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Encapsulation of a Radiolabeled Cluster Inside a Fullerene Cage, $^{177}Lu_xLu_{(3-x)}N@C_{80}$: An Interleukin-13-Conjugated Radiolabeled Metallofullerene Platform

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Synthesis of multimodal nanoprobes for use as diagnostic and therapeutic agents is a goal in the emerging field of nanomedicine.[1] The medical use of radionuclides has evolved and increased during the last 30 years.[2] β-Emitters have the advantage of a relatively short penetration range, thus providing localized ionizing radiation and the possibility of targeted applications. β-Emitters show promise in the treatment of a wide variety of conditions ranging from joint pain to tumors.[3] Delivery mechanisms for radionuclides have focused on chelating agents.[3] Unfortunately, because of chemical interactions or decay events, the radionuclide or the decay product may be released.

Incarceration of atoms inside fullerene cages (e.g., clusters of several atoms inside an I_h C_{80} cage[4]) provides an ideal delivery platform for nanomedicines, since the metal ion is isolated from the biosystem. Our previous work on trimetallic nitride-templated endohedral metallofullerenes (TNT EMFs) demonstrated the use of $Lu_3N@C_{80}$ as an X-ray contrast agent[5] and $Gd_3N@C_{80}$ as a magnetic resonance imaging (MRI) contrast agent with relaxivities 30−40 times higher than those of commercial MRI agents.[6] Other groups have reported radiolabeled EMFs (R-EMFs), but there is a paucity of reports describing multimodal nanoprobes.[7] For example, Diener and co-workers[7c] have reported the preparation of $^{212}Pb@C_{60}$, an α-emitting potential radiopharmaceutical, but without a targeting modality.

In this communication, we describe the encapsulation of the β-emitter ^{177}Lu in a fullerene cage and demonstrate that the encapsulated $^{177}Lu^{3+}$ ions are not released for a period of at least one half-life (6.7 days). It should be noted that ^{177}Lu has an emission spectrum that includes γ radiation, which is detectable, for example, by SPECT imaging. We also demonstrate that this agent can be conjugated with an interleukin-13 (IL-13) peptide that is designed to target an overexpressed receptor in glioblastoma multiforme tumors.[8]

The TNT EMF was synthesized in a quartz Kräschmer−Huffman electric generator that could be controlled behind a radiation shield.[9] Graphite rods containing Lu_2O_3 and radioactive $^{177}LuCl_3$ were vaporized. The chamber was then remotely washed with a toluene spray, driving the products to the bottom of the quartz reactor, where the soot particles were collected in a filter. The solution was collected and purified through a Merrifield resin column.[10]

The inclusion of the radiolabel was confirmed by high-performance liquid chromatography (HPLC) with UV−vis and radioactivity detectors (see the Supporting Information), with a radiolabeled yield averaged over several experiments of ∼0.02%. The retention times with the two detectors matched those of nonradioactive $Lu_3N@C_{80}$.

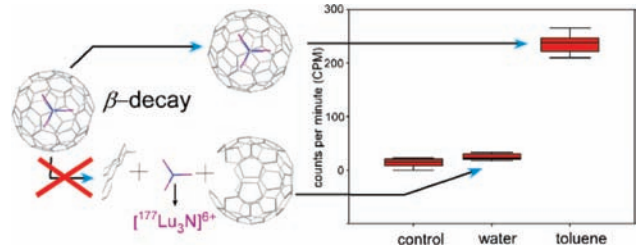

Figure 1. (left) Illustration of possible cage breakdown or retention due to β-decay events. (right) Box plots corresponding to the control, aqueous, and toluene extracts of $^{177}Lu_xLu_{3-x}N@C_{80}$ after 6.7 days.

A necessary characteristic for this nanomedical agent is that the cage remain intact during the radioactive decay. The cage integrity was investigated by allowing a toluene solution containing $^{177}Lu_xLu_{3-x}N@C_{80}$ to equilibrate with water for one ^{177}Lu half-life (6.7 days). Samples were extracted from each solvent layer as well as deionized water control samples and counted on a γ counter (Figure 1). The toluene extract showed a mean of 240 counts per minute (cpm), while the water extract and the control were not significantly different, thus demonstrating that the ^{177}Lu was retained in the toluene solvent layer. This result corroborates findings for other R-EMFs[7] and shows that the cage is robust enough to survive the β-decay process.

In glioblastoma multiforme, the most common and lethal brain tumor in humans (median survival ∼1 year), an IL-13 receptor is overexpressed; this does not occur in normal brain tissue.[8] The receptor for IL-13 in glioma cells is the IL-13Rα2 receptor.[11] Thus, a peptide possessing the binding sequence to the IL-13 receptor and a fluorescent tag [tetramethyl-6-carboxyrhodamine (TAMRA)] was chosen for conjugation to the $^{177}Lu_xLu_{3-x}N@C_{80}$.

$^{177}Lu_xLu_{3-x}N@C_{80}$ was functionalized and conjugated to the IL-13 peptide (Scheme 1) via previously reported methods.[12,13] Successful conjugation was verified by polyacrylamide gel electrophoresis (PAGE) separation and colocalization of signals from each component. In Figure 2 from left to right, the first lane is a visible image of the TAMRA-labeled IL-13 peptide alone, showing the pink color of the TAMRA tag between the blue and yellow loading buffer dyes; lane 2 is a visible image of the $^{177}Lu_xLu_{3-x}N@C_{80}$−TAMRA−IL-13 peptide reaction product run on the same gel; and lanes 3 and 4 are fluorescent and autoradio-

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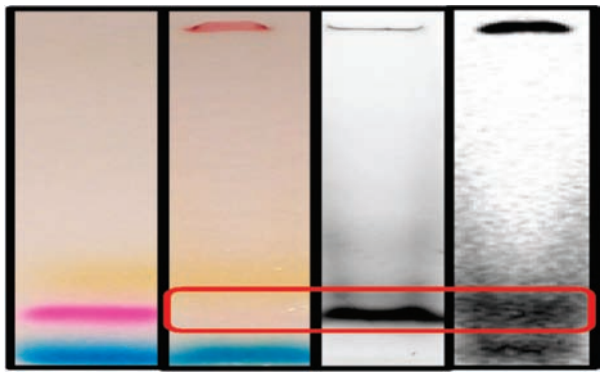
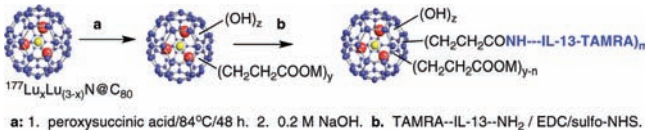


Figure 2. PAGE images from left to right: visible lane for the TAMRA-labeled IL-13 peptide alone and visible, fluorescent, and autoradiograph lanes for the $^{177}Lu_xLu_{3-x}N@C_{80}$−TAMRA−IL-13 peptide. The red rectangle shows the $^{177}Lu_xLu_{3-x}N@C_{80}$−TAMRA−IL-13 peptide conjugate.

Scheme 1. Functionalization and Conjugation of $^{177}Lu_xLu_{3-x}N@C_{80}$ with TAMRA-Labeled IL-13 Peptide ($z \approx 26$, $y \approx 16$, $n = 1, 2$)



a: 1. peroxysuccinic acid/84°C/48 h. 2. 0.2 M NaOH. **b.** TAMRA--IL-13--NH_2 / EDC/sulfo-NHS.

graph images, respectively, of lane 2. While the visible location of the $^{177}Lu_xLu_{3-x}N@C_{80}$−TAMRA−IL-13 peptide is difficult to see, the fluorescent signal from the TAMRA is clearly seen in lane 3, and a corresponding radioactive signal from the ^{177}Lu is seen in lane 4.

All of the signals in lanes 2−4 (red box) are aligned adjacent to the TAMRA-labeled IL-13 peptide alone in lane 1, with the proper alignment being confirmed by the loading buffer dyes and the residual reaction product that became insoluble and remained at the top of the gel. It is also evident that the conjugation reaction left some unconjugated $^{177}Lu_xLu_{3-x}N@C_{80}$ (bottom, lane 4), which could be removed by HPLC.

In this paper, we have reported the successful incorporation of ^{177}Lu into the endohedral metallofullerene $Lu_3N@C_{80}$ cage using a modified Krätschmer−Huffman apparatus that allows remote preparation and extraction in an atmospherically controlled environment. We have also shown that the radiolabeled ^{177}Lu ions are not readily removed from the fullerene cage and demonstrated potential targeting with a conjugated IL-13 protein. This nanoparticle delivery platform provides flexibility to meet a wide range of other radiotherapeutic and radiodiagnostic applications (e.g., with ^{166}Ho and ^{90}Y).

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Supporting Information Available: Synthetic details and characterization data (HPLC and MS). This material is available free of charge via the Internet at http://pubs.acs.org.

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