, which is less efficient for drug loading. Smaller, more predictable and consistent aggregate sizes observed in hMnO$_2$ as compared to sMnO$_2$ or GO indicate that it may serve as a superior drug delivery candidate in this regard.

### 3.3. Chemical groups and crystal structures of sMnO$_2$, GO, and hMnO$_2$ nanoparticles

MnO$_2$ has several different possible crystallographic forms as shown in figure 3(A). The solid nanoparticles closer represent the α-MnO$_2$ structure, representing a nanoparticle-type structure, with typical peaks present around 20 = 25°, 35°, 37°, and 55° [10, 13]. However, MnO$_2$ hollow nanoparticles did not show the characteristic peaks with the α-MnO$_2$ structure, but there are some small peaks present around 20 = 12°, 25°, 37°, and 65°, which implies that the synthesized hMnO$_2$ are amorphous, representing nanosheets [10, 13]. This similarity to nanosheets further confirms the idea that MnO$_2$ nanosheets have formed around SiO$_2$ to develop a hollow amorphous structure. GO had the distinct (001) plane present with a characteristic peak at around 20 = 12° [19].

FTIR was completed to confirm that MnO$_2$ was formed. To confirm MnO$_2$ formation, hMnO$_2$ nanoparticles were compared against sMnO$_2$ nanoparticles, as the FTIR spectra
whereas degradation is seen as the point at which yellowing, or oxidation occurs, resulting in no possibility for a reverse reaction. Dissolution likely occurs at a faster rate in hollow nanoparticles due to their much higher surface area than solid nanoparticles. The faster degradation seen in hMnO$_2$ as compared to sMnO$_2$ due to increased surface area further proves that the shell of the hMnO$_2$ is porous and the hollow nanoparticles have an internal wall. This internal surface area is likely being exposed to degradation solution, resulting in fast degradation. The inner and outer surface areas of the hollow nanoparticles can be potentially used to load more drugs compared to solid nanoparticles.

Both MnO$_2$ nanoparticles likely dissolve and degrade faster than GO due to the redox reaction that occurs. Certain organic species are known to reduce metal oxides, producing oxidation products that may be yellow in color [7]. Ascorbic acid dissolves in water, and at pH levels like those within the body, the prevailing hydroxyl group is the ascorbate monoanion, which can become reduced quickly by metal, oxidation still occurs, producing a yellow color, but it occurs at much slower rates of oxidation. Since GO does not contain a metal, so this process occurs much slower. Ascorbic acid can autoxidize without a metal catalyst, but the scarcer ascorbate dianion is used in this process. Lower levels of this dianion and absence of a metal catalyst cause much slower rates of oxidation. Since GO does not contain a metal, oxidation still occurs, producing a yellow color, but it occurs at a much slower rate, which is reflected in figure 4(D) [7]. PBS is simply an electrolytic solution, so no redox reaction or yellowing occurs.

Glutathione is another antioxidant that reduces MnO$_2$, so degradation occurs in the same manner but not as quickly as in ascorbic acid. When MnO$_2$ is reduced, glutathione is oxidized to form glutathione disulfide (GSSG) as shown in equation (2) [25].

\[
\text{MnO}_2 + 2\text{GSH} + 2\text{H}^+ \rightarrow \text{Mn}^{2+} + \text{GSSG} + 2\text{H}_2\text{O}. \quad (2)
\]
Figure 4. (A) Photos of hMnO$_2$ nanoparticles, sMnO$_2$ nanoparticles, and GO samples after 7 d of degradation testing and centrifugation in 1 × PBS, 20 mg ml$^{-1}$ glutathione (GSH), and 20 mg ml$^{-1}$ ascorbic acid (AA). Percentage weight loss of (B) hMnO$_2$ nanoparticles, (C) sMnO$_2$ nanoparticles, and (D) GO samples after 7 d of degradation testing.

In both cases with glutathione and ascorbic acid, the Mn$^{2+}$ ion and water is produced [7, 24, 25]. Water is easily able to leave the body, and Mn$^{2+}$ can be absorbed into the bloodstream and then transferred to the liver [26]. From the liver, the Mn$^{2+}$, along with bile, goes to the intestine where it can then be excreted from the body [26].

Hollow nanoparticles’ fast degradation rates show superior biodegradability over its MnO$_2$ nanoparticle and GO sheet counterparts, which is a benefit for drug delivery applications. In this study, degradation of hMnO$_2$ occurred very quickly, but a change in geometry, such as increased wall thickness (potentially formed by simply adding additional layers through subsequent KMnO$_4$ additions and autoclaving), would allow for the adjustment and manipulation of degradation rate if needed. This manipulation ability is less likely in carbon-based drug delivery systems, such as those made of GO, as much degradation does not seem to occur even after several days. As cancer cells tend to express higher intracellular glutathione levels than normal cells do [12], hMnO$_2$ nanoparticles have the potential to be degraded faster inside cancer cells than normal cells, allowing for the release of drugs inside these cancer cells. This function would allow for potential targeted drug delivery.

3.5. Suspension stability of nanoparticles

In this study we used several media to test the stability of nanoparticles. In DI water, qualitative settling tests (figure 5(A)) showed that GO (i) settles out very quickly, compared to sMnO$_2$ nanoparticles (ii) and hMnO$_2$ nanoparticles (iii). After just 4 h, heavy aggregation occurred in GO, and the large aggregates settled to the bottom of the vial. Aggregation did
not occur in MnO₂ nanoparticles as readily in the other two samples, and they were very similar in terms of settling. Both MnO₂ samples did show some settling after 4 h.

Settling tests continued with hollow nanoparticles to further observe and understand their behavior in colloidal suspensions. To further improve the suspension of MnO₂ hollow nanoparticles, a settling test was done with multiple dispersants, as shown in S.1. Human epithelial cell media was used to better mimic a physiological environment. All suspension aids were inferior to no suspension aid addition (S.1, 1), except for XG (S.1, 4). XG performed the best and was mostly still suspended after 24 h. XG is a hydrocolloid, a cross linked polymer that swells upon exposure to water [27]. Improved colloidal suspension is most likely due to the nanoparticles getting trapped inside the pores of cross-linked polymer chains. In this settling test, XG was added to the cell media first, and then MnO₂ was added. In this manner, it is likely that larger MnO₂ aggregates may have formed upon addition and were too large to fit inside the XG pores, resulting in some settling of MnO₂.

A different mixing procedure was adopted and tested with the chosen dispersant, XG, to make the best possible colloidal suspension. The next test involved testing hollow nanoparticles in both RPMI-1640 and the same human epithelial cell media with and without XG to understand suspension stability in both cancer and healthy cell environments (as subsequent testing would be completed with these cell media) (figure 5(B)). MnO₂ was sonicated first in cell media, and then XG was added and stirred aggressively. This allowed for less aggregation and better suspension. XG improves suspension in both human epithelial cell media (figure 5(B), ii) and RPMI-1640 (iv) compared to without XG (i, iii). This is confirmed from zeta potential measurements shown in figure 5(D), which shows zeta potential absolute values above 10 mV, indicating incipient stability [28]. Stability in both types of media is still not perfect, likely due in part to the many proteins present in cell media.

One last settling test (shown in figure 5(C)) was completed to compare sMnO₂ nanoparticles (i, ii) to hMnO₂ nanoparticles (iii, iv) in 1 × PBS with and without XG to confirm that hollow nanoparticles could stay suspended in an appropriate media for potential intravenous delivery. Results showed that PBS was a better suspension medium than both DI water and cell media, and hollow nanoparticles with XG stayed suspended for much longer than sMnO₂ nanoparticles with XG. Even after 5 d, the majority of hollow nanoparticles with XG (iv) were still suspended, while all other samples had mostly settled out (figure 5(C)). Hollow nanoparticles form a much more stable colloidal suspension than sMnO₂ nanoparticles do, and XG improves this affect. This is likely due to the decrease density of hollow nanoparticles, resulting in a more buoyant material. Zeta potential measurements, shown in figure 5(E), were taken in these samples in PBS. sMnO₂ samples with and without XG formed some larger aggregates that settled out too quickly for accurate zeta potential measurements. The measurements present in figure 5(E) were of the particles that did not settle quickly. Hollow nanoparticles are superior to nanoparticles in that less aggregation occurs initially and overall. Figure 5(E) also shows that PBS is a preferable suspension medium, as hollow nanoparticles alone and with XG have zeta potential absolute values above 20 mV, indicating improved stability over what was observed in cell media [28]. XG also leads to higher zeta potential values.

Zeta potential measurements were also performed to compare hollow nanoparticles to hollow nanoparticles with dopamine coatings, as shown in figure 5(F). The dopamine coating did not significantly change the zeta potential of MnO₂ hollow nanoparticles. Compared to the high values with the addition of dispersant, the low zeta potential absolute values indicate that hollow nanoparticles alone in media, without any suspension aid, coagulate rapidly. An increase in zeta potential absolute value is necessary for a stable suspension [28].

3.6. Uptake of hollow nanoparticles in cancer cells

To observe the potential uptake of nanoparticles in cells, we immobilized a fluorescent dye onto the surface of hMnO₂ nanoparticles. This dye also acted as a drug model to prove whether hMnO₂ can be a potential drug delivery vehicle. Figure 6 shows the results of the fluorescence test completed with healthy epithelial cells and cancer cells and different MnO₂ hollow nanoparticles. Imaging shows that cancer cells seem to take up hollow nanoparticles, as green fluorescence is readily seen mimicking the shape of cancer cells in hMnO₂ with dopamine. This is not seen in hMnO₂ nanoparticles without a coating. No green fluorescence was observed for this sample. This lack of fluorescence may indicate that fluorescent dye does not adsorb onto uncoated hMnO₂ nanoparticles, as it was likely removed during the washing steps of the procedure. The fluorescent dye can be considered as a model for drug loading, and the lack of dye present during imaging confirms that a dopamine coating is needed in order to load therapeutic drugs onto or into MnO₂ hollow nanoparticles. To confirm that the green fluorescence in the other samples was coming from the green fluorescent dye and not dopamine, imaging was also run on a dopamine-coated sample without dye, and no fluorescence was seen, proving that dopamine does not fluoresce, and all fluorescence seen is from the green fluorescent dye immobilized on the dopamine-coated surface (figure 6). Hollow nanoparticles and/or dopamine may also kill cancer cells, as imaging shows significantly fewer cancer cells present in the control sample without MnO₂ than that in the cancer cells samples treated with MnO₂ or with MnO₂ and dopamine. As shown in figure 6 bright field cancer cell images, the cell morphology is different from the cells without treatment, indicating that the cells may be undergoing cell death and due to the possibility that MnO₂/dopamine may be toxic to cancer cells. Much larger, abnormally shaped cells can be seen in the MnO₂ with dopamine-only sample images. These abnormally shaped cells were found nearby excess MnO₂ that could not be washed off of the well plate, indicating that an excess, or overdose, of MnO₂ particles may be toxic to cancer cells. The inability to wash off all MnO₂ sample from the well plate is likely due to MnO₂ adsorption to cells.

Figure 6 also portrays fluorescence imaging of healthy epithelial cells. Unlike with cancer cells, the number of cells with
and without treatment seems to be fairly consistent, indicating that MnO\textsubscript{2}/dopamine may not be toxic to healthy cells in the same way it is to cancer cells. Green fluorescence imaging shows fluorescence of almost the same locations as DAPI fluorescence does, and there is not much fluorescence showing up surrounding the nucleus. The green fluorescence does not
Figure 6. Bright field, DAPI, and green fluorescence images of KYSE30 cancer cells and control groups of HEC: cells only, cells treated with hMnO$_2$ nanoparticles only, and cells treated with dopamine-coated hMnO$_2$ nanoparticles.

The green fluorescence shown in figure 6 may not be nanoparticle uptake and may instead be adsorption of nanoparticles. It is important to note that the term ‘uptake’ may refer only to adsorption on cell surfaces, and not imbibition. Further studies need to be performed to confirm these results.

Bright field imaging of healthy human mesenchymal stem cells treated with MnO$_2$ hollow nanoparticles coated with dopamine (S.2) shows that healthy stem cells also took up MnO$_2$ hollow nanoparticles; however, there was much more MnO$_2$ stuck at the bottom of the well plate, and imaging shows MnO$_2$ aggregates covering the entire surfaces of the healthy cells. This may indicate adsorption, but not imbibition, which is similar to what was seen in healthy cells in figure 6 when no fluorescence was seen mimicking the shape of the cells. Slower (or lack of) imbibition of hollow nanoparticles in healthy cells, compared to cancer cells, can indicate that hollow nanoparticles may preferentially target cancer cells in a cancer and healthy cell co-culture or more importantly, in the body where cancer and healthy cells co-exist. The reasons behind these observations remain unknown. More experiments in next steps may need to further investigate.

It has been confirmed that MnO$_2$ hollow nanoparticles can target both cancer and healthy cells, with cancer cells imbibing more nanoparticles and healthy cells adsorbing larger aggregates, but these hollow nanoparticles may preferentially and more efficiently target cells and kill cancer cells.

3.7. Cell viability

An MTT assay was done to understand how cancer and healthy cells respond to MnO$_2$ hollow nanoparticles with coatings, as well as degraded products. Figures 7(A) and (B) show consistent growth of the control sample for both cancer and healthy cells over 3 d, with more growth seen in cancer cells, which is to be expected. The addition of MnO$_2$ hollow nanoparticles, regardless of coating, causes a decrease in the number of cells, indicating that the hollow nanoparticles have some toxicity to both types of cells. There is, however, a greater decrease seen in cancer cells than in healthy cells, but the number of healthy cells may not be enough to view a proper trend. There are not significant differences between MnO$_2$ hollow nanoparticle groups with/without dopamine coating.

XG causes an initial decrease in cells, but then leads to large growth after the first day. This phenomenon may be due to the
Figure 7. MTT assay results (at 570 nm wavelength) of (A) healthy human epithelial cells and (B) KYSE30 cancer cells seeded with hMnO$_2$ nanoparticles with various coatings and degradation samples.

hydrocolloidal nature of XG which leads it to imbibe water [27]. XG would imbibe cell media when in contact with it, leading to more media between crosslinks, and therefore less media immediately available to cells. This may lead to an initial decrease in cell count. Cells may later be able to migrate in and around XG molecules, using it as a scaffold, which would allow for increased growth of cells.

Ascobic acid and glutathione increase growth of both cancer and healthy cells. From day 1, the addition of ascobic acid or glutathione with or without degraded MnO$_2$ (and therefore Mn$^{2+}$ ions) improves the growth of healthy cells, whereas Mn$^{2+}$ ions with ascobic acid or glutathione inhibit cell growth on day 1. After the first day, growth increases again for cancer cells. This shows that cancer cells may be negatively impacted by Mn$^{2+}$ ions, contrary to how healthy cells initially respond. The rapid increase in growth may be due to the depletion of Mn$^{2+}$ after day 1, if certain cells already took in the ions.

Ascobic acid and glutathione are antioxidants [7, 25], which is likely why they show an increase in the number of cells overall. If MnO$_2$ alone degrades to any extent, it may oxidize, which is not good for cells. If an antioxidant is present, MnO$_2$ is reduced, and it prevents oxidation from occurring, leading to a much healthier environment for cells [7]. This may explain why MnO$_2$ alone is worse for cells compared to either of the antioxidants alone or with degraded MnO$_2$.

MnO$_2$ does aggregate after some time in cell media, as described previously, and some aggregation did occur during the incubation period prior to the MTT assay. It is possible that aggregation caused blanketing over cells, and potential suffocation, leading to cell death. Therefore, it cannot be confirmed that MnO$_2$ alone is toxic to cells, as aggregation may inhibit cell growth. A separate MTT assay was run with cancer cells seeded with beta-tricalcium phosphate (β-TCP) nanoparticles to test this theory (S.3). β-TCP is not toxic to cells, but it does aggregate. The results showed a lower cell count for the cells seeded with β-TCP compared to the control without it. This indicates that nanoparticle aggregation may inhibit cell growth. Therefore, further studies should be completed to confirm whether MnO$_2$ alone is toxic to cells.

Current results demonstrated that hMnO$_2$ has promise as a platform for drug delivery systems. The coating on the surface has the potential to immobilize drugs for the application of drug delivery. More research would be required to further develop the potential of hMnO$_2$ in biomedical applications, especially for drug delivery systems.

Degradation studies were conducted to compare the MnO$_2$ hollow nanoparticles to other potential candidates for drug delivery systems. The hollow nanoparticles had faster dissolution rates, indicating superior biodegradability. Qualitative and quantitative settling tests (using DLS and zeta potential) showed enhanced colloidal stability of MnO$_2$ hollow nanoparticles over GO sheets and MnO$_2$ solid nanoparticles, indicating less aggregation and lower density. These properties lead to improved colloidal stability of the drug delivery system, which is beneficial during administration to the patient. XG showed a substantial improvement in the colloidal stability of MnO$_2$ hollow nanoparticles, so it was chosen to aid in the dispersion and suspension of hollow nanoparticles for subsequent cell testing.

Healthy cells had more aggregates of MnO$_2$ hollow nanoparticles adsorbed on the outer cell walls (and less inhibition of nanoparticles) than the cancer cells did, suggesting that healthy cells may be slower to take up nanoparticles than cancer cells, but more studies can be completed to confirm this. Additional studies can also be completed to confirm whether the particles are being imbibed, or simply adsorbed, by any type of cell.

Cell metabolic studies confirm that MnO$_2$ aggregation may inhibit the growth of all cells and potentially more toxic to cancer cells than healthy cells. More studies should be done to
confirm this. Degraded MnO2 with antioxidants initially positively impact healthy cells and negatively impact cancer cells, but over time it shows a positive impact on both.

4. Conclusions

There exists a need for biocompatible and biodegradable targeted drug delivery systems on the nanoscale. In this study, MnO2 hollow nanoparticles were successfully fabricated using SiO2 nanoparticle templates at high heat and pressure. Electron microscopy, XRD, and FTIR confirmed the production of a hollow, high surface area, spherical cluster of δ-MnO2 structured sheets.

hMnO2 nanoparticles have higher surface area for drug loading, faster degradation rates for drug release, and improved colloidal stability as compared to other potential drug delivery candidates, and they can be coated, loaded with a drug, and target cells. These characteristics suggest that MnO2 hollow nanoparticles could be prime candidates as targeting drug delivery systems for cancer treatment.

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References

[1] Shaji A and Zachariah A K 2017 Surface area analysis of nanomaterials Thermal and Rheological Measurement Techniques for Nanomaterials Characterization (Cambridge, MA: Elsevier) pp 197–229
[2] Guo X, Li J, Jin X, Han Y, Lin Y, Lei Z, Wang S, Qin L, Jiao S and Cao R 2018 A hollow-structured manganese oxide cathode for stable Zn-MnO2 batteries Nanomaterials 8 301
[3] Tripathi A, Saraf S and Saraf S 2015 carbon nanotropes: a contemporary paradigm in drug delivery Materials 8 3068–100
[4] Patra J K et al 2018 Nano based drug delivery systems: recent developments and future prospects J. Nanobiotechnol. 16 71–67
[5] Mohamadi S and Hamidi M 2017 The new nanocarriers based on graphene and graphene oxide for drug delivery applications Nanostructures for Drug Delivery pp 107–47 (Cambridge, MA: Elsevier)
[6] Yang L, Chueh S T D, Li Y, Patel M, Rathnam C, Dey G, Wang L, Cai L and Lee K-B 2018 A biodegradable hybrid inorganic nanoscaffold for advanced stem cell therapy Nat. Commun. 9 3147
[7] Du J, Cullen J J and Buettner G R 2012 Ascorbic acid: chemistry, biology and the treatment of cancer Biochem. Biophys. Acta (BBA) Rev. Cancer 1826 443–57
[8] Wu M, Hou P, Dong L, Cai L, Chen Z, Zhao M and Li J 2019 Manganese dioxide nanosheets: from preparation to biomedical applications Int. J. Nanomed. 14 4781–800
[9] Chen J, Meng H, Tian Y, Yang R, Du D, Li Z, Qu L and Lin Y 2019 Recent advances in functionalized MnO2 nanosheets for biosensing and biomedicine applications Nanoscale Horiz. 4 321–38
[10] Ghosh D, Bhandari S and Khastgir D 2016 Synthesis of MnO2 nanoparticles and their effective utilization as UV protectors for outdoor high voltage polymeric insulators used in power transmission lines Phys. Chem. Chem. Phys. 18 32876–90
[11] Fernández M, Javid F and Chudasama V 2018 Advances in targeting the folate receptor in the treatment/ imaging of cancers Chem. Sci. 9 790–810
[12] Gamcsik M P, Kasibhatla M S, Teeter G D and Colvin O M 2012 Glutathione levels in human tumors Biomarkers 17 671–91
[13] Devaraj S and Mushchandraiah N 2008 Effect of crystallographic structure of MnO2 on its electrochemical capacitance properties J. Phys. Chem. C 112 4406–17
[14] El-Toni A M, Habila M A, Labis J P, Aloothan Z A, Alhoshan M, Elzatary A A and Zhang F 2016 Design, synthesis, and applications of core–shell, hollow core, and nanorattle multifunctional nanostructures Nanoscale 8 2510–31
[15] Sinet N, Sallem F, Mirojet C, Nury T, Sahoo S K, Millot N and Kumar R 2019 Polydopamine modified superparamagnetic iron oxide nanoparticles as multifunctional nanocarrier for targeted prostate cancer treatment Nanomaterials 9 138
[16] Huang N, Zhang S, Yang L, Liu M, Li H, Zhang Y and Yao S 2015 Multifunctional electrochemical platforms based on the michael addition/schiff base reaction of polypophamine modified reduced graphene oxide: construction and application ACS Appl. Mater. Interfaces 7 17935–46
[17] Huangfu X, Jiang J, Ma J, Liu Y and Yang J 2013 Aggregation kinetics of manganese dioxide colloids in aqueous solution: influence of humic substances and biomacromolecules Environ. Sci. Technol. 47 10285–92
[18] Halim A, Luo Q, Ju Y and Song G 2018 A mini review focused on the recent applications of graphene oxide in stem cell growth and differentiation Nanomaterials 8 736
[19] Johra F T, Lee J W and Jung W G 2014 Facile and safe graphene preparation on solution based platform J. Ind. Eng. Chem. 20 2883–7
[20] He D, Peng Z, Gong W, Luo Y, Zhao P and Kong L 2015 Mechanism of a green graphene oxide reduction with reusable potassium carbonate RSC Adv. 5 11966–72
[21] Huang M, Zhang Y, Li F, Zhang L, Luoff R S, Wen Z and Liu Q 2014 Self-assembly of mesoporous nanotubes assembled from interwoven ultrafinin binresite-type MnO2 nanosheets for asymmetric supercapacitors Sci. Rep. 4 3878
[22] Anon 2015 ATR-FT-IR spectra of sodium carbonate (Na2CO3) Database of ATR-FTIR spectra of various materials
[23] Moon S A, Sathiyamoorthi E, Alkotaini B, Kim B S and Salunke B K 2015 Biological synthesis of manganese dioxide nanoparticles by Kalopanax pictus plant extract JET Nanomaterials 9 220–5
[24] Khan Z, Kumar P and Kabir-Ud-Din 2005 Kinetics of the reduction of water-soluble colloidal MnO2 by ascorbic acid J. Colloid Interface Sci. 290 184–9
[25] Deng R, Xie X, Vendrell M, Chang Y-T and Liu X 2011 Intracellular glutathione detection using MnO2-nanosheet-modified upconversion nanoparticles J. Am. Chem. Soc. 133 20168–71
[26] Williams M, Todd G D and Roney N 2000 3.4.4 elimination and excretion Toxicological Profile for Manganese (Atlanta, GA: U.S. Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry)
[27] Saha D and Bhattacharya S 2010 Hydrocolloids as thickening and gelling agents in food: a critical review J. Food Sci. Technol. 47 587–97

[28] Nimesh S, Chandra R and Gupta N 2017 Advances in Nanomedicine for the Delivery of Therapeutic Nucleic Acids (Duxford: Elsevier/Woodhead Publishing) pp 46–47