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Research paper

Fibrin glue system for adjuvant brachytherapy of brain tumors with ^{188}Re and ^{186}Re-labeled microspheres

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Abstract

Brain tumors such as glioblastoma reappear in their original location in almost 50% of cases. To prevent this recurrence, we developed a radiopharmaceutical system that consists of a gel applied immediately after surgical resection of a brain tumor to deliver local radiation booster doses. The gel, which strongly adheres to tissue in the treatment area, consists of fibrin glue containing the β-emitters rhenium-188 and rhenium-186 in microsphere-bound form. Such microspheres can be prepared by short (2 h or less) neutron activation even in low neutron flux reactors, yielding a mixture of the two β-emitters rhenium-188 ($E_{max} = 2.1$ MeV, half life = 17 h) and rhenium-186 ($E_{max} = 1.1$ MeV, half life = 90.6 h). The dosimetry of this rhenium-188/rhenium-186 fibrin glue system was determined using gafchromic film measurements. The treatment efficacy of the radioactive fibrin glue was measured in a 9L-glioblastoma rat model. All animals receiving the non-radioactive fibrin glue died within 17 ± 3 days, whereas 60% of the treated animals survived 36 days, the final length of the experiment. Control animals that were treated with the same amount of radioactive fibrin glue, but had not received a previous tumor cell injection, showed no toxic effects over one year. The β-radiation emitting rhenium-188/rhenium-186-based gel thus provides an effective method of delivering high doses of local radiation to tumor tissue, particularly to wet areas where high adhesive strength and long-term radiation (with or without drug) delivery are needed.

Keywords: Brain tumor; Brachytherapy; Fibrin glue; Microspheres; Rhenium radioisotopes

1. Introduction

According to the American and Canadian Cancer Societies, 1.6% or almost 25,000 of all newly diagnosed cancers in North America are brain cancers [1,2]. In addition to these primary cancers, the spread of systemic cancer to the brain is a common complication for cancer patients. Such brain metastases are diagnosed in an additional 170,000 patients per year [3].

Typical treatments for primary brain tumors and singular brain metastases include maximal surgical resection followed by radiation therapy and chemotherapy. Local irradiation [4] and/or local chemotherapy with polyanhydride disks slowly releasing 1,3-bis[2-chloroethyl]-1-nitrourea (BCNU) (=Gliadel™) is a recommended follow-up procedure after the surgery. Both treatment methods, however, seem to be of limited value. Although survival appears to improve with higher radiation doses [5], an increase in late normal tissue damage has limited the maximum dose that can be given with conventional techniques (external beam irradiation) to 60 Gy. For the BCNU patches, it seems that limited diffusion prevents complete tumor eradication. In the first controlled clinical trial, the median survival increased modestly from 23 to 31 weeks [6].

Many malignant brain tumors recur within a short time. Most recent epidemiological data give a median survival of 40.9 weeks for glioblastoma multiforme, the most com-

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monly diagnosed primary brain tumor [7]. The fact that more than 80% of the glioma recurrences are within 2 cm of the site of origin makes brachytherapy, the implantation of radioactive sources directly into a tumor or into the tumor bed after its surgical removal, a very promising treatment. The treatment range is radioisotope dependent, and the surrounding brain can thus be spared. Although radioactive colloids mainly in the form of ^{32}P have been applied to brain tumors for many years [8], brachytherapy research is still in its early stages [9]. Recent promising methods include the permanent implantation of radioactive ^{125}I seeds [10] and the temporary treatment of resected tumors with a ^{125}I-filled balloon (Gliasite™) that can be inserted with a catheter and is removed a few days later [11].

In our work, we wanted to optimize both the shape of the delivery vehicle and the treatment range of the drug. First, the shape of wafers as used for the BCNU system somewhat limits the areas that can be treated. A more conformal pharmaceutical would be one that could take on any shape, such as a gel, and that would adhere strongly to tissue in the treatment area. We decided on fibrin glue as the gel-like delivery vehicle because of its strong bioadhesive properties, even under water. Furthermore, fibrin glue is biocompatible, non-toxic, biodegradable, and available in the form of an FDA-approved two-component system [12]. Second, the therapeutic treatment range of BCNU is dependent on diffusion, and thus influenced by the physiology of the distinct tumors [13]. Radioisotopes applied to the surface of the brain tumors are not affected by such factors as a blood–brain barrier, blood flow, and interstitial tumor pressure, their range depends solely on their physical decay characteristics. For this reason, we decided to prepare radioactive fibrin glue by embedding the therapeutic β-emitting radioisotopes ^{186}Re and ^{188}Re in microspheres.

Until recently, fibrin glue has primarily been used for surgical applications, to glue tissues together and prevent bleeding [14]. In the last few years, the first drug delivery applications using fibrin glue as a vehicle have appeared. Nishimoto et al. incorporated the antiinfective agent amikacin into a combined fibrin glue/polyurethane graft, from where it was slowly released [15]. Similarly, Moon et al. locally applied fibrin glue containing losartan, an inhibitor of the renin-angiotensin system useful for hyperplasia inhibition in restenosis, through a cardiac catheter to coronary arteries during balloon angioplasty [16]. Fibrin glue also served as a delivery vehicle for different growth factors. They include vascular endothelial growth factor (VEGF) [17] or fibroblast growth factor (FGF-1) [18] for the induction of vascular graft and angioplasty site cell regrowth, and glial cell line-derived neurotrophic factor (GDNF) [19] for the prevention of chronic brain damage caused by cerebral ischemia.

In this paper, we are presenting dosimetric, toxicity and *in vivo* efficacy data for a fibrin glue-based radiopharmaceutical.

2. Materials and methods

2.1. Microspheres

Glass microspheres containing 30% rhenium oxide were made by flame spheronization as previously described [20] (Fig. 1). Twenty milligrams of the 25–35 μm microspheres was packed in specially made 4 cm long high-purity quartz vials (Crown Glass Company Inc., Somerville, NJ) which were closed with tightly fitting aluminum caps. The vials were neutron-activated at the Ohio State University reactor in Columbus, Ohio, USA, for 1 h at a neutron-flux of 1.5×10^{13} n/cm^2/s, yielding 541 MBq of ^{188}Re and 93 MBq of ^{186}Re. The microspheres were simultaneously sterilized by the more than 0.5 MGy of γ-irradiation produced during neutron activation in the reactor. This radiation dose was more than 20 times larger than the usual dose used for γ-sterilization [21].

2.2. Fibrin glue

Fibrin glue was prepared using either a Tissucol® kit according to the manufacturer's description (Immuno AG, Vienna, Austria) or the Tisseel® VH kit (Baxter, Deerfield, Illinois, USA). The Tissucol® kit consists of two components contained in separate syringes sitting side by side (Fig. 2). The two components were combined at a 1:1 ratio in the syringe tip applicator (needle) during application to the target area and immediately started to gel. Component A consisted of 70–110 mg/ml of fibrinogen and 3000 KIU/ml of the fibrinolyis inhibitor aprotinin that were combined and dissolved at 37 °C using a sterile technique. Component B was a mixture of thrombin (50 I.E. per ml) and $CaCl_2$. The radioactive microspheres could be added to either component. However, adding them to component A was preferable and thus was chosen for the final application.

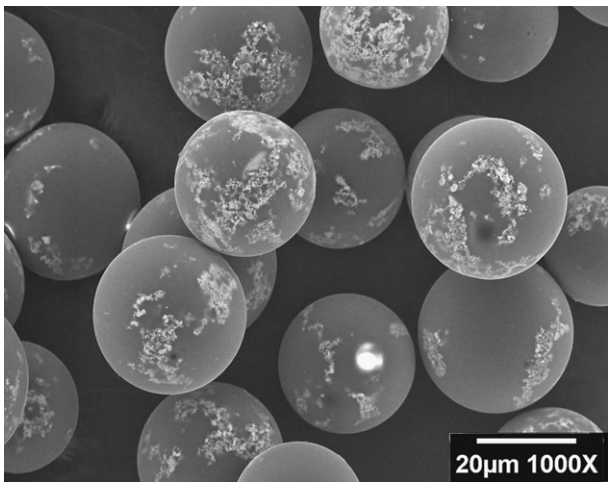

Fig. 1. Homogeneously sized glass microspheres containing 30 wt% of rhenium oxide. The white areas indicate higher rhenium concentrations than the surrounding areas.

Fig. 2. Syringe for the application of the radioactive fibrin glue.

2.3. Testing of adhesiveness

The adhesiveness of the radioactive fibrin glue layer was tested by applying a 2 mm thick layer to the surface of an entire dog brain, binding the brain to a petri dish and fixing everything upside down in the middle of a saline-filled bowl. The bowl was kept in a slowly shaking water bath at 37 °C for 1 week. This study with a dog brain, as well as the experiments with tumor carrying rats described below, was approved by the Animal Care and Use Committee of the Cleveland Clinic Foundation.

2.4. Dosimetry

Gafchromic film, a thin, almost colorless polyester sheet embedding a chromophore that turns dark blue under the influence of radiation [22], was calibrated with doses ranging from 0 to 200 Gy using the 6 MV photons from a Varian Clinac 2100-C (Varian Oncology Systems, Palo Alto, CA) linear accelerator. Twenty-five pieces of 0.26 mm thick, $3 \times 3\ cm^2$ gafchromic film type MD-55 (Nuclear Associates, Carle Place, NY) were carefully placed on top of each other in a 6.6 mm deep indentation in the middle of a $10 \times 10\ cm^2$ and 2 cm thick piece of a "solid water" phantom made from RMI 457 (Gammex RMI, Middleton, WI). The phantom surface was covered with a 7 μm thick piece of mylar and then a 1 mm thick piece of $10 \times 10\ cm^2$ solid water with a central $5 \times 5\ cm^2$ hole. This hole was homogeneously filled with a precalculated amount of fibrin glue containing radioactive glass microspheres with 28.8 MBq of ^{186}Re and 72.5 MBq of ^{188}Re at the beginning of the film irradiation. The radioactive film was exposed to the microspheres in the fibrin glue for 18.18 h, allowed to rest in the dark overnight and was then scanned together with the calibration pieces of $1 \times 1\ cm^2$ on a 600 dpi expression 636 scanner with transparency unit (Epson, Torrance, CA). A calibration curve was constructed from the calibration pieces and applied to the $3 \times 3\ cm^2$ gafchromic film pieces. All the pixels from each piece of gafchromic film were averaged and a radiation dose fall-off curve constructed. The dose given until complete decay of the radioactive microspheres was then calculated by integrating the two radiation components separately over the experiment length, applying the dose fall-off curves determined for each radioisotope in a separate experiment (see inset; [23]) and extending dose deposition over 10 half-lives of ^{186}Re (38 days).

2.5. Animal experiment

Sprague–Dawley rats weighing approximately 300 g were divided into three groups. Group A, the control group, consisted of 6 animals which received 9L-tumor cells followed one week later by 0.5 mg of non-radioactive microspheres in fibrin glue. Group B, the treatment group, consisted of 5 animals which received 9L-tumor cells followed one week later by 0.5 mg of radioactive microspheres (1.85 MBq of $^{188}Re/^{186}Re$ at a ratio of 3:1 at the time of implantation) in fibrin glue. Group C, the toxicity group, consisted of 10 animals that received no tumor cells, but the same amount of radioactive microspheres as the treatment group.

After anesthesia with 60 mg/kg ketamine plus 7.5 mg/kg xylazine, the animals were placed into a stereotactic head frame and a small hole drilled through the skull 3.5 mm caudal to bregma and 3.5 mm to the left of midline. Twenty microliters of saline with 100,000 exponentially growing 9L glioblastoma cells was injected at a depth of 2.5 mm into group A and B animals. The hole in the skull was sealed with bone wax and the incision closed using staples. One week later, the incision was reopened under anesthesia and 0.5 mg of radioactive glass rhenium microspheres injected through the previously drilled hole to the same depth. To allow for the small injection amounts, the microspheres were suspended in 10 μl of fibrinogen and injected into the brain, immediately followed by the injection of 10 μl of thrombin, thus forming fibrin glue in situ. Group A rats received 0.5 mg of non-radioactive glass microspheres, Group B received 0.5 mg of microspheres with 1.85 MBq of $^{188}Re/^{186}Re$ (ratio 3:1). The holes in the skull were sealed with bone wax and the incision closed using staples. The amount of radioactive microspheres received was verified by placing the entire animal into a dose calibrator (Radcal Corp., Monrovia, CA, USA).

The animals were weighed and observed for grooming behavior daily. After 36 days, all surviving animals of group B were sacrificed by pentobarbital injection. Their brains were excised, cut on a microtome, stained with H&E and cresyl violet (Nissl substance stain), and an immunocytochemical reaction performed for glial fibrillary acidic protein (GFAP; a highly sensitive marker for neurodamage induced by radiation or trauma to astrocytes). The resulting histology slides were evaluated for signs of necrosis, tumor extent, and signs of inflammation.

3. Results

A radioactive fibrin glue of reproducible consistency was obtained when the radioactive microspheres were mixed into the fibrinogen component of the fibrin glue, but not when mixed into the thrombin component. The higher viscosity of the fibrinogen component probably helped to produce more reliable results in terms of homogeneity. This in turn also seemed to give a more exact and reproducible clotting time of the fibrin glue. Upon expulsion through

the double-syringe injector, the fibrinogen-microsphere suspension mixed reliably with the thrombin component and then gelled within a few seconds on the surrounding tissue. The mixing produced relatively homogeneously distributed ^{186}Re/^{188}Re-microspheres (Fig. 3). To test the adhesive strength of the fibrin glue to the target tissue, it was spread on a piece of dog brain and hung upside down in a bowl of saline. After 1 week, all the fibrin glue was still adhering to the brain's surface, and no pieces had fallen off (Fig. 4).

A dose fall-off profile centrally perpendicular to a fibrin glue layer containing radioactive microspheres is shown in Fig. 5. The radiation dose was delivered relatively homogeneously, with a standard deviation of less than 7% over the graphed range.

The use of the radioactive fibrin glue in rats with implanted 9L-glioblastoma also confirmed that the radiation dose could be delivered to the target brain tumors. The injection of radioactive rhenium microspheres in fibrin glue significantly altered survival among the three groups ($p < 0.001$) (Fig. 6). Untreated animals died after a median of 17.5 days, with no survivors, whereas 3 of 5 treated rats were still alive after 36 days, which was the end of the experiment. Pair-wise log-rank comparisons indicated that survival in the control group was significantly lower than both in the treatment group ($p < 0.05$) and the toxicity group ($p < 0.001$).

Examination of the stained brain tissue sections revealed some dead tissue, astrocytes, gliosis, and infarction reaction near areas of injection, all of which are typical for the damage applied to the brain tissue during the stereotactic surgery. The accumulation of a small number of macrophages was observed, but the reaction around the intact spheres themselves was minimal and confirmed good biocompatibility of glass microspheres and fibrin glue. GFAP expression in the group that received tumor cells and radiation treatment seemed slightly increased, as compared to the control group, indicating that the high radiation doses produced limited radiation necrosis. Attempts at quantifying this effect with image analysis software, however, yielded no significant results.

The third group of animals, which received an initial injection of saline without tumor cells, followed a week later by radioactive microspheres, was kept alive for one year and then underwent the same histological tests as the animals that had the 9L tumor cells implanted. The histological findings were similar to the other groups, showing

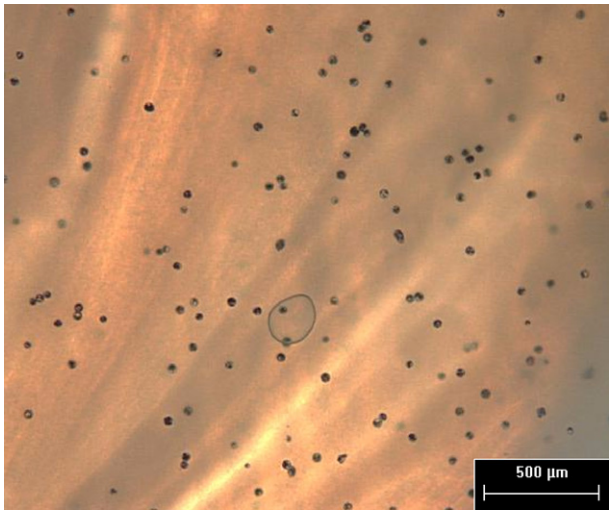

Fig. 3. Microsphere distribution in the gelled fibrin glue under a light microscope.

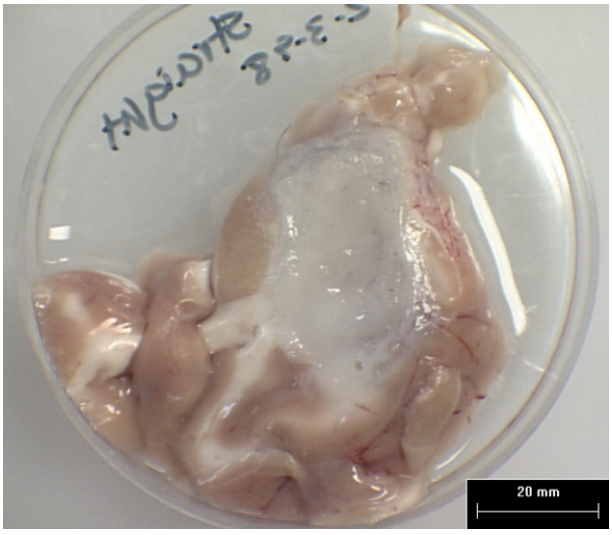

Fig. 4. Adhesiveness test. This picture was taken one week after spreading a 2 mm thick layer of radioactive fibrin glue (central white area) onto a piece of brain tissue and shows no detachment of the fibrin glue.

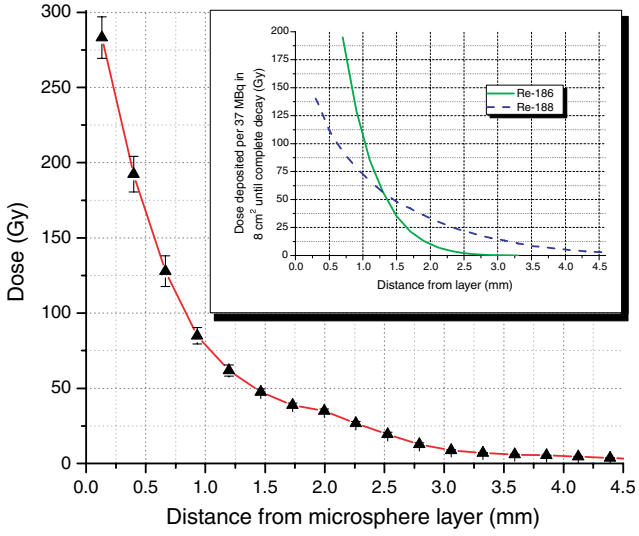

Fig. 5. Dose accumulated from a 1 mm thick and 25 cm^2 large fibrin glue layer containing ^{186}Re (28.8 MBq) and ^{188}Re (72.5 MBq) microspheres. The dose fall-off was measured perpendicular to the center of the fibrin glue layer. The inset shows an earlier measured dose fall-off from a 2-dimensional layer of radiolabeled microspheres containing either ^{186}Re or ^{188}Re, in order to highlight their different radiation range [23]. For the measurements, 37 MBq of activity was homogeneously distributed over 8 cm^2 on top of gafchromic film pieces.

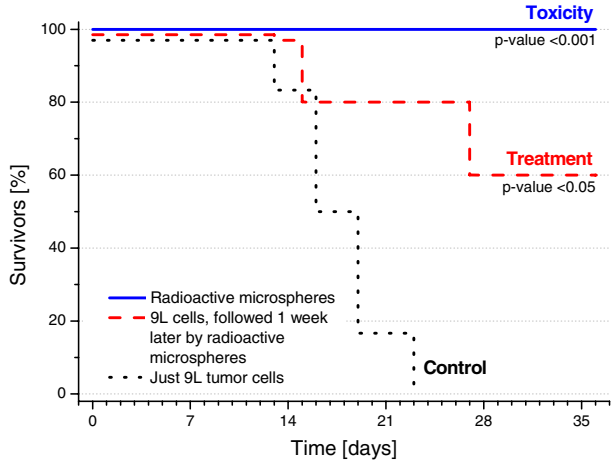

Fig. 6. Survival of rats with 9L-glioblastoma brain tumors treated with non-radioactive microspheres (control, $n = 6$) or radioactive microspheres (treatment group, $n = 5$). The toxicity group received radioactive microspheres in fibrin glue, but no prior glioblastoma cells ($n = 10$).

some response to the lesional damage in the form of dead tissue, astrocytes, Nissl bodies, gliosis, and some infarction reaction near areas of injection. Some macrophages were also observed but not a large number. Some histology sections contained bone fragments, which could be expected due to the method employed to make the holes. Some of the bone chips could have fallen in when the lesion was made or been pushed in when the injection was given. There was not much reaction around the intact microspheres themselves. Furthermore, the GFAP staining, a highly sensitive marker of neurotoxin-induced central nervous system (CNS) injury, showed no obvious necrosis, in spite of the fact that a radiation dose of more than 50 Gy was delivered to the brain tissue at a depth of 1 mm.

4. Discussion

Our experimental findings show $^{188}Re/^{186}Re$ fibrin glue to be a promising vehicle for the delivery of local radiation. The radioactive fibrin glue is not only effective as a local cancer treatment, as shown in Sprague–Dawley rats with implanted deadly 9L glioblastoma cells, but also was found to be biocompatible. Although the rhenium glass microspheres incorporated into the radioactive fibrin glue are not biodegradable, there was very little reaction of the immune system to their implantation. No chronic inflammation and only very small amounts of necrosis were detected, based on the GFAP immunostaining. The glass microspheres behaved as expected in a bioinert way. Overall, it was determined that the tissue responses were appropriate to the damage inflicted.

The preparation of radioactive fibrin glue only requires one more step than making non-radioactive fibrin glue. It is prepared by suspending the dry, radioactive $^{186}Re/^{188}Re$-microspheres received from a nuclear reactor into a syringe containing the fibrinogen component of an FDA-approved fibrin glue kit, adding the thrombin solution into a second syringe, and mounting both into a special holder (Fig. 2). The radioactive microspheres do not settle rapidly in the fibrinogen solution and can be kept ready for injection at room temperature for at least an hour. Ejecting the fibrinogen suspension and thrombin solution through a "mixing connector" produces a viscous gel that quickly sets into an elastic coagulum. This rapid reaction is both an advantage and disadvantage, since it limits the distribution of the radiopharmaceutical to the application site, but requires practice, good technique, and fast handling to guarantee a homogeneous and directed application of the fibrin glue. To help overcome these difficulties, the providers of fibrin glue kits support the end user with improved application methods of fibrin glue. For example, the premature clogging of the fibrin glue in the applicator can be completely prevented by having the two components mix only at the location of fibrin glue deposition. This technique has been demonstrated using a spray pistol [24]. Another method uses a syringe filled with fibrinogen in solution with or without mixed-in drugs. The solution (or suspension) is pushed through a small (sterile filter-like) cartridge with immobilized thrombin. During the flow-through, the fibrinogen is activated to fibrin, and the fibrin monomers leaving the syringe cross-link rapidly to form fibrin glue with the mixed in drug [25].

For the delivery of brachytherapy, it is important that the chosen radioisotopes stay at the intended location until complete decay and do not translocate and harm normal tissue and non-target organs. Fibrin glue performed this function very well. It adhered very strongly to wet tissues, was able to support high density glass microspheres with a density of 2.9 g/cm^3 while maintaining their homogeneous distribution, and did not break down from possible radiation damage. Fibrin glue prevented any movement of the radiopharmaceutical, even under very wet conditions. This is essential for applications in the brain, where cerebrospinal fluid immediately fills the entire excision cavity after surgical removal of brain tumors. The adhesion of fibrin glue to brain matter was highly superior to that of commonly used bioadhesive compounds. The well-known mucoadhesive compounds tested (data not shown) included poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene)-*g*-poly(acrylic acid) (Smart Hydrogel™ [26]), gelatin sponge (Gelfoam™, Avitene® [27]), oxidized regenerated cellulose (Surgicel®), the reverse phase thermal gel Poloxamer 407, sodium carboxymethylcellulose, methylcellulose, and sodium alginate [28]. Leaving the mucoadhesive coatings submersed, thus mimicking the clinical situation, completely hydrates the gels, and is followed by their slow breakdown starting within minutes or at latest hours. Under these circumstances, all of the above-mentioned bioadhesive materials seriously degraded and never stayed longer than an hour on the submersed test surfaces (data not shown). The fibrin glue layer, in turn, was still intact after 7 days of submersion, with no signs of shedding of the matrix material.

In order to prepare a radioactive fibrin glue film, the chosen radioisotope must be incorporated into the film in appropriate form. Unlike in drug delivery, slow release of radioisotopes is neither required nor desired, since free radioactivity in general behaves differently from the applied radiopharmaceutical and might deliver excessive radiation doses to non-target tissues. For this reason, we chose rhenium glass microspheres which are bioinert and mechanically and chemically stable. The activation of the rhenium glass microspheres can be done in a low neutron flux nuclear reactor, normally within less than an hour. Rhenium as a neutron-activatable element thus has a distinct advantage over yttrium contained in the FDA-approved Therasphere™. The activation of the glass yttrium microspheres requires long activation times at high neutron fluxes due to ^{89}Y's small cross section of $0.001 + 1.28$ barns vs. 112 and 74.6 barns for ^{185}Re and ^{187}Re, respectively. Stored in lyophilized form inside a quartz vial, rhenium microspheres can be activated directly and shipped the day before use, resulting in very reasonable associated costs. Before reactor-activated rhenium radioisotopes can be used, it is necessary to use a multichannel analyzer to determine the activity of each of the radioisotopes in the mixture and to calculate the dosimetric properties of the product. This step can be avoided by using ^{188}Re from a ^{188}W/^{188}Re-generator [29]. Appropriate ^{188}Re-labeled carriers for incorporation into fibrin glue include colloids such as sulfur rhenium colloids [30] or radioactive microspheres such as hydroxyapatite particles [31], albumin particles [32], carbon-coated iron particles [33], magnetic silica-coated nanoparticles [34], or biodegradable poly(lactic acid) microspheres [35]. An advantage of the radioactive glass microspheres over these particles is their minimal leakage of radioactive rhenium. Until complete decay, which is 36 days for ^{186}Re, less than 0.5% of the radioactivity is released in the form of perrhenate [20].

Applying radiation in the form of a layer, and not for example filled into a balloon such as in the Gliasite Radiation Therapy System [11], has the advantage of minimizing the amount of radioactivity used. Furthermore, it is possible to directly use a γ-camera with ^{99m}Tc settings for the imaging of the tumor site. The main γ-lines of ^{186}Re and ^{188}Re at 137 and 155 keV are only slightly below and above ^{99m}Tc's γ-line. ^{99m}Tc is the most commonly used diagnostic radioisotope.

Radioactive fibrin glue also has radiobiological treatment advantages. The healthy brain consists of slowly dividing or static cells that together form a late-reacting, relatively radio-resistant tissue. A brain tumor in turn consists of rapidly proliferating cells that are susceptible to radiation damage and have little ability to repair sublethal and potentially lethal radiation damage. Thus, the cell survival curve for tumor cells has a shallower shoulder than the curve for healthy, late-reacting brain cells [36]. The shallower curve and the dose-rate effect of brachytherapy give it distinct advantages over external-beam radiation therapy. Brachytherapy allows hypoxic cells to become reoxygenated during treatment, causes proliferating tumor cells to accumulate in the radiosensitive phase and impairs the ability of the tumor to repair sublethal damage under hypoxic conditions [37]. Using ^{186}Re/^{188}Re-microspheres, a radioisotope-dependent therapeutic depth of about 4 mm can be reached (Fig. 5), although therapeutic doses should probably only be prescribed to 2.5 mm in order to limit necrosis on the fibrin glue surface. It will have to be shown in clinical trials whether the radiation range from a ^{186}Re/^{188}Re fibrin glue layer is sufficiently large to prevent the recurrence of brain tumors.

In further studies, a longer experimental time for the treatment group should be considered to demonstrate definitive treatment outcome, long-term radiation effects, and detailed immune responses. The length of survival time was too short for significant necrosis to occur. The fact that all control rats survived until sacrifice, however, is a good sign that the radioactive fibrin glue injections were not overly toxic or life threatening.

Radioactive fibrin glue seems to be just as effective, if not more effective, than the chemotherapeutic drug 5-fluorouracil (5-FU) which has been tested in a very similar nitrosurea-induced brain tumor model by Menei et al. [38]. The authors encapsulated 5-FU into microspheres made from the biodegradable polymer poly(lactide-coglycolide) (PLGA). They then stereotactically injected the microspheres containing 10 mg of 5-FU per kg into a C6 glioblastoma implanted 7 days earlier. After 36 days, their slow release formulation microspheres, like ours, showed a 60% survival, while the fast release formulation microspheres seemed less effective with about 35% of the animals still alive on day 36. Their later experiment [39] with another nitrosurea-induced glioblastoma cell line F98 using a different polymer for 5-FU delivery, poly(methylidene malonate), was a little less effective, showing a 45% survival on day 36 after the injection of slow release microspheres containing 8.8 mg of 5-FU/kg.

5. Conclusions

Radioactive fibrin glue containing ^{188}Re/^{186}Re represents an effective method of delivering high focal doses of radiation. The dose fall-off is rapid and this method might thus be particularly useful for patients with minimal residual disease. The use of rhenium β-emitters prevents the irradiation of normal brain tissue more than 3–4 mm away from the fibrin glue layer. Normal brain tissue can thus be spared and the application of radioactive fibrin glue may be possible even in critical areas such as near the optical nerve. Furthermore, this local treatment may be useful in patients with previous radiation treatment. Clinical trials in brain tumor patients will have to show what the necessary radiation doses are and if the treatment depth is sufficient to treat all left-over tumor cells and prevent recurrence of brain tumors.

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