131I radioactive self-expandable metallic stents for the therapy of malignant biliary obstruction

Meirong Liu1, Mengfei Hou, Qinghe Wu, Chunfu Zhang*
Shanghai Jiao Tong University, Shanghai 200030, China.
1meirong_l6@sjtu.edu.cn; 2cfzhang@sjtu.edu.cn

Abstract. Biliary stent implantation has become a standard approach for patients with unresectable malignant biliary obstruction. Unfortunately, in-stent restenosis after stent implantation still occurs because of tumor overgrowth. The majority of studies show that radioactive stents harbor both the obstructed biliary tract-dilating and tumor-suppressing functions. In this study, we designed a kind of novel 131I-labeled radioactive self-expandable metallic stent (SEMS) that combines the mechanical dilation forces induced by stent expansion and 131I intraluminal brachytherapy, which was prepared by first coating the SEMS with poly (diallyl acrylate) (PDA) and then labeling with iodine-131. The designed stents would not only have a longer patency time but also is able to inhibit biliary compression caused by tumor growth. The PDA coating was robust and flexible. The 131I labeling rate was about 60%, and after a 16-day test of radiochemical stability of 131I, the radioactive stability was around 80%. In addition, the radiochemical stability of 131I could be improved by PDA recoating post-131I labeling. In conclusion, we designed and fabricated a kind of novel 131I-labeled radioactive self-expandable metal stents by labeling 131I on the PDA-coated stent. We provide a new idea of radioactive stents design, which would extend the patency time of the stent while reducing the rate of stent restenosis after implantation.

1. Introduction
Malignant biliary obstruction (MBO) is a common disease in clinic, which is commonly caused by cholangiocarcinoma and pancreatic cancer as well as metastatic disease. Cholangiocarcinoma (CCA) is a primary hepatic malignancy, including intrahepatic, perihilar, and distal extrahepatic tumors. Among them, hilar cholangiocarcinoma accounts for approximately 50% of CCA [1] MBO usually inhibits bile from the liver into the duodenum, which is accompanied by symptoms such as painless jaundice and pruritus. Unfortunately, due to the lack of obvious early symptoms, MBO is commonly diagnosed at advanced ages. As a result, only 10%-20% of MBO patients can accept resection [2], [3]. Even following surgical resection, the 3- and 5-year survival rates remain low (18-52% and 5-31%, respectively) [4], [5]. For these patients, biliary stent implantation has become an important palliative treatment to palliate symptoms such as pruritus, pain, jaundice and cholangitis [6]. However, there are many cases of tumor ingrowth, tumor overgrowth, epithelial ingrowth, and biliary clogging. Moreover, conventional stents can only facilitate drainage but have no antitumor effect. Consequently, the frequency of stent restenosis is about 50% within six months after stent implantation, which has become a critical factor negatively affecting the prognosis of cancer patients [7]. For this reason, it is essential to prepare a kind of radioactive stent that serves both tumor treatment and drainage to increase stent patency duration and the survival rate of patients.

With the development of interventional radiology, intraluminal brachytherapy (ILBT) provides new opportunities for the therapy of MBO. There are three main methods for the design of existing radioactive stents:(1) 125I seed strands; (2) "Bundled" biliary stent; (3) Integrated 125I seed stent. In
2018, Chen [8] et al. developed an integrated $^{125}$I seed stent. They demonstrated that inserting a radioactive biliary stent is more effective in reducing jaundice symptoms, inhibiting tumor growth and prolonging the stent patency, as well as prolonging patient survival when compared to a conventional stent in patients with unresectable MBO. However, the above stents also have obvious disadvantages: they are expensive, additionally, only several radioactive seeds attached to stent, resulting in poor homogeneity of radiation and low efficacy.

PDA has received wide attention for biomedical applications due to its biocompatibility and ease of modification. In 2007, inspired by mussels, Lee [9] et al. discovered that diallyl acrylate can be oxidized and spontaneously self-polymerized under alkaline conditions, and then forms PDA on virtually any surfaces, including noble metals, oxides, polymers, semiconductors and ceramics. In addition, thanks to the catechol group on the PDA surface, PDA can be used for many functions according to different purposes, making it an extremely versatile platform.

Intraluminal brachytherapy provides a high localized dose of radiation to the tumor and reduces the volume of irradiated normal tissues. Therefore, it is highly favored in the treatment of biliary obstruction. Iodine-131 is a β-decay radionuclide with a half-life of 8.02 days. It emits β-rays (99%) and γ-rays (1%). The maximum energy of β-rays is 0.6065 MeV and the main γ-ray energy is 0.364 MeV. $^{131}$I has broad application in clinical radiation therapy. For example, Zhao [10] et al. coated single-walled carbon nanotubes (SWNT) with PDA and further modified it with polyethylene glycol. Then, the radionuclide $^{131}$I was labeled on SWNT@PDA-PEG, combining tumor nuclear imaging and radioisotope therapy.

Herein, a novel radioactive stent was developed involving PDA coating followed by radionuclide $^{131}$I labeling. PDA coating was ultra-thin (nm level), and there was no PDA degradation within 60 days when incubated at 37°C in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS), which demonstrated PDA could remain stable over a long period. Moreover, the PDA-coated stent was labeled with iodine-131 for ILBT, which exhibited excellent radiolabeling yields and radiochemical stability. Our study provides a new idea for radioactive stents design, then reduces the rate of stent restenosis after implantation as well as extends the patency time of the stent.

2. Materials and methods

2.1. Synthesis of PDA coated stent

The stents were cleaned by sonication in isopropyl alcohol and deionized water for 10 min each. PDA-coated stent was prepared by dipping the SEMS in diallyl acrylate solution (2 mg/mL of diallyl acrylate in 10 mM of Tris Buffer solution at pH = 8.5). Then, the reaction mixture was exposed to the air and stirred for 4 h at room temperature.

2.2. Synthesis of iodine-131 labeled stent

The stents were soaked overnight in 5 mM NaIO$_4$ solution and then washed with deionized water for 5 minutes. Iodine-131 was labeled by the chloramine T method. Chloramine T oxidizes iodine ions into iodine or high-valent iodine ions. To label PDA-coated stent with radioisotope iodine-131, iodine-131 (100 μCi) and non-radioactive sodium iodide (150 mCi) and chloramine T (0.88 mg) was added into PBS (0.5 mL, pH=7.5), the PDA-coated stent was then put and shook for 10 min. The radiolabelling yield was measured by a radioactivity meter (HD-175A, Heyiqitx, Beijing, China).

2.3. Recoated with PDA and NaIO$_4$ oxidation after labeling iodine-131

The stent was recoated with PDA (2 mg/mL for 1 h) after labeling, and the coating procedure was the same as above. Then used 5 mM NaIO$_4$ solution to oxidize the outer PDA overnight.

2.4. Radiochemical stability of iodine-131 labeled stent

To examine the radiochemical stability of the final $^{131}$I radioactive self-expandable metallic stents under physiological conditions, the $^{131}$I radioactive SEMS were immersed into DMEM + 10% FBS for different periods of time (1, 2, 4, 8, 16 day). The radioactive stability was calculated by dividing the radioactivity retained on the $^{131}$I radioactive SEMS to that of sample.
2.5. Statistics
All experiment data expressed are the calculated mean ± standard deviation (SD). A student’s t test was used for differences of statistically significant evaluation, and p < 0.05 was regarded as significant differences between groups.

3. Results and discussion

3.1. Preparation of PDA-coated stent
PDA coating was synthesized according to the methods described previously\(^9\). Specifically, PDA-coated stent was prepared by dipping the SEMS in diallyl acrylate solution for different time (2 mg/mL of diallyl acrylate in 10 mM of Tris Buffer solution at pH = 8.5). Then, the reaction mixture was exposed to the air and stirred for 4 h at room temperature. Diallyl acrylate solution then turned to brown, and finally to black while polymerization was occurring with prolonged time (Figure 1). It was seen from Figure 2 that the surface of PDA-coated stent lost its original metallic luster and turned to black. The experimental results initially demonstrated that the PDA was successfully coated on the stent.

![Figure 1. The color change of diallyl acrylate solution after stirring for different time: (a) 0 min; (b) 20 min; (c) 40 min; (d) 1 h; (e) 2 h.](image)

![Figure 2. Physical map of the stent. (a) Stent size (left: bare-metal stent; right: PDA-coated stent); (b) The surface color contrast of bare-metal stent (left) and PDA-coated stent (right).](image)

3.2. XPS characterization of PDA coating
As shown in Figure 3, compared to the bare stent, the XPS results of PDA-coated stent showed that the PDA coating generated the characteristic carbon (phenolic) and nitrogen peaks (amine group) (C1s peak at around 285 eV and O1s peak at 532 eV). Meanwhile, XPS spectra in the bare stent group revealed the presence of Cr, Mo elements. However, it should be noted that no metallic Cr or Mo were observed in the XPS spectra after PDA coating, indicating the formation of a polymer coating of 10 nm or more in thickness, which was consistent with the previous report\(^9\). These peaks indicated successful coating of PDA on the stent. We also characterized the stent after labeling iodine using XPS. From its XPS spectrum, it can be seen that in addition to the peak of PDA, a characteristic binding energy peak of iodine appears, suggesting that iodine could be labeled onto the stent through the PDA coating. The nitrogen-to-carbon signal ratio (N/C) is 0.114 and 0.115 for PDA coated stent and iodine labeled stent, respectively, which is similar to that of the theoretical value (N/C = 0.125), implying that the coating is derived from diallyl acrylate polymerization.
Figure 3. XPS full spectrum. (a) XPS spectrum of bare stent; (b) XPS spectrum of stent coated with PDA; (c) XPS spectrum comparison of bare stent (Control) and stent coated with (PDA g; (d) XPS spectrum of the stent after cold labeling with iodine.

3.3. SEM characterization of PDA coating

To evaluate the flexibility of PDA coatings, we bend the PDA-coated stent and observed the bent part by SEM. The results were displayed in Figure 4 and indicated that even undergoing severe bending, there are no breakages and peeling off in the PDA coating, which implied the PDA coating had excellent flexibility. At the same time, the PDA-coated stent has a rougher surface due to the presence of a coating while the bare stent struts have a smoother surface appearance.

Figure 4. The surface morphology of the stent after being immersed in 2 mg/mL diallyl acrylate for (a) 40 min, (b) 1 h, (c) 1.5 h, (d) 2.5 h.

Since multiple coatings of PDA will be conducted to enhance the radiolabeling stability, the stent was coated with PDA for several times (2 mg / mL for 1 h), then characterized by scanning electron microscopy. As shown in Figure 5, even after four times of PDA coating, the stent did not exhibit any shedding or coating breakages after severe bending. We assessed the surface morphology of PDA-coated stent in comparison to an uncoated bare stent via SEM. The results showed that an increase in surface roughness was found after the coating deposition, which implied the PDA coating
had excellent flexibility. Collectively, our results indicated that PDA coating has excellent stability and flexibility, which could adapt to the severe deformation process during biliary stent implantation.

![Control, 1 time, 2 times, 3 times, 4 times](image)

**Figure 5.** Scanning electron micrographs of the stent immersed in 2 mg/mL diallyl acrylate for 1 hour each time, coated with PDA 1-4 times.

### 3.4. Degradation of PDA coating

To examine whether the PDA coating will be degraded, PDA-coated nanoparticles were prepared, then PDA coated nanoparticles were incubated in DMEM + 10% FBS. The degradation of PDA was monitored by transmission electron microscope by measuring the thickness of the coating layer (About 200 nanoparticles). As shown in Figure 6, the thickness of PDA coating after immersing in DMEM+10% FBS for 60 days nearly remained unchanged compared with the initial thickness (about 32 nm). The experimental results demonstrated that there were no PDA degradation reactions occurring, indicating that the PDA coating had good stability.

![Thickness distribution map of PDA coating](image)

**Figure 6.** Thickness distribution map of PDA coating. (a) The initial thickness of PDA; (b) the thickness of PDA after immersing in DMEM+10% FBS for 60 days; (c) Significant difference analysis of PDA; (d) Transmission electron micrograph of PDA-coated nanoparticles (initial); (e) Transmission electron micrograph of PDA-coated nanoparticles after immersing in DMEM+10% FBS for 60 days.

### 3.5. Labeling and characterization of iodine-131

Iodine-131 was labeled by the chloramine T method. The labeling efficiency of iodine-131 and the radiochemical stability in DMEM+10% FBS were evaluated. The iodine labeling efficiency was 50%, and it was increased to 60% when further oxidization of PDA with sodium periodate (NaIO₄), making it further crosslinked.
3.6. PDA coating and oxidation after labeling
To improve the radiochemical stability of iodine-131, the stent was recoated with PDA after labeling, and the coating procedure was the same as above. Results showed that further PDA coating achieved moderate improvement for radiochemical stability of $^{131}\text{I}$, which was about 10% higher than the stents that did not wrap PDA again (Figure 7). The radiochemical stability of iodine-131 was then further improved by using sodium periodate to oxidize the outer PDA, which was more stable than the conventionally physical absorption method [11]. These results indicated that the radiochemical stability of iodine-131 was further improved after oxidation of the outer PDA coating with NaIO$_4$.

![Figure 7](image)

Figure 7. Radiochemical stability of iodine-131. (a) Change of radiochemical stability of $^{131}\text{I}$ after recoated with PDA after the previous labeling; (b) Change of radiochemical stability of $^{131}\text{I}$ after oxidation of the outer PDA coating with NaIO$_4$.

4. Conclusion
In summary, we fabricated a novel $^{131}\text{I}$-labeled radioactive self-expandable metal stents. PDA was self-polymerized under alkaline conditions on the surface of the stent. Iodine-131 was labeled onto the PDA coating by electrophilic attack on the ortho carbon of the catechol group of PDA. In addition, radiolabeling yields and radiochemical stability of $^{131}\text{I}$ were further improved by recoating with PDA and then oxidation with NaIO$_4$. The radioactive stent had excellent radiochemical stability and could achieve long-term tumor-killing purpose. Therefore, we provided a new method for radioactive stent preparation, which would reduce the rate of stent restenosis after implantation and extend the patency time of the stent.

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