Neutron-Activatable Radionuclide Therapy Using Mesoporous Carbon Nanoparticles
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Purpose
Radiation therapy is the standard of care for 60-70% of cancer patients, either alone or in conjunction with other therapies (Georgakilas 2013). For the purpose of achieving targeted systemic radionuclide delivery with minimum handling of radioactive materials, we investigated the use of a carbon-based nanocarrier for delivering neutron-activatable radionuclides to treat gynecologic cancers. Utilizing a neutron-activation strategy, the stable isotope Ho-165 was loaded into the nanocarrier and subsequently irradiated in a nuclear reactor to produce a nanocarrier with a radioactive isotope (Ho-166). One limitation of this strategy was that large amounts of heat are generated during the neutron-activation process; this results in the degradation of conventional nanocarriers (liposomes and polymeric micelles) (Di Pasqua, Yuan et al. 2013). To overcome this issue and improve current systemic radiation therapy, we here suggested using a novel nano-sized drug delivery carrier: mesoporous carbon nanoparticles (MCNs). These carbon nanocarriers possess several advantages over other nanocarriers including heat-resistance, low density, high metal adsorption capacity and low toxicity.

Methods
MCNs were synthesized from a phenol formaldehyde resin by the soft-template method (Fang, Gu et al. 2010). The first approach of surface modification of MCNs was oxidation with strong acids (3:1 ratio of sulfuric and nitric). Another strategy of surface modification was attaching 1,2-distearoyl-sn-glycero-3-phospho-ethanolamine-N-[methoxy(polyethylene glycol)-3000 (phospholipid-PEG) onto MCNs because hydrophobic MCNs could rapidly bind to phospholipids with short sonication (5-10 minutes). When holmium was loaded onto MCNs, a simple physical adsorption method was sufficient as opposed to using a chelating agent. The loading of [Ho-165]- (2,4-pentanedione holmium(III)) into MCNs was evaluated with various analytical techniques: inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer Nexion 300-D), energy-dispersive X-ray spectroscopy (EDS; Oxford INCA Energy TEM 250) and γ-counting (PerkinElmer 2470 Wizard γ-counter). The in vitro cytotoxicity of MCNs and PEGylated MCNs was tested by CCK-8 assay in a human ovarian cancer cell line (A2780). As a preliminary test of stability under the neutron-activation, MCNs and PEGylated MCNs were irradiated for one hour in the PULSTAR nuclear reactor (reactor power: 1 MW; thermal neutron flux: 5.5*10EE12 neutrons/cm2*s) and their morphology and structural integrity were observed by scanning electron microscopy (SEM; Hitachi S-4700) and transmission electron microscopy (TEM; JEOL 100 CX II).

Results
Successfully synthesized MCNs were ~150 nm in diameter, were monodisperse (PDI: 0.152) and possessed a neutral surface charge (zeta potential: -1.6 mV). Image analysis of MCNs showed spherical morphology and about 100 nm-sized particles in both SEM and TEM. EDS confirmed that MCNs were only comprised of carbon (~90 %) and oxygen (~10 %). The average pore size of the MCNs was estimated to be ~2.6 nm by 1H-NMR. To endow their dispersibility in biological media, surface modification on MCNs was necessary because of their hydrophobicity. By inducing numerous carboxyl groups on the surface of MCNs, the zeta potential of MCNs changed from almost neutral charge (-1.6 mV) to ~40 mV and retained a narrow size distribution. Phospholipid-PEGylated MCNs showed slightly increased size (~180 nm) and lower PDI (0.146). The maximum holmium loading was assessed by several analytical tools: 10.4 (w/w %) by EDS, 18.3 (w/w %) by ICP-MS and 12.1 (w/w %) by γ-counting. Both MCNs and PEGylated MCNs maintained their spherical morphology and structural integrity after 1 hour of neutron activation. Since we wanted the cytotoxicity of 166Ho-MCNs to come from the radioactivity and not from the nanocarrier, we anticipated that all A2780 cells would survive until exposed to 166Ho. As expected, the in vitro cytotoxicity test showed that stable [Ho-165]-MCNs and PEGylated MCNs were not toxic to A2780 cells after an incubation period of 48 hours.

Conclusion
MCNs were synthesized for use as a potential radionuclide carrier. Surface modified MCNs showed desirable physical characteristics. The maximum loading of Ho on MCNs was ~12 %. These particles retained their structural integrity following neutron irradiation. Non-radioactive holmium (Ho-165) loaded MCNs did not show the cytotoxicity in a human ovarian cancer cell.