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A novel carrier of radionuclide based on surface modified poly-(lactide-*co*-glycolide) nanofibrous membrane

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ABSTRACT

Electrospun nanofibrous membrane is an approved drug carrier. However, the radionuclide carrier used an electrospun membrane is rare. In this study, Poly(lactide-*co*-glycolide)(PLGA) nanofibrous nonwovens were prepared through electrospinning technology, and then surface modification of the nonwoven was performed to stably conjugate the radioisotope with the fibrous membrane. A novel PLGA nanofibrous nonwoven conjugated with radioactive yttrium ^{90}Y for tumor internal radiotherapy was prepared for the first time. Evaluation of the stability of the radioisotope indicated that the leakage of ^{90}Y from the PLGA membranes can be neglected after 24 h incubation in saline. The retention of ^{90}Y on the PLGA membrane was 75% when five half lives of ^{90}Y expired and the vast majority of radioactive decay had occurred. This labeled nanofibrous membrane function as a novel radio-medical appliance with excellent surface hydrophilic and mechanical properties that can be directly implanted into the lesions not only to locally kill the cancerous cells but also to play the anti-adhesion role at where surgical procedures have been made to remove the tumor tissue.

1. Introduction

Cancer has been one of the major causes of death in the world. Great steps are being taken every day in pharmaceutical nanotechnology towards changing the scale and methods of radioisotope delivery for a better future of tumor radiotherapy [1]. To date, the used carriers of radionuclide include chiefly, among others, liposomes [2,3], microparticles [4,5], nanoparticles [6,7], micelles [8], dendrimers and hydrogels [9–12]. However, although there is no doubt the effection in tumor radiotherapy following the introduction of above drug carriers, there are still some disadvantages that can not be neglected. It is well known that liposome and micelle is not enough stable in vivo. Therefore, the leakage of drug is a hidden threat to normal organ. The difficulties in preparation, sterilization or storage also restrict the wide usage in tumor therapy of liposome, hydrogels, dendrimers, micro- and nanoparticles. Moreover, almost all of the above drug carriers have been administered by embolization via a catheter or serial injections with a needle, therefore, they can not be used in the in-situ tumor radiotherapy or chemotherapy.

In a typical case of tumor treatment, the tumors are normally surgically removed, and then an adjuvant radiotherapy or chemotherapy is normally suggested. Therefore, the radiotherapy in-situ would be more desired via implanted directly the drug into lesion after the surgery. Meanwhile, anti-adhesion are the urgent problem as occurs with the surgery. Medical fibrous membrane is one of the most important forms in the biomaterial fields due to its easy operation in surgery. It is well established that the electrospun membranes have many attractive advantageous features including higher specific surface area, higher porosity, and much lower density [13–17]. This makes it an ideal implant carrier as radiopharmaceutical with minimum side-effect. Moreover, Chu et al. [18,19] presented nano-structured electrospun poly(lactic acid-*co*-glycolic acid) (PLGA) membranes for prevention of postsurgery-induced abdominal adhesions. Therefore, it would be desirable to use the electrospun membrane binding radionuclide for the in-situ adjuvant radiotherapy. The PLGA membranes are easy to handle on the lesion after tumor surgery operation. The electrospun membranes are expected to perform the radiotherapy and the anti-adhesion functions. However, in the open literatures, there is

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a dearth of work that deals with the conjugation of radioisotope to the electrospun nanofibrous membrane for the potential application in tumor radiotherapy.

PLGA has been well documented for its excellent biodegradability, biocompatibility and non-toxicity. Therefore, PLGA has gained the approval of US Food and Drug Administration [20]. In this work, we prepared tumor-targeting nano-scale carrier from electrospun PLGA membranes through surface modification. This carrier, with a stable structure, would not only convey the radioactive nuclide quantitatively to the tumor tissue by directly embedded at the lesions, but play an anti-adhesion function at where surgical procedures have been made.

2. Experimental

2.1. Materials

Medical-grade poly(D,L-lactide-*co*-glycolide) (PLGA, M_w 100,000 g/mol, molar ration of lactide-to-glycolide = 75:25) was purchased from Chengdu Organic Chem Co. Ltd (China). Gelatin (GE, Approx. 220 Bloom, viscosity 4.8 mPas) was purchased from Sanhesheng Gelatin Co. (Wenzhou, China), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was synthesized as described in reference [21]. Diethylenetriaminepentaacetic acid dianhydride (DTPA, Flulka) and $^{90}YCl_3$ solution (radioactivity is 20.35 TBq/L, Perkin Elmer) were used directly. All the using water was deionized (R = 18.2 MΩ) by a Millipore water purification system.

2.2. Preparation of PLGA membranes

PLGA nanofibers were prepared by electrospinning a 10 wt% PLGA solution in the blend solvents of *N*,*N*-dimethyl formamide (DMF) and acetone (the volume ratio is 1:1). PLGA nanofibers were collected on a target drum that was placed at a distance of 8 cm from the syringe tip (inner diameter 84 μm). A voltage of 20 kV was applied to the syringe tip by a high voltage power supply, and the flow rate of the solution was 20 μL min^{-1}. The nanofibers were dried in a vacuum for 24 h at room temperature to remove the remaining solvent.

2.3. Surface modification of PLGA membranes

Firstly, the 1.5 cm × 1.5 cm PLGA electrospun membranes were immersed in 0.075 M sodium hydroxide (NaOH) solution for 30 min at 0 °C, then the hydrolyzed membranes were washed with plenty of distilled water at 0 °C. Secondly, the hydrolyzed PLGA membranes were immersed in 5 mL of DMTMM aqueous solutions (100 mM) at 4 °C overnight to activate carboxyl (−COOH) groups on the surface of PLGA membranes, then the membranes were rinsed thoroughly with distilled water, and then incubated in 5 mL of GE aqueous solutions at a concentration of 2 g L^{-1} for 12 h at 4 °C. The PLGA-g-GE membranes were washed with plenty of distilled water to remove physically absorbed gelatin. Thirdly, DTPA was anchored onto the amine-functionalized PLGA membrane surface. In a 25 mL dry flask, 20 mg of DTPA was added to 10 mL of 0.05 M bicarbonate solution. PLGA-g-gelatin membranes were further incubated in the above DTPA solutions at room temperature for 1 h. After rinsed with distilled water and then dried in a vacuum for 24 h at room temperature to remove the remaining solvent, PLGA-g-GE-DTPA nanofibrous membranes were obtained finally.

2.4. ^{90}Y labeled PLGA membrane

PLGA-g-GE-DTPA nanofibrous membranes were prepared with the above mentioned method. The PLGA-g-gelatin-DTPA membranes (2 cm × 1.5 cm) were immersed in 100 μL of saline solution, then 20 μL of $^{90}YCl_3$ buffer solution (pH = 5.4, 1.221 mC_i) was added into the saline solution. The labeling reaction was carried out at 30 °C for 1 h, and then the radiolabeled PLGA membranes were washed three times with saline through ultrasonic surge, and then incubated in 1000 μL saline. The radioactivity of the radiolabeled PLGA membranes was measured to evaluate the labeling yield (a ratio value of the radioactivity of labeled PLGA membrane to the original reactivity of ^{90}Y buffer solution used for the labeling reaction).

2.5. Stability evaluation of radionuclide on the PLGA membrane

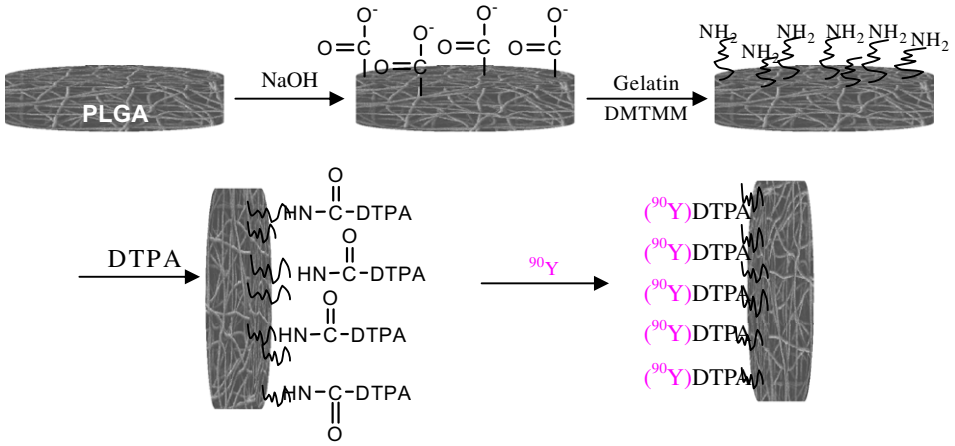
The ^{90}Y labeled PLGA membranes were incubated in 1000 μL saline at room temperature, 20 μL upper liquid of the incubated solution containing ^{90}Y labeled PLGA membranes was diverted into a new tube at regular intervals, and a same volume of saline was subsequently supplemented into the incubated solution. The activity counts in all the tubes and the final PLGA nanofibrous membranes were simultaneously measured to evaluate the stability of the ^{90}Y on the PLGA membranes The retention of ^{90}Y in the PLGA membrane, a ratio value of the remainder amount of ^{90}Y in the membrane to the overall amount of ^{90}Y including in the all of incubated solutions and the PLGA membranes was used to describe the stability of ^{90}Y. The morphologies of the incubated ^{90}Y labeled PLGA membrane were also characterized after incubation in saline at room temperature for 63 d.

2.6. Characterization

The morphologies of the membranes were observed using scanning electron microscope (SEM, JEOL JSM-6700F, Japan) at an accelerating voltage of 5 kV. Each sample was sputter-coated with platinum for analysis. The surface chemical composition of the PLGA nanofibrous membranes was investigated by high resolution X-ray photoelectron spectroscopy (XPS). XPS data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300 W AlKα radiation. The base pressure was about 3×10^{-9} mbar. The angle of analyzer was directed at 90° to the sample's surface. All binding energies were scaled to the C_{1s} photoelectron peak at 284.6 eV. Radioactivity was measured by radiometer (CRC-l5R, Capintec Co., US), and activity counts were obtained by γ auto-counts instrument (1470-002, Perkin Elmer, US). The reported contact angle value represents an average of at least five independent determinations at different sites. Both original PLGA nanofibers and modified PLGA nanofibers were evaluated for a stress–strain response using a tensile testing machine (Series IX Automated Materials Testing System, Instron Co., US). Nanofibrous membranes were cut in a dumb-bell shape with a cross section of 20 mm prior to the tensile test. The load was fixed at 100 N with a stretch velocity of 10 mm/min. The tensile modulus was calculated from the stress-versus-strain curve.

3. Results and discussion

PLGA is one of synthetic polyesters with good biocompatibility, biodegradability and proper degradation rate which is often comparable with the healing time of the damaged human tissues [22,23]. However, due to the inert chain of PLGA without active groups for the conjugation of isotope, surface modification of PLGA become necessary and important for the precise control of the radiodosage of the isotope on the membranes. As shown in Scheme 1, the radiolabeling procedure consists of four steps. Firstly, the electrospun PLGA nanofibrous membranes were hydrolyzed by immersion in NaOH aqueous solutions. Secondly,



Scheme 1. The procedures of ^{90}Y labeled PLGA electrospun membrane.

the hydrolyzed PLGA membranes containing newly formed carboxylic groups were reacted with gelatin (GE) to introduce multi amino functional groups. Thirdly, bifunctional conjugant DTPA was bonded to the surface of the fibers. Finally, the radionuclide was firmly conjugated to the membranes by conjugation reaction with DTPA.

Fig. 1a shows the morphology of the electrospun PLGA fibrous membrane. The morphology of PLGA membrane after hydrolysis reaction (Fig. 1b), GE grafting reaction (Fig. 1c) and Y conjugation reaction (Fig. 1d) retained persistence without any significant damage.

After the hydrolysis reaction, functional groups, such as carboxyl groups (PLGA-COOH) and hydroxyl groups could be formed on the PLGA fibers. Then GE with multi-amine groups was grafted to the PLGA membrane (PLGA-g-GE) after PLGA-COOH was activated with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), which has been shown to be a suitable dehydrolysis reagent for forming peptide bonds by reacting amino groups with carboxyl groups even in solid phase synthesis [21]. XPS measurements were used to characterize the elemental changes on the PLGA membranes. It was found that only carbon and oxygen signals, corresponding to C_{1s} (binding energy, 284.6 eV) and O_{1s} (binding energy. 532 eV), were present in the spectrum of pristine PLGA membrane (Fig. 2). However, a new peak corresponding to N_{1s} (binding energy, 400 eV) appeared on the spectrum of PLGA-g-GE membrane. The average density of grafted primary amino groups in the PLGA membranes was 1.14×10^{-4} mol cm^{-3} according to the calculation method from reference [24]. Bifunctional conjugants that involve metal ion binding sites were covalently bonded to the substrate materials. The covalent attachment of DTPA to the PLGA-g-GE membrane was carried out by formation of amide linkages between the primary amino group of PLGA-g-GE and one of five carboxyl groups of DTPA. Here, the amount of

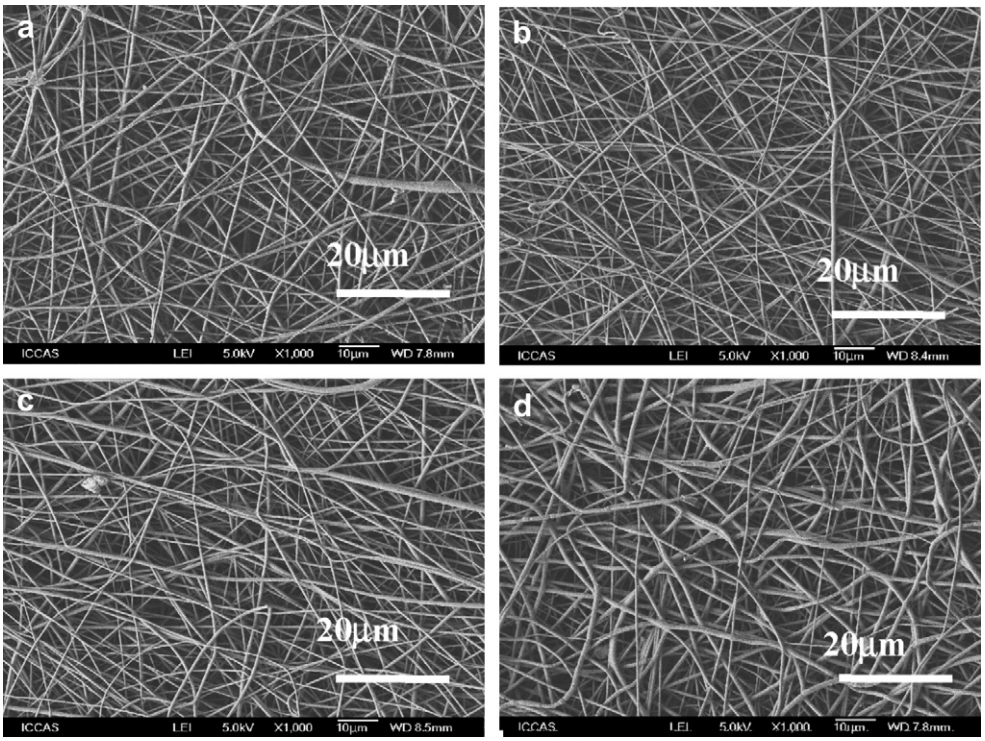


Fig. 1. SEM images of PLGA membranes: (a) Pristine PLGA electrospun membrane; (b) Hydrolyzed PLGA electrospun membrane; (c) PLGA-g-GE electrospun membrane; and (d) Y conjugated PLGA electrospun membrane.

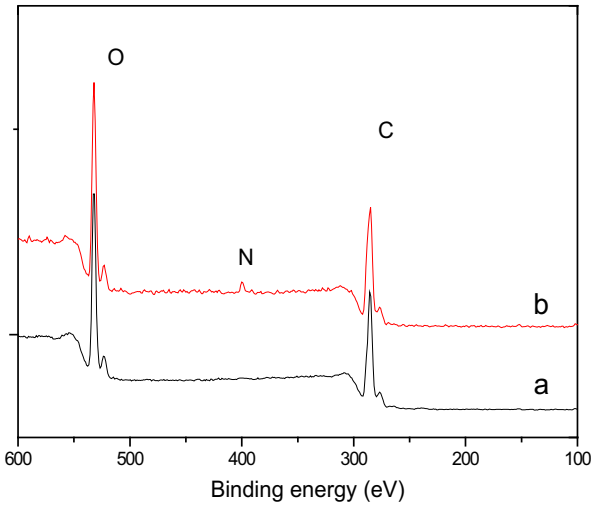


Fig. 2. XPS spectra of PLGA membranes:(a) Pristine PLGA and (b) PLGA-g-GE membrane.

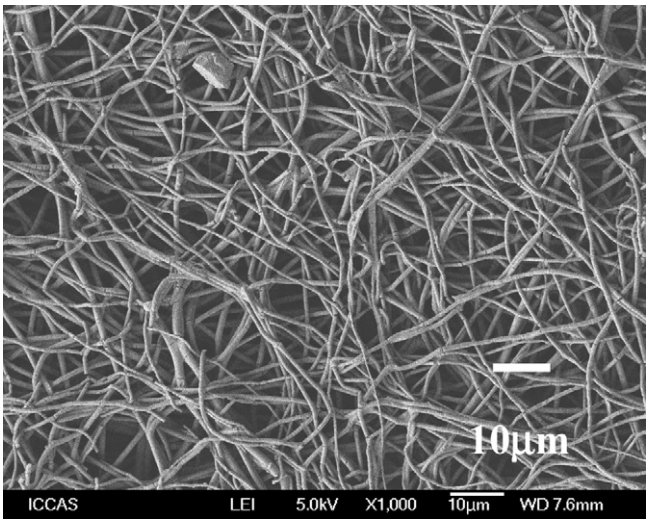


Fig. 4. SEM images of ^{90}Y labeled PLGA membrane after incubation in saline at room temperature for 63 d.

functional group of DTPA and the conjugated metal in the PLGA membranes can be adjusted by controlling the reaction condition including the amount of grafted GE amount and the provided content of radionuclide.

For internal brachytherapy, the emitted radiation should be of high energy and short range, which could ensure the energy emitted will be deposited into the target immediately around the implant material and not into other normal tissues. Of particular suitability of use in this form of treatment is the isotope of yttrium (^{90}Y). ^{90}Y decays with a half-life of 64 h, while emitting a high energy pure beta radiation with the emitting range of 4–11 mm in water. Here, 20 μL of $^{90}YCl_3$ buffer solution (pH = 5.4) containing specific activity of 1.221 mCi was used to react with PLGA-g-GE-DTPA membranes with the area of 3 cm^2. After rinsed with saline following the labeling reaction, the radioactivity of the PLGA membrane is 1.038 mCi. The results showed that the labeling yield of ^{90}Y reached 85%, which is an attracting and pretty high percentage in radio-chemistry. Therefore, it provides large space to adjust the right amount of radioisotope labeled on the membranes to answer the need of the tumor by adjusting the reaction condition, such as the hydrolysis time, the grafting amount of amine and the input of the radionuclide. The stability of the ^{90}Y on the PLGA nanofibers was evaluated in this work (Fig. 3). The activity counts of the upper incubate solution were too low to be measured after 24 h incubation at room temperature, indicating that the leakage of ^{90}Y from the labeled PLGA membrane can be neglected. The retention of ^{90}Y on the PLGA membrane was 75% when five half lives of ^{90}Y expired and the vast majority of radioactive decay had occurred [25]. These results indicated that the stability of radionuclide ^{90}Y on the PLGA membrane is satisfactory and the ^{90}Y labeled PLGA membranes has the potential application in tumor brachytherapy as radioactive materials.

As radioactive materials, the structures should also have sufficient persistence period, thus assuring that the radionuclides remain localized at the target before the radioactivity can be neglected for the normal tissue [26]. The predefined persistence period of the bioabsorbable structure should usually be substantially longer than the half-life of the radionuclides, usually being at least two times longer, preferably being four times longer. In general, the ^{90}Y carrier should possess a longer degradation or elimination time, and normally greater than 320 h (near five half lives) [25]. In this study, the morphology of ^{90}Y labeled PLGA membrane was characterized after 63 days. Fig. 4 demonstrated that although a few fibers showed some cracks, the structure of the membrane was kept very well even when more than twenty half lives past.

PLGA is a hydrophobic biopolymer and this nature often leads to some handicaps as implant device. For in vivo applications, the biomaterials are expected to be hydrophilic for a better

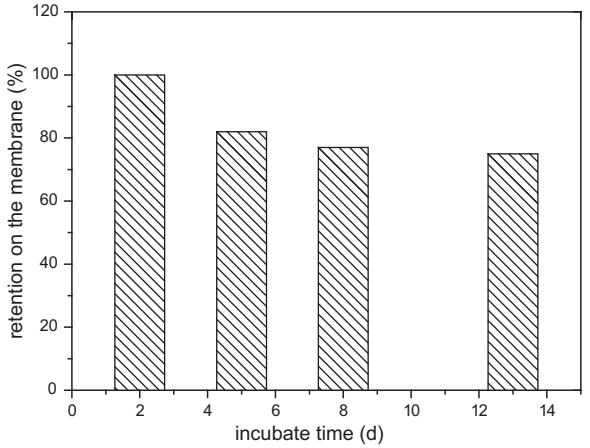


Fig. 3. Evaluation of stability of radionuclide on PLGA electrospun membranes.

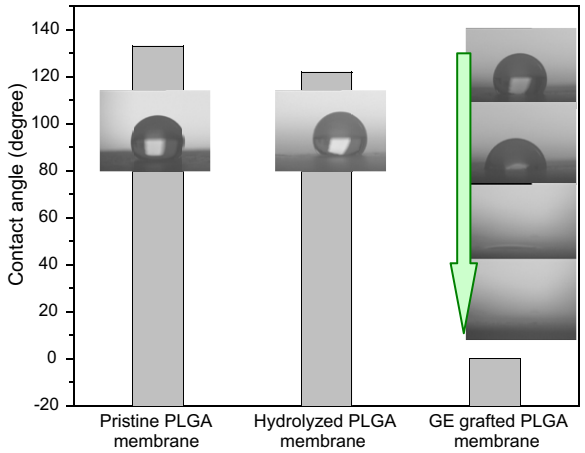


Fig. 5. Evaluation of the wettability of PLGA based membranes.

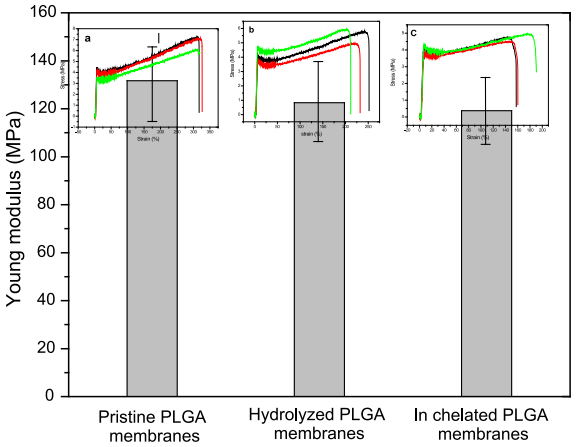


Fig. 6. Mechanical tensile moduli of the origin and modified PLGA nanofibrous membranes.

biocompatibility [27]. In this work, after the chemical modification, the wettability of the PLGA membranes changed accordingly (Fig. 5). The contact angle images of PLGA-g-GE membranes were measured after 20 s interval. It could be seen that the hydrophilicity of the PLGA membranes had no significant changes after alkali treatment, which also demonstrated that only a few available reaction sites could be used for the following reaction. However, the static contact angle of the PLGA-g-GE membranes decreased nearly to 0 from the original 122°. GE is a hydrophilic macromolecule, which contains many polar groups. Therefore, grafting GE to the PLGA surface could improve the wettability of the PLGA membrane.

Mechanical strength is an important factor to be considered for applications as implant devices. In most cases, it is preferable to have the material's mechanical strength close to that of the target tissue to offer sufficient mechanical support during the tissue-rebuilding processes. Young's moduli of the pristine PLGA nanofibrous membrane, hydrolyzed PLGA membrane and Y attached PLGA membrane is 117.2–140.3 MPa, 105.6–125.7 MPa and 105.1–129.0 MPa, respectively (Fig. 6). It is reported that strength of the cartilage is characterized by a Young's modulus of about 130 MPa [28]. Therefore, the mechanical properties of the modified PLGA nanofibrous membranes could satisfy the mechanical requirements as the implant devices.

4. Conclusion

In summary, we fabricated the PLGA ultrafine fibrous membranes by electrospinning. Then the radionuclide was stably bound to the membranes by surface modification. The labeled biodegradable electrospun membrane has good stability in saline, hydrophilic surface and excellent mechanical properties. This carrier size can be tailored to match the tumor size and the amount of radioisotope can be bound quantitatively on the membranes according to the need of the tumor. This offers an attractive method of radiolabelling the electrospun nanofibrous membranes for local brachytherapy. The radiolabeled PLGA electrospun membrane may be used to not only kill the cancerous cells, but can be used as an anti-adhesion membrane.

Acknowledgments

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