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Histologic study of effects of radiation synovectomy with Rhenium-188 microsphere

Shyh-Jen Wang[a,*], Wan-Yu Lin[a], Min-Nan Chen[b], Jung-Ta Chen[c], William-L Ho[c], Bor-Tsung Hsieh[b], Henton Huang[b], Lie-Hang Shen[b], Gann Ting[b], Furn F. Knapp Jr[d]

[a]*Department of Nuclear Medicine, Veterans General Hospital, Taichung and National Yang-Ming University, 160 Taichung Harbor Road, Section 3, Taichung 40705, Taiwan*
[b]*Institute of Nuclear Energy Research, Taiwan*
[c]*Department of Pathology, Veterans General Hospital, Taichung, Taiwan*
[d]*Oak Ridge National Laboratory, Oak Ridge, TN, USA*

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Abstract

Rhenium-188 microsphere is a relatively new radiation synovectomy agent developed for the treatment of rheumatoid arthritis. It has been shown that the levels of unwanted extra-articular radiation are negligible with this agent. A histologic study was conducted to assess the effect of radiation synovectomy on synovium and articular cartilage after intra-articular injection of various doses of Re-188 microspheres into the knee joints of rabbits. Intra-articular injection of Re-188 microspheres into rabbit knee joints resulted in mild reactive inflammation and thrombotic occlusion of vessels which subsided rapidly. Sclerosis of subsynovium could be seen 12 weeks after injection. No evidence of damage to articular cartilage was noted. There was no significant difference in the articular pattern after injection of 0.3 or 0.6 mCi Re-188 microspheres. This study suggests that a treatment dose of Re-188 microspheres causes transient inflammation of synovium without any detectable damage to the articular cartilage of knee joint.

Keywords: Radiation synovectomy; Rhenium-188 microsphere; Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis causes chronic synovial inflammation which can lead to pannus formation and eventual destruction of the articular cartilage, followed by progressive loss of joint function and significant disability. Radiation synovectomy has been developed as an alternative to surgical synovectomy for the treatment of rheumatoid arthritis. This procedure consists of an intra-articular injection of beta-emitting radiopharmaceuticals into a joint to counteract and control synovial inflammation [2]. This technique has been used extensively in Europe for more than 30 years. However, it has not gained widespread acceptance in the USA. The main disadvantage of radiation synovectomy is the unacceptably high radiation dose delivered to nontarget organs due to leakage (5%–25%) of radioactivity from the treated joints [11]. Radioactive leakage from the treated joints can be minimized in three ways: (1) immobilizing the treated joint for the first 48 hours after administration, (2) using radioactive particles of an appropriate size (1–20 um), (3) choosing a radioisotope with a short half-life [1,4,10, 11]. It is therefore possible to overcome the problem of radiation leakage by using a short-lived radionuclide and relatively large particulate carrier [8].

Rhenium-188 (Re-188) is carrier-free and is available from an in-house generator system. Re-188 is suitable for treatment of the knee owing to its deep tissue penetration (maximum 11 mm, average 3.8 mm) and relatively short physical half-life (16.9 hours). In 1998, we labeled microspheres with Re-188 and analyzed their biodistribution after intra-articular injection into the knees of rabbits. The biodistribution data revealed very low radioactivity in all organs, which suggests that leakage of radiotracer from the knee is negligible [9]. We think that the Re-188 microsphere has good potential for clinical application. However, there are still some questions that need to be answered, such as the effect of Re-188 microspheres on treated joints. Thus, in this study we investigated the effects of Re-188 micro-

* Corresponding author. Tel.: +886-4-3741349; fax: +886-4-3741348.
E-mail address: sjwang@vhgtc.vghtc.gov.tw (S.-J. Wang).

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spheres on rabbit knee joint tissue using histological methods.

2. Materials and methods

2.1. Preparation of Re-188 microsphere

148 MBq of Re-188 was added to 20 mg vacuum dried microsphere (Aminex A-27, Bio-Rad Scientific Company, Richmond, California, USA), and mixed with a mixer for 15 min. 200 mg of SnCl2 anhydride and 1 ml of 0.2 N HCl were added and mixed again for 5 min. The content was boiled on a hot plate for 30 min. After centrifugation, the supernatant was removed. Vials of Re-188 microsphere were reconstituted as required with resuspension in aliquot normal saline.

2.2. In vitro *stability test*

Aliquots of Re-188 microsphere were added to tubes containing 10 ml of synovial fluid, which was drawn from the inflamed joints of patients with rheumatoid arthritis. The tubes were stoppered and mixed continuously on a rotator. At intervals of 5 hrs, 1 day, 2 days and 3 days, the tubes were removed and centrifuged at 500 g for 5 min. Aliquots of the supernatants were counted by a gamma counter, and the precipitate was readjusted to original volume and returned to the rotator. All counts were corrected for radioactive decay, and expressed as a percentage of the total radioactivity measured at the beginning of the experiment.

2.3. Animal studies

Sixteen mature male New Zealand white rabbits, each weighing approximately 3 Kg, were used in this study. After anesthesia by intramuscular injection of 3 ml of ketalar (Parke-Davis) and 0.3 ml of Rompum 2% (Bayer), 0.2 ml sterile suspension containing 0.3 or 0.6 mCi Re-188 microspheres, with particle size 15 $\pm$ 2 μm, were injected via an anterior subpatellar approach into the left knee joint of each rabbit. Eight rabbits received a dose of 0.3 mCi. The other eight received a dose of 0.6 mCi. The animals, two from each dosage group, were sacrificed at 2, 4, 12, and 24 weeks after injection. Knee joints of sacrificed rabbits were dissected and sectioned for histologic examination. The joints were code numbered and studied by a histologist blind to the data.

3. Results

In vitro stability tests revealed that the labeling efficiency of Re-188 microsphere was still greater than 98% over a 3-day period. Two weeks after the injection of Re-188 microspheres into knee joints, the synovial membrane showed round cell infiltrate (Fig. 1A) consisting of plasma cells, histiocytes and fibroblasts. Synovial villi contained leukocytes and round cells. A striking feature was the occlusion of numerous capillaries within the synovilli (Fig. 1B). Some capillaries were recanalized. The mesothelium of the synovium showed focal proliferation. After 4 weeks, most of the inflammatory and reactive changes had decreased (Fig. 2), and there was an increase in fibrocytes and collagen fiber. Microsphere particles were distributed in the synovium. At 12 weeks after injection of Re-188 microspheres, the cellular infiltration of subsynovium markedly subsided (Fig. 3), and there was focal epithelialization of synovial mesothelium. Focal fibrotic vessels and an increase in interstitial fibrous tissue could be seen between fat cells of joint capsule. After 24 weeks, both sclerosis of subsynovium and regeneration of the mesothelium were even more marked than after 12 weeks (Fig. 4A). There was no evidence of damage to articular cartilage (Fig. 4B). There was no significant difference in articular pattern after injection of 0.3 or 0.6 mCi Re-188 microspheres.

4. Discussion

Radiation synovectomy has been used as an alternative in the treatment of rheumatoid arthritis for more than 40 years. However, it has not been widely adopted because of leakage of radioactivity from the treated joints, limited availability and high costs. The main advantage of Re-188 is convenience of preparation, since it can be produced by a generator system. The long physical half-life of the W-188 parent (69 days) gives the generator system a long shelf-life and results in a lower cost of production of Re-188 [3]. The short half-life of Re-188 can effectively reduce the hazard of systemic radiation secondary to leakage. The microspheres used in our study were made up of an anion-exchange resin (acetate form) with a particle size of 15 $\pm$ 2 μm. Acute toxicity study showed that the intraperitoneal LD_{30} for Re-188 microspheres in Sprague-Dawley rats is 1000 mg Kg^{-1}. No symptoms of toxicity were recorded in rats receiving an intraperitoneal injection of less than 300 mg Kg^{-1}. The administered dose of microspheres is 4.45 mg Kg^{-1} (well below the toxic dose) when a dose of 740 MBq Re-188 microspheres (99.9 mg of microsphere) is given to a 60-Kg adult.

After intra-articular injection of Re-188 microspheres, Re-188 microspheres were retained in the knee throughout the study. According to our previous study [8], the mean retention percentages of radioactivity in the arthritic knees, determined by gamma camera imaging, were 98.7 $\pm$ 1.3%, 94.6 $\pm$ 3.6%, and 93.6 $\pm$ 4.4%, at 1 hr, 24 hr and 48 hr, respectively. For a synovial surface of 250 cm^2, the estimated doses is about 116 mGy/MBq to synovial surface of knee after the intra-articular injection of Re-188 microspheres into the rabbit's knee. The estimated doses to other organs are very low. The estimated dose is 0.04 mGy/MBq

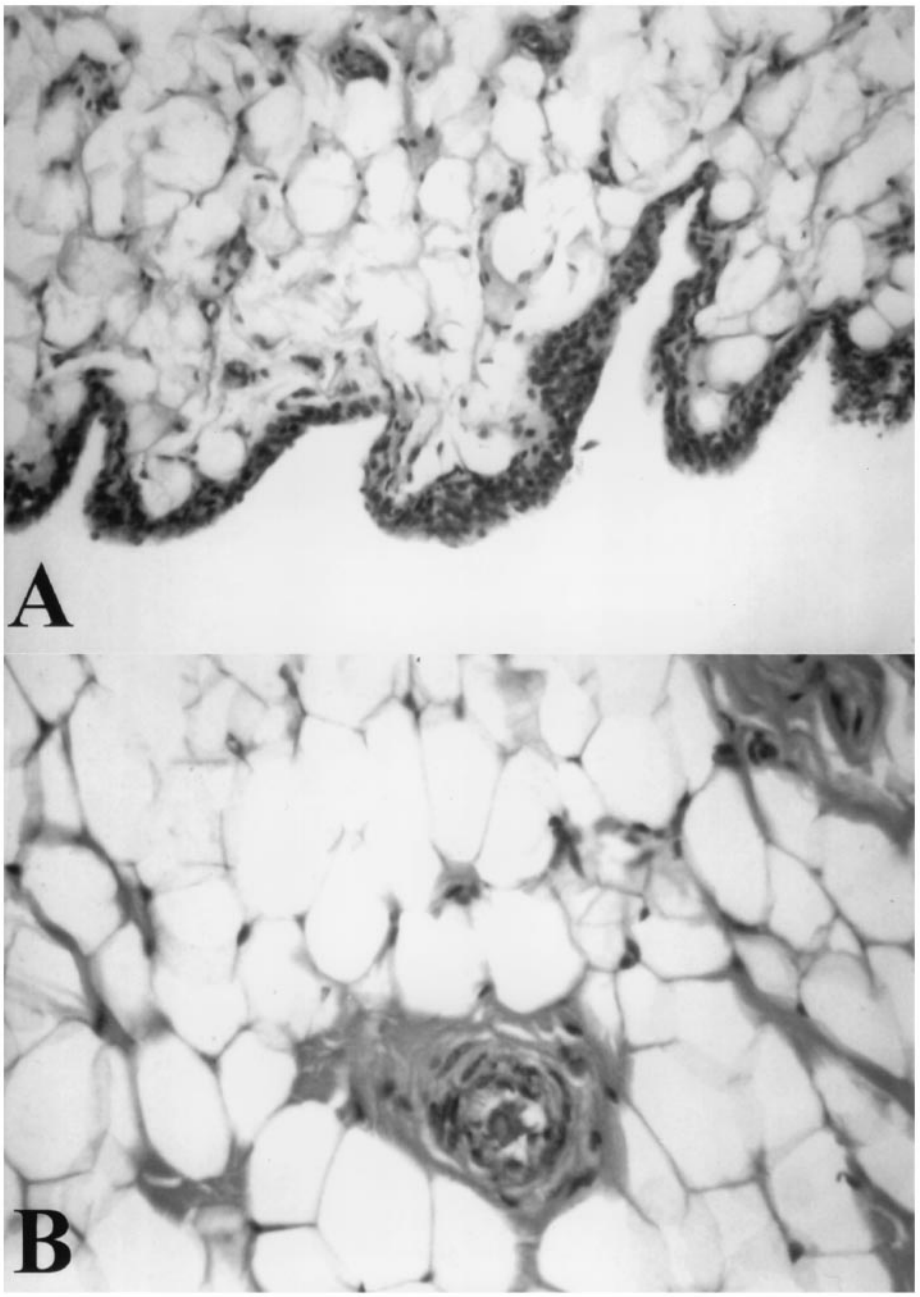

Fig. 1. Histological findings of knee joint at two weeks after intra-articular injection of Re-188 microspheres (hematoxylin and eosin, ×100). (A) Synovium of knee joint was infiltrated with round cells. (B) Occlusion of a capillary within the synovial villi was noted.

to the liver, 0.045 to the lung, 0.045 to the kidney and 0.059 to the red marrow. When a 370 MBq Re-188 microspheres would deliver more than 4292 cGy (rad) to the synovial surface, while the radiation doses to other organs such as lung, kidney and red marrow are very low (less than 2 cGy). In this histologic study, intra-articular injection of Re-188 microspheres into rabbit knee joints resulted in mild reactive inflammation which subsided rapidly. There was an increase in fibroblasts and fibrotic changes in the subsynovium and synovial vessels. This process was probably enhanced by thrombotic occlusions of vessels and capillaries. In a study by Pavelka et al., they evaluated the histological changes of colloidal 90 yttrium on knee joint tissues of rabbits. Within two weeks after the injection of colloidal 90 Y silicate into the knee joints, the synovial membrane showed round cell infiltrates consisting of plasma cells, histiocytes, fibroblasts, and isolated deposits of fibrin. In addition, thrombotic occlusion of numerous capillaries within the synovial villi was also noted. All the inflammatory process subsided rapidly and replaced by an increase of fibroblasts and by fibrotic changes in the subsynovium and synovial vessels [5]. The reactive inflammation and throm-

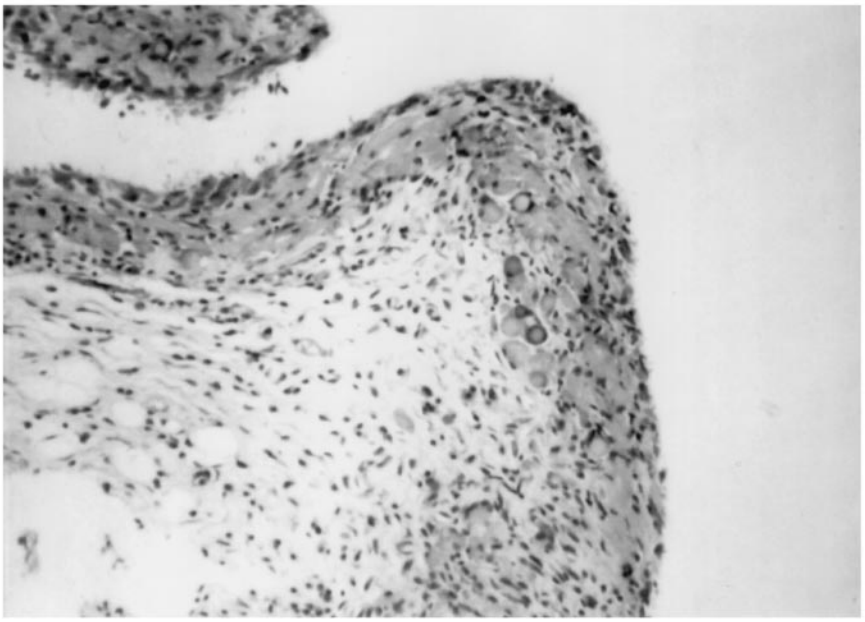

Fig. 2. Histological findings of knee joint at four weeks after intra-articular injection of Re-188 microspheres (hematoxylin and eosin, ×40). The inflammatory and reactive changes markedly decreased. An increase in fibrocytes and collagen fiber was noted.

botic occlusion of blood vessels are considered to result from the radiation effects. The occlusion of the capillaries of the synovial membrane and in fibrosis of the subsynovial layer reducing the filtration and resorption rate of the synovial fluid. This is a possible explanation for the favorable therapeutic effects of radiation in a chronic inflammatory joint effusion. There was no evidence of damage to the articular cartilage over a 24-week observation period. This result is in agreement with the findings of Pirich et al. [6]. Clinically, most patients complained of a mild increase in knee discomfort after intra-articular injection of radiopharmaceutical. A few patients developed an increased knee effusion with associated discomfort [7]. The mild reactive inflammation and thrombotic occlusion of blood vessels may provide a good explanation for the mild adverse reaction. Since the inflammation and thrombotic occlusion of

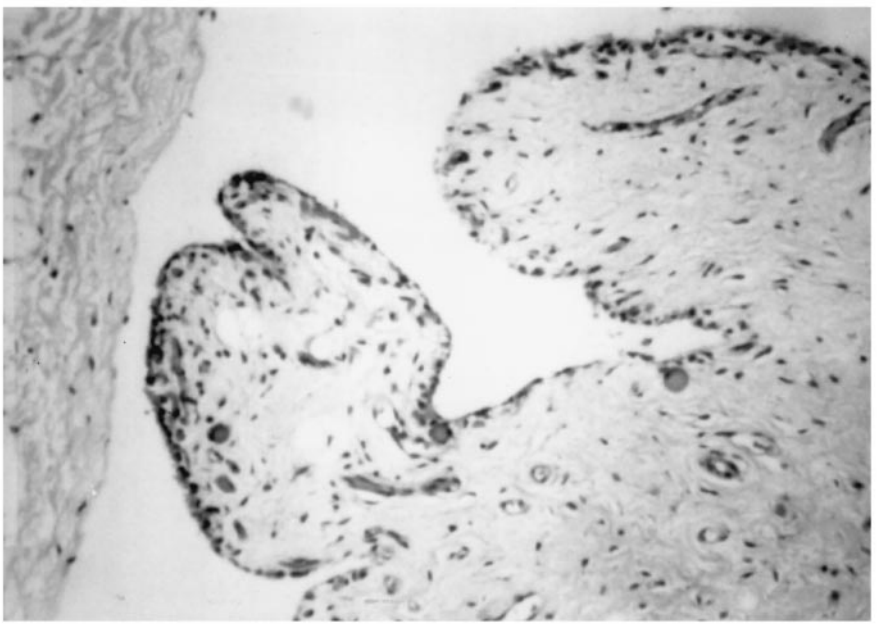

Fig. 3. Histological findings of knee joint at 12 weeks after intra-articular injection of Re-188 microspheres (hematoxylin and eosin, ×40). Cellular infiltration subsided. Focal epithelialization of synovial mesothelium was visualized. Focal fibrotic vessels and an increase in interstitial fibrous tissue could be seen between fat cells.

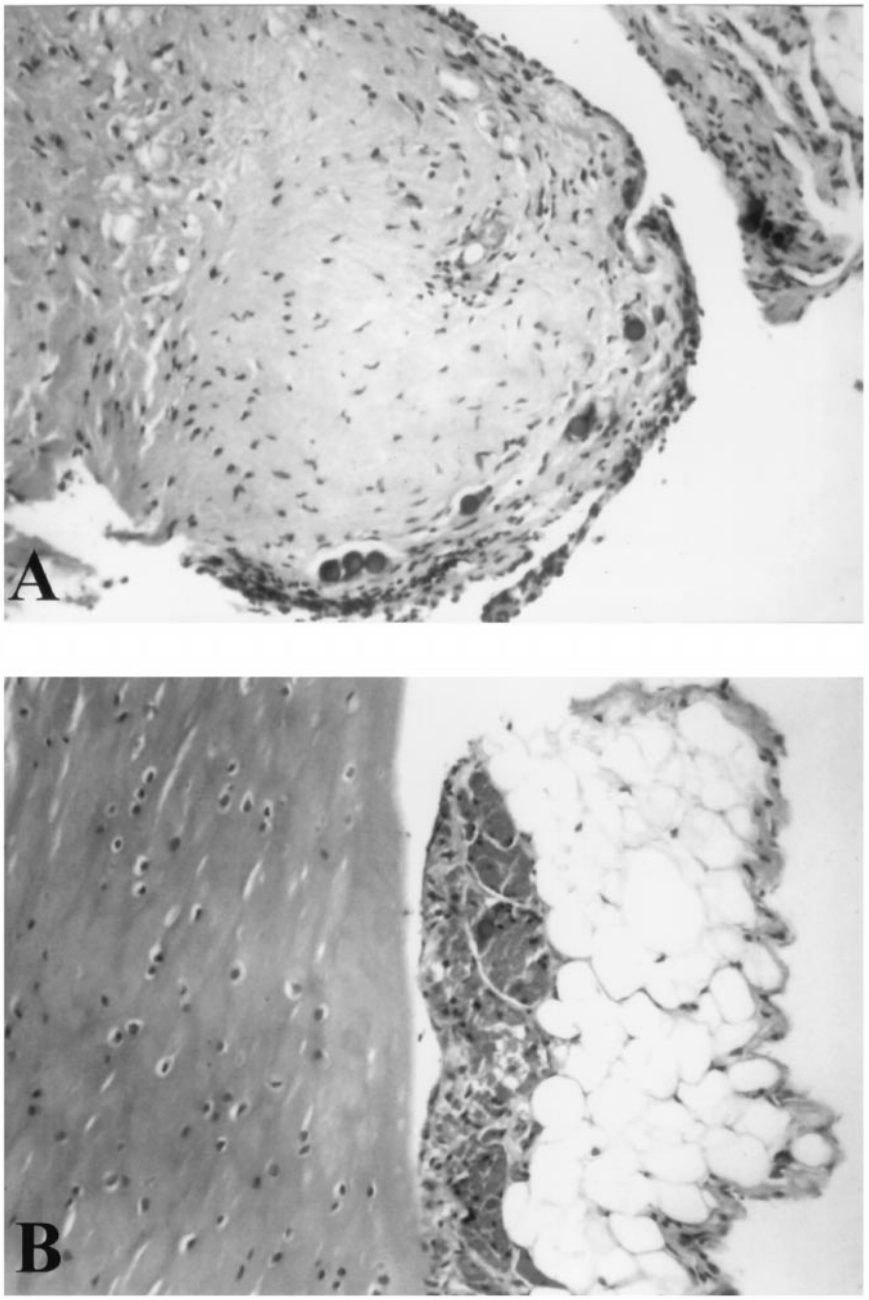

Fig. 4. Histological findings of knee joint at 24 weeks after intra-articular injection of Re-188 microspheres (hematoxylin and eosin, ×40). (A) Sclerosis of subsynovium and regeneration of the mesothelium were more marked. (B) No evidence of damage to the articular cartilage was noted.

vessels resolved rapidly, the mild increase of clinical discomfort should also be temporary. This is indeed the case. From the information accumulated, Re-188 microsphere appears to be a very safe radiation synovectomy agent.

Dysprosium-165 ferric hydroxide [Dy-165 Fe(OH)3] macroaggregates has been proven to be useful in the treatment of rheumatic synovitis [7]. Table 1 shows the comparison between Re-188 microspheres and Dy-165 Fe(OH)3 macroaggregates. There are several significant advantages to using Dy-165 Fe(OH)3 as the agent for radiation synovectomy. The half-life of Dy-165 is as short as 2.3 hours

Table 1
Comparison of characteristics between Re-188 microspheres and Dy-165-Fe(OH)3 macroaggregates in the application for radiation synovectomy

	Re-188 microspheres	Dy-165-Fe(OH)3 macroaggregates
Particle size	11–19 um	3–5 um
Half-life of radioisotope	16.9 h	2.3 h
Maximal beta energy	2.12 MeV	1.3 MeV
Maximum tissue penetration	11 mm	5.7 mm
Leakage percentage at 48 hours	6.4% [9]	1.2% [8]

and its maximal beta energy is 1.3 MeV with a maximum tissue penetration of 5.7 mm [7]. The half-lives of both Re-188 and Dy-165 are short when compared to other radiopharmaceuticals for radiation synovectomy such as Y-90 and Au-198 radiocolloids. The relative short half-life allows the treated patients discharged from the hospital soon, even within a day. In addition, if the rate of leakage from the knee to other tissues is slow, the short half-life should significantly reduce the integrated radiation absorbed dose received by the rest of the body. Actually, it is difficult to determine an ideal half-life for radiation synovectomy agent. Many factors should be taken into account for an radioisotope with ideal physical half-life, such as biological half-life of the radiopharmaceutical in the target organ, leakage rate from the target organ to systemic organs. . . etc. Long physical half-life may not be a withdrawal for a radiotherapeutic agent if its biological half-life in target organ is long. Based on our study, the 17 hours half-life of Re-188 would not be a disadvantage since the leakage of the microspheres from the treated knee is minimal. The maximum beta energy of Dy-165 is 1.3 MeV with a maximum soft tissue penetration of 5.7 mm, which may closely approximates the thickness of inflamed knee synovium [7] and shown to effectively reduce inflammation in an animal model of antigen-induced arthritis [8]. The maximum soft tissue penetration of Re-188 is 11 mm with an average of 3.8 mm, which is significantly longer than that of Dy-165. Again, it is very difficult to determine an ideal soft tissue penetration in radiation synovectomy. Stronger beta energy may provide better radiation effect in thicker synovium and reduce the recurrence rate in the future. However, a long soft tissue penetration of beta particle may also cause an unnecessary damage to the underneath tissue. We consider Re-188 is safe in the treatment of synovitis since no evidence of damage to the underneath articular cartilage was noted in our study. The short half-life of Dy-165 poses one significant disadvantage. Its short half-life necessitates being close to a nuclear reactor capable of producing the material; this poses a problem of availability and accessibility. On the contrary, Re-188 is available from an in-house generator system similar to the current Tc-99m generator. Re-188 can be obtained from a W-188/Re-188 generator, which makes it very convenient for clinical use [9].

In conclusion, Re-188 microspheres are safe, readily available and potentially inexpensive therapeutic agents for synovectomy. Further studies are warranted to clarify the therapeutic efficacy of Re-188 microspheres.

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