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Radioactive lutetium metallofullerene $^{177}Lu_xLu_{(3-x)}N@C_{80}$ - PCBPEG derivative : a potential tumor-targeted theranostic agent

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A radioactive metallofullerene $^{177}Lu_xLu_{(3-x)}N@C_{80}$ was firstly synthesized by means of neutron irridation on $Lu_3N@C_{80}$. After modification by methoxypolyethylene glycol amine, *in vivo* investigation on tumor-bearing mice was performed. The results reveal favorable affinity toward tumor, suggesting that obtained $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG would be promising for tumor diagnosis and therapy.

Since the first discovery in 1985,[1] fullerenes have been raised great interests in biomedicine due to their unique physical and chemical properties. Specially, endohedral metallofullerenes expanded the applications of fullerene family by endowing with the specialities of carbon cage and endohedral metal atoms.[2] In biomedical science, endohedral metallofullerenes have showed great potential as magnetic resonance imaging (MRI) contrast agents,[3,4] antioxidant[5], drug delivery nanocarriers[6] and antitumor agents.[7,8] As for radiomedicine and radiotherapy, the carbon cage could effectively prevent the inside metal atoms/atomic clusters from escaping to the surrounding environment, providing an obvious advantage over the traditionally used chelating agents, which are likely to release free metal cations *in vivo*.[9] Up to date, there are lots of metal isotopes have been successfully encapsulated in fullerene carbon cage.[10,11,12,13] Moreover, the outside carbon cage of endohedral metallofullerene could be modified easily and variously, making endohedral metallofullerene a promising radio-delivery platform.

For example, Koichi et al. prepared the first endohedral radioactive metallofullerene $^{159}Gd@C_{82}$ by neutron flux to activate $Gd@C_{82}$ in 1994, and the obtained $^{159}Gd@C_{82}$ proved to be stable as the usual normal endohedral metallofullerene.[14] Dawson et al. prepared another kind of radioactive endohedral metallofullerenes $^{166}Ho_x@C_{82}$ using the same method.[15,16,17] After modification to be water-soluble, the $^{166}Ho_x@C_{82}$ derivatives were studied *in vivo* and suggested to accumulate mostly in the liver of mouse. Michael et al. reported the preparation of $^{212}Pb@C_{60}$ by allowing the ^{212}Pb to recoil into C_{60} in 2007. The subsequent *in vivo* study showed that the malonic ester derivatives of $^{212}Pb@C_{60}$ did not accumulate in bone post-administeration, in contrast to results from the polyhydroxylated ones.[18] These results suggest that different modification on the carbon cages may lead to different metabolic properties.

The radionuclide ^{177}Lu, with its appropriate half-life period (6.67 d) and γ/β-emitting energy, is an ideal candidate in radiotherapy and radiodiagnosis. As a result, much more attention has been widely taken in clinical applications. However, most of them focus on the metal complexes, which may bring about side-effects during γ/β-emitting process and metabolic process. Metallofullerenes containing ^{177}Lu, however, would have potential in developing new type of safe and efficient radiopharmaceutic species. Specially, the trimetallic nitride template metallofullerene $Lu_3N@C_{80}$ would be more efficient than the other fullerene families since it can carry three metal atoms in one carbon cage. Michael and co-workers successfully developed a new radioactive endohedral metallofullerene $^{177}Lu_xLu_{(3-x)}N@C_{80}$ by doping $^{177}LuCl_3$ into the graphite rods containing Lu_2O_3 in a quartz Kräschmer-Huffman electric generator.[19] A series of studies showed that the $^{177}Lu_xLu_{(3-x)}N$ cluster was stable in C_{80} cage in a period of one half-life. After modification with an interleukin-13 peptide, the $^{177}Lu_xLu_{(3-x)}N@C_{80}$ is suggested to be a promising radiolabeled metallofullerene platform for tumor targeting, tumor diagnose and therapy. However, considering the low productivity of metallofullerenes and the potential radioactive pollution in the production process, a more efficient and fast production method should be developed. In addition, due to the low yield, the *in vivo* information about the metabolic and tumor-targeting properties of the endohedral ^{177}Lu

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metallofullenene is not available yet, further investigation should be necessary.

In this communication, we describe the preparation of $^{177}Lu_xLu_{(3-x)}N@C_{80}$ by direct irradiation of $Lu_3N@C_{80}$ with a neutron flux, which is much cleaner and more efficient than previously reported method.[19] After neutron activation, the $^{177}Lu_xLu_{(3-x)}N@C_{80}$ was isolated and purified by high performance liquid chromatography (HPLC). After a series of modification for biocompatibility, *in vivo* study was explored. The results demonstrated that mPEG(5000)-NH_2 modified $^{177}Lu_xLu_{(3-x)}N@C_{80}$ showed an outstanding tumor-targeting properties. Importantly, the obtained agent could be metabolized in a suitable rate, which is vital in radiomedical application.

The endohedral metallofullerene $Lu_3N@C_{80}$ was synthesized in a quartz Kräschmer-Huffman electric generator as previously reported.[20] Benefiting from 2.6% ^{176}Lu in the natural lutetium and the big scattering cross section of ^{176}Lu, and the modest tolerance of carbon cages under the neutron irradiation as well, the $Lu_3N@C_{80}$ could be partially activated by the neutrons and converted to $^{177}Lu_xLu_{(3-x)}N@C_{80}$. In our study, the $Lu_3N@C_{80}$ powders were irradiated by neutrons with a flux of $6x10^{13}$ $n{\cdot}cm^{-2}{\cdot}s^{-1}$ for 1 h. The activated sample was redissolved by toluene and the solution was filtered to remove the insoluble substance. HPLC in combination with UV-Vis and γ-ray detectors was used to confirm the successful preparation of radiolabelled $^{177}Lu_xLu_{(3-x)}N@C_{80}$ (Figure 1).

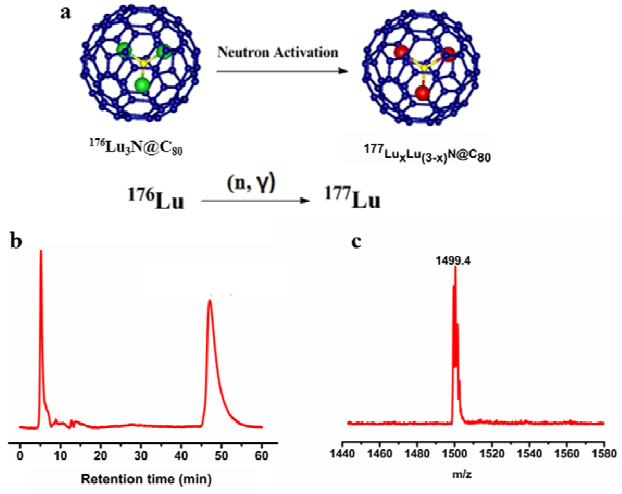

Figure 1. (a) Illustration of activating $Lu_3N@C_{80}$ by neutron flux. (b) Chromatogram of the isolated $^{177}Lu_xLu_{(3-x)}N@C_{80}$. (c) MALDI-TOF MS of purified $^{177}Lu_xLu_{(3-x)}N@C_{80}$.

As the polyethylene glycol (PEG) has exhibited good biocompatibility and effectiveness *in vivo* to avoid the phagocytosis of reticuloe endothelin system (RES),[21,22] lots of studies have used this star molecule to modify nanomaterials. Herein, the $^{177}Lu_xLu_{(3-x)}N@C_{80}$ was firstly modified to $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBM as reported,[23] and then hydrolyzed by HCl/AcOH (v/v = 3/1) for conjugation with mPEG(5000)-NH_2 under a reflux condition. After purification by a sephadex G-25 chromatography, the obtained $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG solution was concentrated for *in vivo* study (***Figure 2 and Figure S10).***

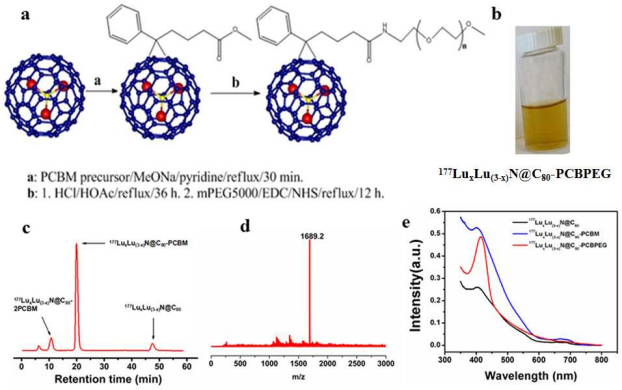

Figure 2. (a) The scheme of the functionalization of $^{177}Lu_xLu_{(3-x)}N@C_{80}$ with mPEG(5000)-NH_2. (b) The photograph of prepared $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG solution. (c) The HPLC profile of isolation of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBM in toluene. (d) MALDI-TOF MS of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBM. (e) UV/Vis-NIR spectra of purified $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG in water and its precursors $^{177}Lu_xLu_{(3-x)}N@C_{80}$ and $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBM in toluene.

Animal experiments were performed according to Chinese law and accepted international standards in biomedical research, and permission of the Beijing Administration Office of Laboratory Animal was obtained. The Ethical Guidelines (http://www.rsc.org/Publishing/Journals/guidelines/EthicalGuidelines/ExperimentsInvolvingLiveSubjects/index.asp) that involves the use of live animals or human subjects were obeyed as well. A total of 24 BALB/c female mice weighing 16-20 g were used in the *in vivo* study, and each mouse was inoculated with a S180 sarcoma. After a week of feeding, the tumor weights were between 80 - 120 mg, then the mice were randomly divided into 6 groups. The $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG (in physiological saline) was then injected to the caudal vein of mice with a dose of $5.0x10^4$ Bq. At different time points (0.5, 1, 5, 12, 24, 48h) of post-injection, the 6 groups of mice were sacrificed successively. Different tissues and organs of mice were harvested and weighted, and the radioactivity was measured by an automatic gamma counter. The biodistribution of ^{177}Lu at 0.5, 1, 5, 12, 24 and 48 h post-injection of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG in female mice were shown in Figure 3.

The results reveal that the agent has long blood circulation time (***Figure S11***) than the other reported radiolabeled metallofullerene derivatives with short life-time *in vivo*.[16, 18] This should probably contribute to methoxypolyethylene (mPEG), which is helpful to assist nanoparticles to escape the endothelial system and prolong the materials' blood circulation time. As a result, the enrichment of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG in tumor increases continuously and reaches the maximal (11.96 ± 2.55 %ID/g) at 24 h post-injection. Interestingly, the biodistribution of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG in tumor site is even higher that in liver at 24h post-injection, demonstrating its tumor-targeting property. Moreover, with the prolonging of time, the tumor to muscle ratio of relative content increases from ~3.5 (at 0.5 h post-injection) to ~26.8 (at 48h post-injection), again suggesting its unique tumor-affinity property. This might benefited from the particle size (ca. 144nm) which could give nanomaterials the property of tumor passive targeting, what's more, the long blood circulation time also leaded to the concentration of the material to the tumor. Importantly, further investigation reveals that $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG could be excreted *in vivo* as

confirmed by the results from the 48h post-injection in Figure 3. As we know, this is the first example to explore the fate of radioactive endohedral metallofullerene in tumor-bearing living body, so the acquired information is valuable.

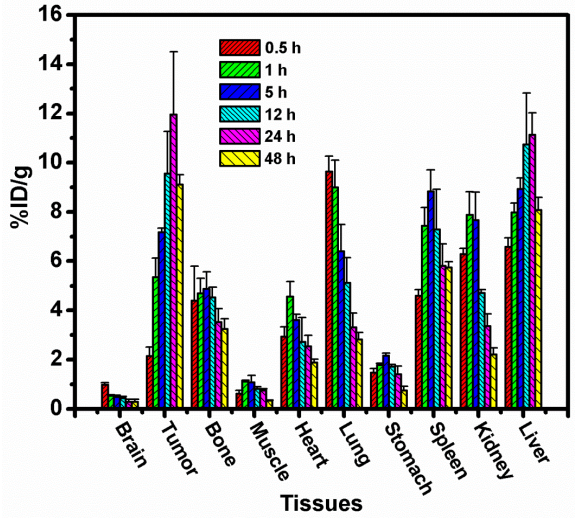

Figure 3. Biodistribution of $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG in the S180-bearing female BALB/c mice.

Conclusions

In this communication, we reported the preparation of endohedral radioactive metallofullerene $^{177}Lu_xLu_{(3-x)}N@C_{80}$ by neutron activation of $Lu_3N@C_{80}$. After modification with PEG5000, the biodistribution of this material in tumor-bearing BALB/c mice was investigated for the first time. The results indicated that $^{177}Lu_xLu_{(3-x)}N@C_{80}$-PCBPEG had a long blood circulation time and outstanding tumor-targeting property, which would have potentials in tumor theranostics. As there was only 2.6% ^{176}Lu in the lutetium metal used in production of $Lu_3N@C_{80}$, the neutron activation efficiency was relatively low, more efforts are taken to verify the actual diagnosis and therapy effect of this material.

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