Porous Carbon Produced in Air: Physicochemical Properties and Stem Cell Engineering

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Aerosol materials synthesis methods[1–9] have been utilized to prepare a distinct platform of spherical composite materials that incorporate sub-100 nanometer features. Nanostructured microspheres (ns-μS) and extended surfaces made up of metal, metal oxides and sulfides such as TiO2[10–14] SiO2,[15–19] Co,[20] CO3O4, CuO, NiO,[21] ZrO2,[22] ZnO,[23,24] Al,[25] Al2O3,[26] BiVO4,[27] MoS2,[28] ZnS,[29,30] as well as polymers[31] and carbon[32–35] have been prepared via ultrasonic spray pyrolysis (USP)[7] methods using molecular precursors to give uniquely structured materials (Figure S1). Here, we report a new, simple and versatile synthetic route to high surface area carbon microspheres that have hierarchical, mesoporous structure (Figure 1); and, we report their physicochemical and biological properties.

Titanium(IV) bis(ammonium lactato) dihydroxide[33] was diluted in water, aerosolized (nebulized) in air, and reacted using USP at two different temperatures (Figure 1). The synthetic result was the formation of TiO2 nanoparticles that assemble into larger micrometer-sized spheres, a well-known outcome for aqueous aerosol synthesis methods.[1–9] The USP product color was gray to black indicating the presence of carbon. This was unexpected since the carrier gas was air and carbon syntheses predominantly occur under inert gas conditions.[36] A reasonable initial hypothesis was that at 850 °C, a core-shell structure resulted; a shell of TiO2 solidified while carbon was being incorporated into a TiO2 matrix towards the center of the microsphere. The TiO2 shell, more than likely, acted as a barrier resulting in an oxygen free interior core condition and introduced a sub-micrometer level temperature gradient.

The formation of a TiO2-carbon core structure (Figure 1.2) was confirmed via post-USP HF etching experiments. When chemically treated with 10% hydrogen fluoride (HF) for several hours, the core density of the 850 °C sample decreased. A sample made at 1000 °C, in contrast, displayed no apparent structural changes since the core TiO2 was more crystalline, and therefore etch resistant, and the carbon was burnt away (Figure 2, XRD data in Figures S2, S3). Concentrated HF treatment produced solid carbon spheres (Figure 1b, TEM, C-μS-850). If the reaction temperature was lowered to 400 °C, USP followed by etching produced a hollow carbon structure (Figure 1a, TEM, C-μS-400). Based on these results it was clear that a shell of crystalline TiO2 was formed and a less crystalline TiO2-carbon nanocomposite core was formed as the droplet of precursor solution underwent heating and consolidation. As mentioned earlier, we speculate that the solidified shell acted as a shield, inhibiting oxygen diffusion to the core and thereby reducing the core temperature. The carbon source comes from the TiO2 precursor solution that incorporates monomeric lactate anions (chemical structure in Figure 1). These ligands, coordinated to Ti(IV), chemically polymerize and subsequently undergo anaerobic carbonization inside the TiO2 shell. Lactate-like anions are known to form biodegradable copolymers at elevated temperatures or in the presence of a catalyst (e.g., Sn(IV)).[37]

Our report is a novel example of the formation of carbon microspheres using lactate as a carbon source under oxygenated conditions. There have been countless multistep efforts to create porous carbon[38–41] spheres based on resin co-polymerization processes and soft (i.e., surfactants such as F127 and P123)[42] hard template (i.e., SiO2)[43] approaches under inert gas atmosphere. Our system utilizes a readily available molecular precursor and is highly reproducible, facile, and high yielding. Depending on the reaction temperature, the particle core is HF-soluble (i.e., at 400 °C, the degree of lactate polymerization at the core is low, affording only a shell to form at the interface), multi-phased (i.e., at 850 °C, nearly spherical carbon forms) or phase-separated (i.e., at 1000 °C, non-spherical carbon forms) (Figure 1, Figure 2), as is evident from the formation of spatially defined crystalline TiO2 and carbon microspheres.

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Surface area analyses revealed that C-µS-850 yields mainly sub-3 nm pores (Figure 3) with a $N_2$ adsorption (BET) surface area of 1670 m$^2$/g, a pore volume of 1.06 cm$^3$/g, and an average pore diameter of 2.5 nm. The full isotherm curve is a cross between types I and IV, which means that the spheres have both micro- and mesoporosities (Figure 3). FT-IR analysis shows the presence of carboxylate and/or hydroxy functionality and C–H groups (Figure 3d) but after 50% HF treatment C-µS-850 does not incorporate any detectable Ti species (Figure 3e). We believe that the less crystalline TiO$_2$ nanoparticles present in the core act as an in situ sub-3 nm (hard) template (Figure 4a–f; enlarged in Figure S4, S5) with the formation of polylactide and subsequent (incomplete) carbonization. This new one-pot core-shell phase separated approach can lead to other intriguing structures such as meso- and macroporous hollow TiO$_2$ spheres (Figure S6).

Microtome/TEM experiments (Figure 4) of a USP TiO$_2$-carbon 850 °C sample undisputedly show that the shell is comprised of strongly crystalline TiO$_2$ nanocrystals compared to the core (Figure 2, Figure 4a–f). Selected area electron diffraction (SAED) analyses show that highly crystalline TiO$_2$ phases are seen only in the shell and not the core (Figure 4a–f; enlarged in S4, S5). In contrast, the distinctive presence of void spaces was observed in the 1000 °C sample (Figure 4g–l; enlarged in

Figure 1. Hollow, Porous, and nanostructured Carbon Microspheres (C-µS). Schematic routes for (a) hollow carbon (C-µS-400) and (b) porous carbon (C-µS-850). Respective transmission electron microscopy (TEM) images are on the far right.

Figure 2. Etching experiments on microspheres prepared only with a Ti(IV) source. The TEM images show the microspheres before (left) and after etching (right) for TiO$_2$-carbon µS prepared at (a) 850 °C and (b) 1000 °C. Please refer to the main text and Supporting Information for additional details.
Figure 3. Physicochemical properties of porous C-µS-850. (a) Nitrogen adsorption-desorption full isotherm curve is a cross between type I and IV. Data are consistent with reported systems.\cite{36,40,42,43} (b) TEM of analyzed carbon sample. (c) TEM close-up showing 2–3 nm pores (red box in b). (d) FT-IR analysis result. (e) EDX analysis result. Details are in the main text.

Figure 4. Microtoming, TEM, and SAED experimental results for USP TiO$_2$µS. Ti(IV) lactate at 850°C: Whole particle (a) SEM, (b) TEM; high-resolution (c) TEM and (d) SAED of the shell; high-resolution (e) TEM and (f) SAED of the core. Ti(IV) lactate at 1000°C: Whole particle (g) SEM, (h) TEM; high-resolution (i) TEM and (j) SAED of the shell; high-resolution (k) TEM and (l) SAED of the core. 12 nm SiO$_2$ doped TiO$_2$ (µS-1): (m) TEM. 70–100 nm SiO$_2$ doped TiO$_2$ (µS-2): (n) whole particle TEM, (o) core close-up TEM. 12 nm SiO$_2$ and Co(II) doped TiO$_2$ (µS-3): (p) whole particle TEM, (q) shell SAED, and (r) core SAED.
In this case, the void space (Figure 2b, Figure 4h) was not filled with the epoxy resin, which was used to prepare the sample for microtoming. This is an indication that the shell formed at this temperature is practically non-porous. Since rutile phases are much more stable than anatase, additional etching process time was required for total TiO$_2$ dissolution (Figure 2, Figure 4; XRD in S2, S3).

Three different amorphous silica doped TiO$_2$ µS’s (Figure S1) were additionally prepared\cite{13} and their structures were analyzed (Figure 4m–r; enlarged in S9) via TEM in order to further understand the core-shell synthesis. For silica doped titania, µS-1 (12 nm) and µS-2 (70–100 nm), crystallinity was the same throughout the particle (Figure 4m–o; S1) whereas for 12 nm silica and Co(II) doped titania, µS-3, the result was similar to that obtained for the 850 °C USP TiO$_2$ sample. A more crystalline shell formed and then a polycrystalline core formed while Co$_3$O$_4$ formed on the exterior (Figure 4p–r; S1, S9).

Blending of the SiO$_2$ nanoparticles revealed that the aqueous-phase nanostructured TiO$_2$ USP synthesis affords the following three situations (Figure S1). Porous TiO$_2$ µS (Type I) can be synthesized using a precursor mixture of Ti(IV) and silica nanoparticles (5–100 nm). Since silica nanoparticles increase the thermal conductivity within the droplet, a temperature gradient does not readily form, though this depends on the doping level. The core and the shell experience similar temperatures, close to the surrounding gas temperature; we believe that the oxygen level should be the same as well. SiO$_2$ nanoparticles are encapsulated inside a crystalline TiO$_2$ microspherical matrix. Microtome experiments reveal that the crystallinities of the inner TiO$_2$ nanoparticles are similar to those in the shells (Figure 4m–o).

The core-shell microsphere synthesis can be summarized in the following manner (Figure S10). TiO$_2$-carbon core-shell µS (Type I) can be synthesized using a Ti(IV) precursor with an organic counter-anion (i.e. lactate). The shell formation occurs earlier, which creates a TiO$_2$-carbon nanocomposite. The core, which experiences a lower temperature than the shell, eventually solidifies but with less crystallinity. The resulting µS is composed of a strongly crystalline shell and a less polycrystalline core (Figure 4, and Supporting Information S4, S5). The formation of carbon within the core depends on the reaction temperature and concentration, since air will burn carbon at a faster rate in elevated temperatures and low concentrations.

For transition metal ion and nanoparticle doped TiO$_2$ synthesis (Type III), phase separation is induced possibly due to thermal conductivity and oxygen concentration perturbation. Especially in the Co(II) and 12 nm silica doped case,\cite{13} sub-100 nm cobalt oxide nano-islands form on the exterior of the µS and subsequently act as an insulating/shielding layer to prevent heat and mass transport by plugging up pores in the shell. Thus, a shell of strongly crystalline TiO$_2$ forms first, then a less polycrystalline core forms later. Controlling the etching rates should be the same as well. SiO$_2$ nanoparticles are encapsulated inside a crystalline TiO$_2$ microspherical matrix. Microtome experiments reveal that the crystallinities of the inner TiO$_2$ nanoparticles are similar to those in the shells (Figure 4m–o).

Thus, a shell of strongly crystalline TiO$_2$ forms first, then a less polycrystalline core forms later. Controlling the etching rates should be the same as well. SiO$_2$ nanoparticles are encapsulated inside a crystalline TiO$_2$ microspherical matrix. Microtome experiments reveal that the crystallinities of the inner TiO$_2$ nanoparticles are similar to those in the shells (Figure 4m–o).

Table 1. Incorporation of Albumin-FITC and C16-W3K into porous C-µS-850 spheres.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Particle</th>
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<tbody>
<tr>
<td>Albumin-FITC</td>
<td>SBA-15</td>
<td>15</td>
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<tr>
<td></td>
<td>KIT-6</td>
<td>9</td>
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<tr>
<td></td>
<td>FDU-12</td>
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</tr>
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<tr>
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<td>porous Carbon microsphere</td>
<td>98</td>
</tr>
<tr>
<td>C16-W3K</td>
<td>SBA-15</td>
<td>43</td>
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<td></td>
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<td>100</td>
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<tr>
<td></td>
<td>porous carbon microsphere</td>
<td>67</td>
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(DHED: model Alzheimer’s disease therapeutic candidate)\cite{44}, DHED sodium carboxylic (DHED-COONa, a DHED variant), albumin-FITC (a representative blood protein), and peptide amphiphile C16-Trp-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Lys-Ala-Ala (C16-W3K; a bioactive lipid).\cite{45,46} Unlike carbon nanotubes and other carbon materials, our carbon microspheres disperse well in aqueous media. FT-IR analysis of C-µS-850 revealed that carboxylate and/or hydroxy groups are present on the microspheres (Figure 3d) while EDX analysis showed the absence of Ti (Figure 3e); thus, C-µS-850 incorporates areas where polylactide was partially carbonized but without detectable TiO$_2$ nanoparticles. The percent uptake for representative small molecules R6G, ThT, DHED, and DHED-COONa were 100% at 5 µg/mL, which well-exceeds the uptake limit of SBA-15,\cite{47,48} KIT-6,\cite{49,50} FDU-12,\cite{51} and porous TiO$_2$ (USP) microspheres;\cite{13} these latter examples typically uptake not more than 50%.

We envision using the high-surface-area carbon microspheres and hollow microspheres (average diameter ∼1 µm) as binders to control the viscoelastic properties of biomimetic (hydro)gels for the construction of three-dimensional tissue scaffolds in vitro that can also achieve controlled release of bioactive molecules.\cite{54–56} First, we confirmed through mammalian cell uptake experiments using murine microglia cell line BV-2 that our porous carbon microspheres, with or without incorporated molecules, are essentially non-cytotoxic (Figure 5a, b). Second, albumin-FITC incorporating C-µS-850 spheres were tested on human neural stem cell ReNcell VMs (here on ReNcells or hNSCs; Millipore, Inc.)\cite{57} that showed which the carbon spheres are able to enter into hNSCs and deliver proteins without detrimental effects (Figure 5c, d).
Exclusively on RenCels, we conducted long-term interfacing and DNA transfection experiments (Figure 6). hNSCs grown on laminin were treated with C-μS-850 dispersed in sterile nanopure water. Growth factors (i.e., bFGF, EGF) were removed after one day of culture and the differentiation potential was compared. After more than a month of culture, the hNSCs were fixed and stained for specific markers (i.e., GFAP, Nestin, SNCA). Results showed that carbon-sphere-incorporating hNSCs did differentiate (Figure 6a,b) and stopped doubling; the control cells appeared to exhibit similar differentiation capacities while stopping cell division. Both the control (no treatment) and carbon treated cells expressed GFAP, Nestin (Figure 6c,d), and Sox-2 (weak, data not shown), levels identical under a confocal microscope. We also wanted to see the expression of alpha-synuclein (SNCA) proteins that are normally present in neural tissue. The aggregated or mis-folded forms of SNCA are implicated in Parkinson’s disease and there is an increased interest of identifying the role of SNCA.[58–60] We confirmed the presence of SNCA in the nucleus of differentiated RenCels and there was no real difference in the expression area and level (Figure 6c,d). We originally hypothesized that C-μS-850 may allow the amphiphilic SNCA protein to aggregate inside the cell and lead to cell death but the result showed that no SNCA, GFAP, or other proteins incorporated into the carbon spheres. This prompted us to utilized C-μS-850 as hNSC transfection agents. Preliminary results suggest that (modified) C-μS-850 spheres are able to deliver (plasmid) DNA into RenCels (Figure 6e,f) but at a sub-0.01% efficiency. The particular carbon spheres utilized for the successful transfection study were subject to fluorescein modification via surface carboxylate groups but no detectable fluorophore incorporation was observed. The chemically treated C-μS-850 spheres (e.g. peptide chemistry) were washed multiple times with nanopure water before hNSC experiments. Further hNSC transfection studies with carbon spheres are beyond the scope of this paper and we will report additional results in the near future.

In conclusion, studies of the USP TiO2 μS structures[13] clearly show that the internal structure of a reacting droplet nebulized by a high-frequency ultrasound can be tuned. It is already known that cavitating bubbles produced by high intensity ultrasound (i.e., 20 kHz) are several micrometers in size, and have a temperature gradient.[61] Physical property confinement within a few micrometer sized spherical space is fascinating and we expect that further manipulations of this phenomena will be useful, particularly for USP since robust, porous and hollow TiO2 nanostructured microspheres and high surface area carbon microspheres that are water-dispersible and...
biocompatible can be synthesized using this approach in large quantities under oxygenic conditions.

**Experimental Section**

Details are available in the Supporting Information.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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