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Preparation, quality control and biodistribution studies of ^{165}Dy-chitosan for radiosynovectomy

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Abstract. The preparation of ^{165}Dy-labeled chitosan for radiosynovectomy applications is described in this paper. ^{165}Dy ($T_{1/2}$ = 2.33 h) was prepared by irradiation of natural $Dy(NO_3)_3$ at a flux of 3–4 × 10^{13} neutrons/cm^2·s for about 6 h. The irradiation resulted in the production of 11.1 GBq (300 mCi) of ^{165}Dy activity. Emitting gamma ray (94.7 keV) and beta particles (E_{max} = 1.3 MeV, 83%) ^{165}Dy decays to ^{165}Ho. Eight hours after bombardment, the corresponding specific activity was 703 MBq/mg (19 mCi/mg). The irradiated target was dissolved in 0.1 N HCl solution. Radionuclidic purity was ascertained by high resolution gamma spectrometry. Chitosan solution was prepared in acetic acid solution (pH 3). The chitosan solution was labeled with ^{165}Dy to prepare ^{165}Dy-chitosan (^{165}Dy-Chit) complex (labeling yield, > 99% and specific activity ~ 3.7 TBq/mmol). In optimized conditions (pH 3, 35 mg/4 ml chitosan acidic solution, and 370 MBq of ^{165}Dy) Chit was stable after 48 h. Bioevaluation of the prepared ^{165}Dy-Chit was carried out by injecting 37 MBq (1 mCi, 50–100 μl) directly into the knee joints of wild rats. Free ^{165}Dy cation was also injected to study the effect of complex formation on the retention of radionuclide in the administered site. To study the consequence of radioactivity leakage from the administration site, a dilute sample of the complex was injected intravenously into the rats followed by biodistribution studies. It was observed that there was no significant extra-articular leakage of the injected activity over the study period of 24 h post-injection.

Key words: radiosynovectomy • Dy-165 • chitosan • biodistribution

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Introduction

In the treatment of rheumatoid arthritis, a surgical, chemical, or radiation synovectomy (RSV) may be applied. Surgical synovectomy presents the risks of surgery and anesthesia, the need for hospitalization, and a prolonged period of rehabilitation, albeit to a minor degree [4]. In chemical synovectomy, highly toxic agents like osmic acid, alkylated substances like nitrogen mustards, methotrexate, and cobra venom were used initially, but subsequently abandoned because of possible joint tissue damage [14, 19].

RSV is a plausible replacement for surgical and chemical synovectomy, and it is a method capable of easily removing the inflamed region of synovium by beta radiation through the direct injection of radioactive materials. This method has the merits of a simple operation and no post-operative complications.

In the treatment of arthritis by radiation from radioactive materials in the lesions, the material administered to the lesion should be retained only in the lesion, with no leakage.

Since the introduction of RSV in 1952, a large number of radionuclides have been studied and their usefulness and clinical efficacy were analyzed in several clinical trials. The first β-emitting radionuclide for RSV

was colloidal gold, Au-198. Though it was clinically effective, it did not gain widespread use because of an unacceptable whole-body radiation load due to its additional gamma-emission and a high extra-articular leakage into regional lymph nodes [18]. Thus, as early as 1963, yttrium-90 was recommended instead and is still in use for RSV of the knee joint. It is a pure β-emitter with a physical half-life of 2.7 days and a high energy of 2.26 MeV, leading to a mean penetration depth of its particles of 3.6 mm [1].

Due to its maximum tissue penetration of approx. 11 mm, yttrium-90, cannot be used to treat joints smaller than the knee because this could lead to damage to articular cartilage or overlying skin [10]. In radiosynovectomy with Y-90, some very small particles seem to leave the joint and lead to this excessive leakage. Moreover, colloidal solutions of yttrium-90, pH < 6 may contain free Y-90 ions that can easily drain from the treated joint [9]. Besides, the problem of whole-body radiation, leakage of the radionuclides out of the joint leads to a significant reduction of the radiation dose imparted to the synovial surface – which may fall to 60% – thereby reducing the probability of clinical effectiveness [3].

Due to these disadvantages, dysprosium-165 was recommended as an alternative radionuclide for knee joint RSV. It has been used for labeling of ferric hydroxide macroaggregates (FHMA) with a particle size of 1–5 μm [15]. Preclinical studies demonstrated its efficacy in reducing inflammation in an animal model of antigen-induced arthritis and produced a leakage of only 0.1% of the injected activity to the liver and 0.001% to the regional lymph nodes [9]. A clinical study on radiosynoviorthesis with Dy-165 in 108 knee joints of 93 patients with seropositive rheumatoid arthritis revealed good results in 61% of these patients after one year, which is comparable to the results reported for Y-90 [16]. For treatment of joints smaller than the knee, other radionuclides are needed to avoid articular or skin damage.

One of the most important factors affecting the quality of radiopharmaceuticals in RSV is the leakage of radioactive material. If the administrated radioactive materials leak out of the lesion, the radioactive material will spread throughout the body through the blood flow, and will accumulate in other tissues – especially bone marrows, which can be fatal. Therefore, a suitable carrier should be adviced.

A carrier will be ideal if it carrier has a high affinity with radionuclides *in vivo* and *in vitro,* can be evenly distributed in the lesion, and can be absorbed without inflammation in the lesion. Its biological half-life time should be longer than that of radionuclide, and it should decompose and be excreted after decay of the radionuclide [5].

One of the most famous carriers that have been used for radiosynovectomy are FHMA [2, 8, 11]. However, the procedures for preparation are complicated and iron can accumulate in the body and also its presence in the synovial membrane leads to more inflammation.

Another formulation is ^{165}Dy-HMA, (dysprosium hydroxide macroaggregates), prepared by irradiation of natural Dy-HMA in a nuclear reactor that must be controlled precisely to prevent the formation of undesired products of neutron activation [7].

Radioactive chitosan complexes are new internal radiation therapeutic agents. They exist in solution state at acidic pH and in gel state at pH of the human body. Therefore, they do not leak from the lesion. In addition, a radioactive chitosan complex is a natural biocompatible and biodegradable product that can be excreted after decay [7].

There are several reports of successful intra-articular injection of a ^{166}Ho-chitosan complex for the treatment of rheumatoid arthritis of the knee [6, 12, 13, 17]. Song *et al*. [17] have reported magnetic resonance (MR) evaluation of RSV of the knee by means of intra-articular injection of holmium-166-chitosan complex. Song *et al*. have performed a phase I/IIa study on intra-articular injection of a holmium-166-chitosan complex for the treatment of knee synovitis of rheumatoid arthritis. Shin *et al*. also reported the preparation of ^{153}Sm-chitosan for RSV [13].

In this study, development of ^{165}Dy-chitosan is reported. ^{165}Dy is produced by irradiation of a natural target.

Experimental

Materials

Natural Dy $(NO_3)_3 \cdot 5\ H_2O$ (99.9% pure) used as the target for the production of ^{165}Dy was obtained from Alfa Aeasar, Canada. Chitosan (low molecular weight) was purchased from Merck Chemicals, USA. All other chemicals were of analytical reagent (AR) grade and supplied by reputed chemical manufacturers. Radionuclidic purity of ^{165}Dy produced was determined by gamma spectroscopy with an high purity germanium (HPGe) detector, on the basis of the 94.7 keV peak and also beta spectroscopy which were carried out using a Wallac 1220 Quantulus liquid scintillation spectrometer. Radio-chromatography was performed by counting of Whatman no. 1 and no. 2 using a thin-layer chromatography scanner (Bioscan AR2000, Paris, France). Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed.

Production of ^{165}Dy

^{165}Dy was produced by thermal neutron bombardment of natural $Dy(NO_3)_3$ at the Tehran Research Reactor (TRR) for a period of 6 h at a flux of 3–4 × 10^{13} neutrons/cm^2. In a typical procedure 1 mg of Dy was dissolved in HNO_3 and evaporated in a quartz ampule. Then, it was sealed and irradiated in the reactor after placing it inside an aluminum can. The irradiated powder was dissolved in HCl (0.1 M) and this radiochemical form was used for the subsequent studies. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as gamma spectroscopy for the detection of various interfering beta- and gamma-emitting radionuclides.

Preparation of ^{165}Dy-Chit

A stock solution of chitosan was prepared by dissolving it (35 mg, low molecular weight) in 1% acetic acid aque-

ous solution at pH 3 (4 ml). Then, 2 ml of the prepared solution was used for complexation of ^{165}Dy (10 mCi, 370 MBq). The pH of the reaction mixture was adjusted to pH 3 and stirred at room temperature for 10 min to facilitate complexation.

Biodistribution studies of free dysprosium-165 radionuclide and dysprosium-165-labeled chitosan compound

The ^{165}Dy-Chit solution was injected intra-articularly into one knee joint of normal male rats, each weighing 180–210 g (n = 3). A $^{165}DyCl_3$ solution, which contained no chitosan, was injected into the knee joint of the other rats for the control study (n = 3).

In the case of ^{165}Dy-Chit, a volume of 100 µl containing ~ 35 MBq (~ 1 mCi) of radioactivity was injected to each rat knee joint. The intravenous injection of dilute ^{165}Dy-Chit (200 µl, 1 mCi) was also performed to study the consequence of radioactivity leakage from the administered site. The animals were sacrificed at the exact intervals of 2 and 4 h post-injection (see Fig. 4). The tissue and the organs were excised and the activity associated with each organ/tissue was measured in a gamma-spectroscopy system HPGe detector. The uptake by different organs/tissue was calculated from these data and expressed as percentage injected dose per gram (%ID/g).

Results and discussion

Production and quality control of ^{165}Dy

Irradiation of natural dysprosium nitrate was performed at a thermal neutron flux of 3–4 × 10^{13} neutrons/cm^2·s for 6 h at TRR and the radionuclide was prepared according to regular methods with a range of specific activity 18–19 mCi/mg for radiolabeling use. Gamma-ray spectrum of the appropriately diluted $^{165}DyCl_3$ solution showed a major peak at 94.7 keV, which is the photo-peak of ^{165}Dy (see Fig. 1). The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated to obtain the desired pH and volume followed by sterile filtering. The absence of any other photo-peaks in the gamma-ray spectrum indicated that the ^{165}Dy was produced with a radionuclidic purity of > 99%.

Preparation and quality control of ^{165}Dy-Chit complex

The preparation of ^{165}Dy-Chit was performed with regard to the optimized situation reported by Shin *et al.* [13] in which complexation gradually increased with increasing ligand concentration and reaching almost 100% at a value of 35 mg of chitosan in 4 ml of acetic acid (pH 3).

The ^{165}Dy-Chit complex was prepared using 2 ml of the chitosan solution (35 mg in 4 ml acetic acid, pH 3 and 370 MBq of Dy-165 activity (specific activity of complex: 3.7 TBq/mmol). For measuring radiochemical purity and radiolabeling yield, a 1 µL sample of the ^{165}Dy-chitosan complex was spotted on a chromatography paper (Whatman no. 1), and developed in a mixture of methanol/water/acetic acid (4:4:2) as the mobile phase. The R_f values of free Dy-165 and ^{165}Dy-chitosan complex were 0.9 and 0.65, respectively (see Fig. 2).

Biodistribution studies

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (2, 4 and 6 h). Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lungs, skin, and knee samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo-peak per gram of tissue (%ID/g).

Biodistribution studies after intra-articular administration of Dy^{3+} cation in rats

The distribution of injected dose in rat organs up to 6 h after intra-articular injection of ^{165}Dy-chloride

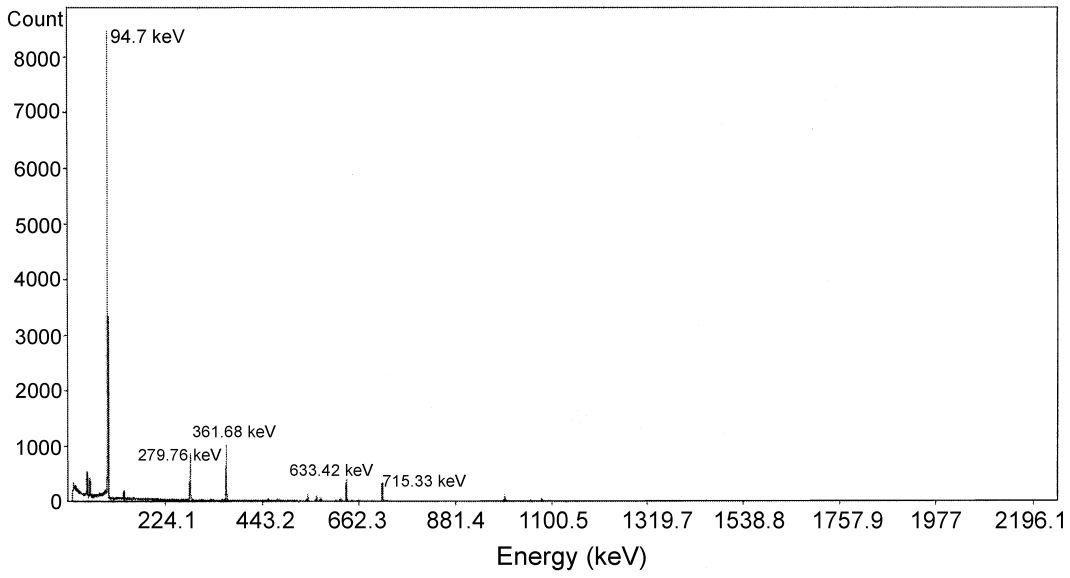

Fig. 1. Gamma spectrum of dysprosium-165.

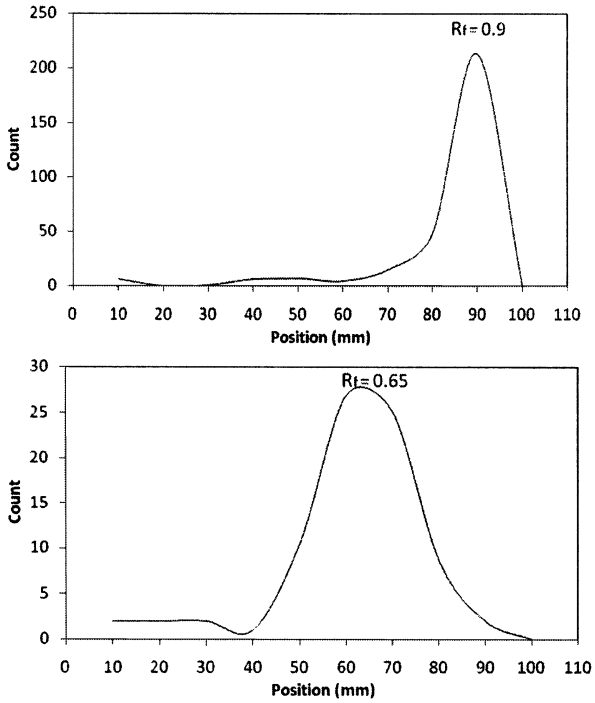

Fig. 2. ITLC chromatograms of ^{165}Dy-$DyCl_3$ (above) and ^{165}Dy-chitosan solution (below) on Whatman no. 1 paper using a methanol:water:acetic acid (4:4:2) mixture.

(1 mCi/100 µl) solution was determined for control studies (see Fig. 3). Based on these results, it was concluded that most of the injected activity was extracted from the circulation and distributed in rat organs.

During the first two hours the injected activity is retained in the site and no significant uptake is observed for other organs. But, as can be seen in Fig. 4, four hours after post-injection, non-negligible uptakes were observed for some organs such as the liver, femur, and kidney. Also, more than 80% of the injected activity leaked out of the site after 6 h.

Biodistribution studies after IV-injection of dilute ^{165}Dy-chitosan

To study the effects of probable leakage of the prepared complex and knowing organs that show notable uptakes of that, dilute ^{165}Dy-Chit (~ 35 MBq, 200 µl) was also injected intravenously to the rats and biodistribution studies were performed.

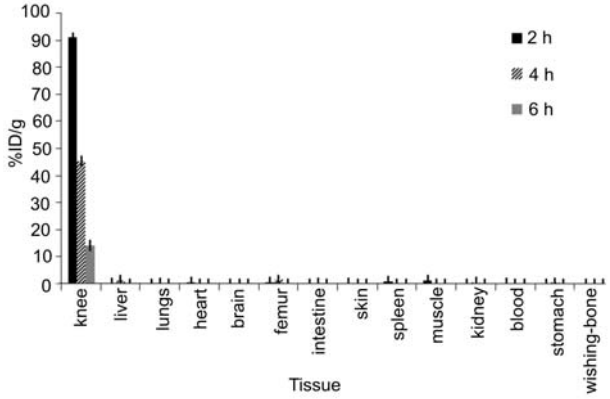

Fig. 3. %ID/g of Dy^{3+} cation after 2, 4 and 6 h of post-injection to the rat knee joint. Each bar presents mean ± SD (n = 3).

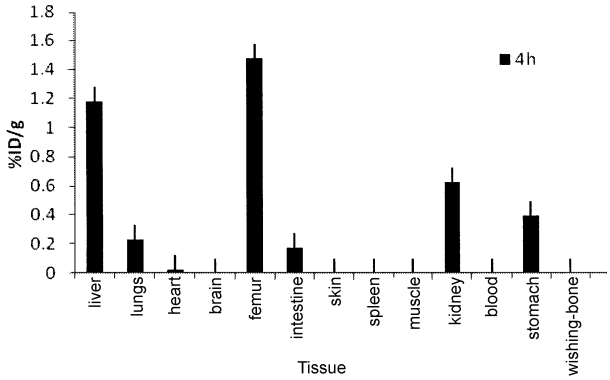

Fig. 4. %ID/g of Dy^{3+} cation after passing almost two half-lives of the radionuclide excluding the knee (administered site). Each bar presents mean ± SD (n = 3).

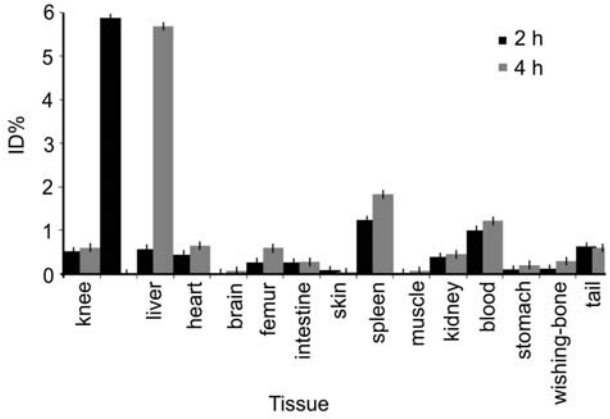

Fig. 5. %ID/g of dilute intravenously injected ^{165}Dy-Chit up to almost two half-lives of the dysprosium radionuclide. Each bar presents mean ± SD (n = 3).

As can be seen from the data in the chart (see Fig. 5), the biodistribution was mainly in the liver, lungs, and spleen. Spleen and liver are two important reticuloendothelial (RE) systems and due to the high molecular weight of the complex, these two organs show relatively high uptakes of the radio-labeled complex.

Biodistribution studies after intra-articular injection of ^{165}Dy-chitosan

The distribution of the injected dose in the rat organs at various time intervals after intra-articular injection of 1 mCi/50 µl of ^{165}Dy-chitosan complex as a percentage of injected dose is presented in Fig. 6. As mentioned above, in the case of any leak from the joint, the complex would accumulate in RE system, unless the complex dissociated at serum pH and Dy^{3+} cation would be formed.

A very small amount of activity was observed in the spleen and liver, two important RE organs, indicating that no major complex leak occurred. Very negligible liver and kidney uptakes were observed, possibly caused by the Dy-165 cation release from the injected joint and not the radio-labeled complex uptake. Figure 7 demonstrates the biodistribution of the compound among the tissues, excluding the injected knee data, to better understand the biodistribution of the leaks from the knee.

The distribution of the radioactivity among tissues, after removing knee joint accumulation data, demon-

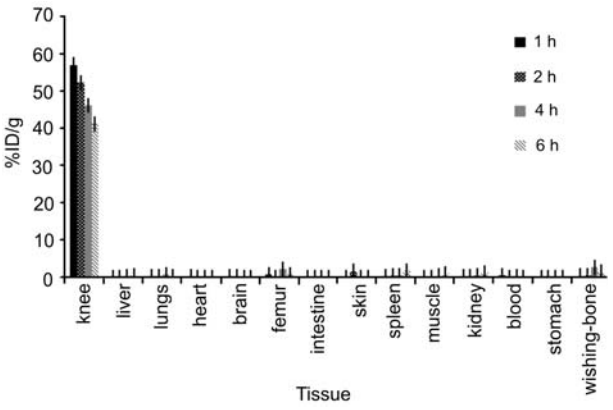

Fig. 6. Distribution of ^{165}Dy-chitosan in wild-type male rats – 1, 2, 4 and 6 h after intra-articular injection of 1 mCi of compound. %ID-percentage of injected dose. Each bar presents mean ± SD (n = 3).

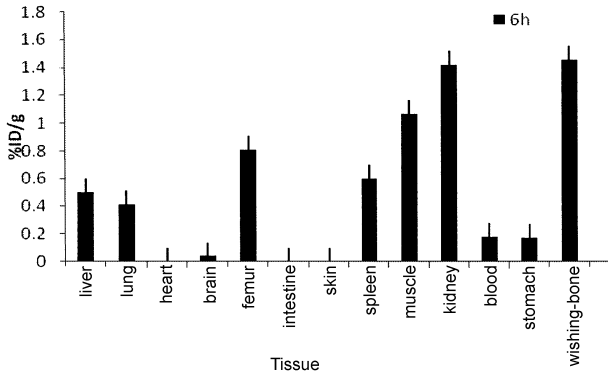

Fig. 7. Distribution of ^{165}Dy-chitosan in wild-type male rats excluding injected knee data 6 h after intra-articular injection of 1 mCi of the compound. %ID-percentage of injected dose.

strates a typical Dy^{3+} cation biodistribution among the tissues. It is believed that the free Dy cation is the only radiochemical species escaping from the knee joint and no ^{165}Dy-Chit complex was found in circulation.

A comparison of activity retentions between the free Dy-165 and ^{165}Dy-Chit is shown in Fig. 8. It is clearly observed that the presence of chitosan works as an efficient carrier for keeping Dy-165 in the administered site. The value of %ID/g is almost invariable for ^{165}Dy--Chit, but a relative 80% drop is observed in the case of free ^{165}Dy, proving the efficiency of chitosan for this purpose.

The preparation of radio-labeled chitosan compounds is feasible and, due to its interesting biological properties, the human studies have been started

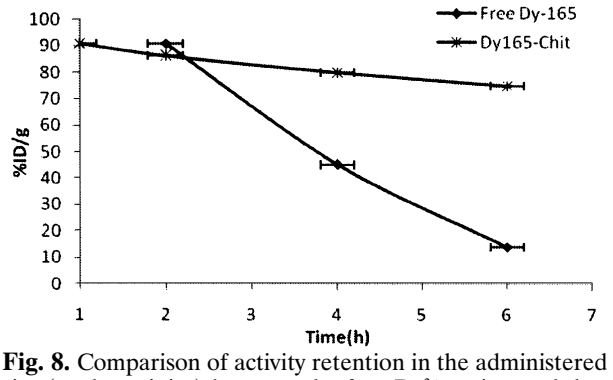

Fig. 8. Comparison of activity retention in the administered site (rat knee joint) between the free Dy^{3+} cation and dysprosium complex.

across hepatocellular carcinomas and radiosynovectomy. The side effects usually imposed by iron macroaggregates are mostly a problem in clinics such as iron toxicity and painful organs, while in this case are rare.

Conclusion

The short half-life of ^{165}Dy (2.3 h) suggests minimal exposure of non-target organs by reduction of leakage. Numerous reports have shown the efficacy and safety of ^{165}Dy for the treatment of chronic synovitis. It is also important to prepare the radiopharmaceutical in a form that, besides having good retention in knee joint, is biodegradable and biocompatible. To guarantee these needs, we decided to use chitosan as ^{165}Dy carrier for radiosynovectomy.

Under optimized conditions, total labeling and formulation of ^{165}Dy-Chit took less than 1 h with a yield of > 99% and specific activity of 3.7 TBq/mmol. The biodistribution of the tracer after intra-articular injection, was checked in wild-type rats up to three half-lives of the radionuclide. A comparison of activity retentions between free Dy-165 and ^{165}Dy-Chit showed that the value of %ID/g is almost invariable for ^{165}Dy-Chit, but a relative 80% drop is observed in the case of free ^{165}Dy, proving the efficiency of chitosan for this purpose.

These studies indicated that ^{165}Dy-Chit shows promising features and warrants further investigation for development as a cost-effective agent for radiosynovectomy. It is ready for therapeutic applications in the country.

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