Cureus

Open Access Review Article

DOI: 10.7759/cureus.29

A Concise Review of the Emerging Applications of Synchrotron- Generated Microbeams in the Treatment of Brain Disorders

Pantaleo Romanelli, Erminia Fardone, Elke Brauer-Krisch, Yolanda Prezado, Alberto Bravin

1.

Corresponding author: Pantaleo Romanelli, radiosurgery2000@yahoo.com

Abstract

Synchrotron-generated X-ray microplanar beams (microbeams) are characterized by the ability to avoid widespread tissue damage following delivery of doses ranging from hundreds to over a thousand Gray. The preservation of tissue architecture following high-dose microbeam irradiation is known as “tissue-sparing effect” and is strictly related to the ability of microbeams to restrict spatially these exceedingly high doses to the beam path with minimal doses spreading outside to the adjacent tissue. Image-guided microbeam radiosurgery has been recently used to generate cortical transections or to induce deep-seated lesions in the rat brain. The ability to generate focal lesions or microscopic transections over the eloquent and non-eloquent cortex in experimental animals is of great interest for the development of experimental models in neurobiology, opening new treatment avenues for a variety of neuropsychiatric disorders originating from focal brain dysfunction. This paper reviews the current state of research on the radiobiological properties of synchrotron-generated microscopic X-ray beams and their emerging microradiosurgical application, with special reference to the treatment of a variety of brain disorders.

Categories: Neurosurgery
Keywords: spine, radiosurgery, cortex, movement disorders, transections, microbeams, synchrotron, brain, epilepsy

Introduction And Background

Stereotactic radiosurgery aims to attain growth-control through the ablation of a neoplastic lesion, to induce the obliteration of vascular malformations or to restore the correct functioning of a neural circuitry by modulating or ablating selected brain nuclei or fiber bundles. The ablation of the tissue contained within a well-defined target is achieved through the precise delivery of several hundreds of ionizing radiation beams sized 4 to 60 mm sent from a wide array of directions and intersecting over the volume selected for ablation with 3 to 5 mm 80% to 20% dose fall-out from the volumetric boundaries of the target. There is growing evidence that synchrotron-generated microscopic beams (microbeams) beams can carry much higher doses to the target as compared to conventional high-energy photons or gamma irradiation. Microbeams beams are characterized by the ability to carry extremely high doses without inducing damage to surrounding tissue. This paper aims to offer a concise review of the state of microbeam research applied to the CNS with special reference to the potential new clinical applications that can originate from this novel approach.

Published 07/14/2011

Review

Synchrotron-generated X-ray beams are tangentially emitted by relativistic electron bunches circulating in the storage ring of a synchrotron radiation facility. The X-ray source is a wiggler (a magnetic structure of alternating poles positioned on a straight section of the storage ring) producing a wide spectrum of photons with an energy range up to several hundreds of kilo electronvolts (keV). The quasi-laminar beam can be spatially fractionated into an array of rectangular microbeams of variable size by means of a multislit collimator [1]. The X-ray fluence is thousands of times higher than that of standard linear accelerators used in conventional radiotherapy. At the European Synchrotron Radiation Facility (ESRF, Grenoble, France), shown in Figure *1*, the dose rate is around 16.000 Gy per second. This dose output is much higher than those available using conventional linear accelerators delivering up to 6 Gy per second. The minimal beam divergence of synchrotron X-rays allows the delivery of extremely high doses to the cells along the penetration path with minimal dose dispersion over the adjacent cells. Depending on the microbeam size, typically between 25 to 75 microns, a quasi-surgical cut involving a width comprising up to 3-4 neuronal cell bodies can be generated along the tissue penetration path (see figure *2*). The delivery of an array of parallel beams generates alternating volumes receiving peak doses of several hundreds of Grays and valley doses of a few Grays (see figure *3*). The beam distance center-to center is variable from 100 to 400 gm, depending on the collimator choice and settings. The biological properties of larger beams measuring up to 0.7 mm (also known as minibeams) have been investigated lately. Minibeams could be generated without a synchrotron and can facilitate clinical use [2, 3]. For an overall description of the dosimetric methods, definitions, and calculations, please refer to the references [4, 5].

How to cite this article
Romanelli P, Fardone E, Brauer-Krisch E, et al. (July 14, 2011) A Concise Review of the Emerging Applications of Synchrotron- Generated Microbeams in the Treatment of Brain Disorders. Cureus 3(7): e29. DOI 10.7759/cureus.29

FIGURE 1: The European Synchrotron Radiation Facility (ESRF), Grenoble, FR. In foreground: the biomedical beamline ID17, external to the synchrotron radiation storage ring.

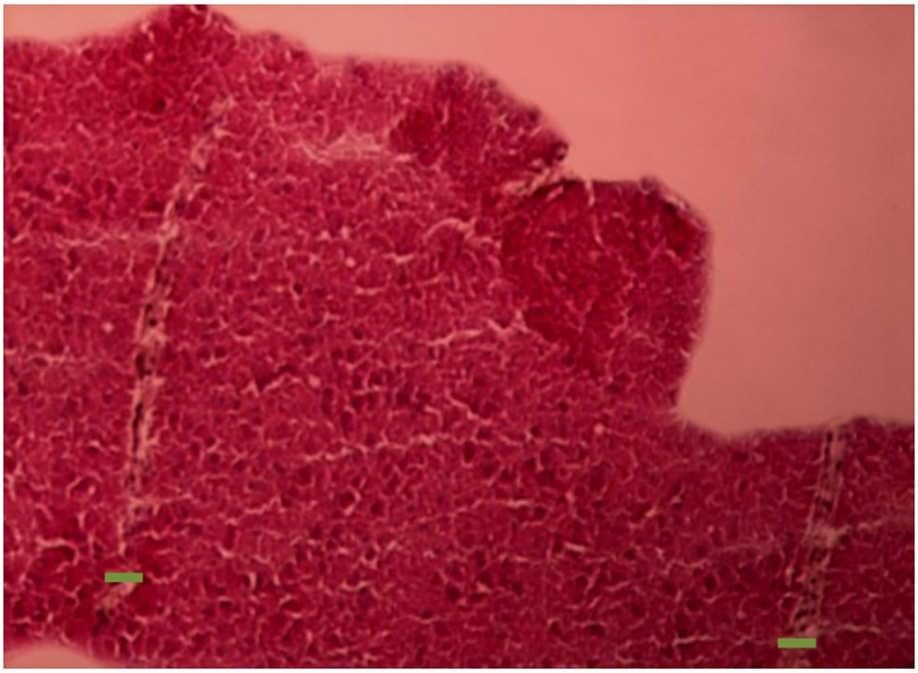

FIGURE 2: Haematoxylin-Eosin staining showing a quasi-surgical cut through the rat cortex 3 months after the delivery a microbeam array (incident dose of 600 Gy, beam thickness: 75µm, spacing: 400 µm).

Scale bars (placed at the bottom of the microbeam paths) : 75 µm

FIGURE 3: A microbeam array dosimetry on gafchromic film.

Dose distribution is characterized by volumes with extremely high peak doses (in the range of several hundreds Gy, color blue) and very low valley doses (less than a few Gy, color white). The fall-out from high to low dose requires few microns. Scale bar: 1 mm

Radiobiology of central nervous system microbeam irradiation

The Central Nervous System (CNS) radiobiology of microplanar beams was first studied about 50 years ago at the Brookhaven National Laboratory (BNL) by Curtis and coworkers. This group was the first to describe the preservation of CNS architecture after incredibly high radiation doses delivered by microscopic deuterium beams. An incident dose of 4000 Gy delivered to the mouse brain by a 25 gm-thin cylindrical microbeam failed to induce radionecrosis, which instead appeared after an incident dose of 140 Gy delivered as a 1-mm thick cylindrical beam [6, 7, 8]. Further work performed in the late nineteen at the National Synchrotron Light Source (NSLS) of the BNL using synchrotron-generated X-ray microbeams, and, later on, at the ESRF, investigated further the tissue tolerance to microscopic beams at doses tens to hundreds time larger than those allowed by conventional macroscopic beams [9, 10, 11, 12].

Unidirectional irradiation using microbeam arrays has been delivered to adult rat brain [11, 13, 15, 16], suckling rats cerebellum [16], piglets cerebellum [1], duckling embryo brain [18], skin and muscle of the mouse leg [19, 20], rat leg [21] and rat spinal cord [12]. The exceptional resistance of the normal-tissue to high dose microbeam irradiation with no evidence of late tissue effects [12, 22, 23, 24] lead to the development of a new concept in radiobiology, the tissue sparing effect, described in the next paragraph.

The tissue-sparing effect

Microbeam irradiation is characterized by minimal side effects on normal tissue adjacent to the irradiated volume. While the cells along the path of penetration of the beams are completely destroyed, the nearby cell bodies remain unaffected with substantial preservation of the tissue architecture. In essence, microbeam irradiation acts like a surgical cut, leaving a scar extending up to the size of the penetrating beams. The lack of normal tissue damage following high dose unidirectional irradiation using arrays of microbeams is referred to as "tissue sparing effect". The tissue sparing effect is bound to the microbeams size: very high doses (up to 4000 Gy) can be delivered through microscopic beams sized 25 to 60 gm with no histological evidence of widespread radionecrosis outside the penetration path. Immunohistochemical studies using

pH2AX show clearly that the neurons hit by the microbeam along his penetration path die almost immediately while the adjacent cells separated by a few microns but outside the high dose volume remain viable (unpublished personal data). Progressively lower doses (but still much higher than conventional radiosurgical or radiotherapeutic doses) are required to avoid tissue damage if ticker beams (100 to 600 gm) are used. Submillimetric beams (sized 0.6 to 0.7 mm) appear to retain the tissue sparing effect allowing to deliver incident doses of 400 Gy to the spinal cord of rats without inducing neurological damage: irradiation of rat spinal cord with four parallel 0.68-mm thick microbeams at 400 Gy in-depth beam dose did not induce paralysis after 7 months in three out of four rats [12]. This study showed not only that a highly radiosensitive structure such as the spinal cord can receive high dose irradiation through a microbeam array without neurologic sequelae but also that a beam width up to 0.68 mm is well tolerated, substantially maintaining the tissue sparing properties of thinner beams.

Microbeam radiosurgery

The ability of microbeam arrays to avoid radionecrosis and to preserve the architecture of the irradiated tissue is mainly attributed to the rapid regeneration of normal microvessels. Only a short segment of the microvascular bed receives ablating doses while the adjacent endothelial cells fall into the valley dose region receiving just a few Gy and can restore quickly the continuity of vascular supply [24]. The wide spatial interface between the unhindered tissue placed in the valleys and the tissue irradiated with peak doses within the microbeam paths facilitates a widespread vascular recolonization of the tissue receiving necrotic doses preventing the dissolution of the architecture of the irradiated tissues [3]. The self-repair of the normal microvasculature through the migration of unaffected cells surrounding the paths of microbeam penetration is considered by most as the basis for this ability of normal tissue to tolerate high dose microbeam irradiation [25, 26]. The tolerance of the vascular bed to high dose microbeam irradiation has been clearly demonstrated by the lack of extravasation of dyes administered to the experimental animals, which remained confined in the vessels after irradiation from 12h until three months following 1000 Gy [27]. This radioresistance phenomenon was not observed in 9L glioma microvessels, confirming the presence of a differential response to between normal and tumour brain tissues in rodents, an effect that can have significant clinical applications [19]. The neoplastic vasculature appears to be unable to replicate the fast repair of the segments hit by the peak dose, facilitating the development of radionecrosis over the irradiated tumor [12, 20, 28].

The unidirectional delivery of microbeam arrays to parts of the body harbouring neoplastic tissue is known as Microbeam Radiation Therapy (MRT). MRT irradiation has been carried out to deliver very high radiation doses into tumours in a single fraction through an unidirectional approach [11, 15]. Most of the experimental activity performed until now has been focused on the study of the effects of arrays of parallel microbeams [4, 5, 9-24, 28-30]. MRT has been applied to several tumor models including intracerebral gliosarcoma (9LGS) in rats [11, 13, 30], murine mammary carcinoma (EMT-6) [19] and human squamous-cell carcinoma (SCCVII) in mouse and rat [9, 20]. Rats bearing the intracranial 9L gliosarcoma irradiated anteroposteriorly with arrays composed of 27 gm thick microbeams spaced 100 gm on-center showed a clear survival advantage after MRT: 4 out of 14 rats irradiated at 625 Gy incident doses were long-term survivors with little brain damage revealed in histopathology [11]. The introduction of stereotactic techniques to deliver arrays of microbeams that interlace over a selected target volume [31, 12] has recently opened a new field of research that is by most considered as an extension of MRT but is much closer to the stereotactic radiosurgery experience.

The authors prefer to name this technique characterized by the stereotactic delivery of microbeams to a selected target volume as Microbeam RadioSurgery (MRS). MRS is currently performed at the ESRF biomedical beamline by: 1) directing beams to the target in a convergent isocentric fashion (creating a hot spot where the dose is enhanced by the overlapping of the beams) or 2) by interlacing 2 to 4 microbeam arrays over the target. MRS has been used at ESRF to ablate selected volumes (such as the subthalamic nucleus, the substantia nigra and the caudate nucleus) into the rat brain using both approaches. The ability to induce precise lesions in the rat brain using an image-guided non-invasive approach opens new ways to create experimental models of disease. For example MRS nigrotomies could offer a novel experimental model to study Parkinson's disease (PD): experiments are underway to assess if MRS can replace or complement other PD experimental models of PD based on the induction of chemical lesioning of the Substantia nigra. A novel way to use microbeam arrays in a quasi-surgical way is currently being developed at ESRF: convergent or parallel arrays of microbeams carrying high doses are placed over selected cortical areas in order to hit tangentially and cut the horizontal axons connecting adjacent cortical columns. Cortical transections are a surgical procedure to parcellize an epileptic focus located in eloquent cortex [32, 33]. Cutting the horizontal axons required for the spreading of epileptic activity is an effective way to control the seizures without inducing neurologic dysfunction. Synchrotron-generated microbeams can be used to create cortical transections in rats offering a chance to study the tolerance of CNS to this technique.

This novel experimental application of microbeams provides a new and attractive tool to modulate cortical function by transecting the fibers connecting the cortical columns. Aside from the tight dosimetry, the relative low energy of the microbeams (continuous X-ray spectrum ranging from 50 to -350 keV, mean energy -100 keV) makes them well suited to treat superficial targets within the cortex. Microbeam transections, either placed over neocortical seizure foci or through the hippocampus, could prove to be an

excellent tool to be added to the current radiosurgical techniques used to control seizures. A series of experiments are currently planned at ESRF aiming at the development of an experimental model of microbeam cortical transections in epileptic rats with the goal of verifying the ability of microbeam cortical transections to control seizures without damage to the eloquent cortex irradiated. Figures *4*, *5* illustrate the irradiation room and stereotactic set-up at ESRF.

FIGURE 4: Irradiation room at ESRF, located at about 45 meters from the synchrotron radiation wiggler source.

The sample positioning system is a Kappa-goniometer, that combined with an X-ray on-line X-ray detection system, allows for a submillimetric identification of the target. On the picture, a water tank for dosimetry is positioned on the Kappa goniometer.

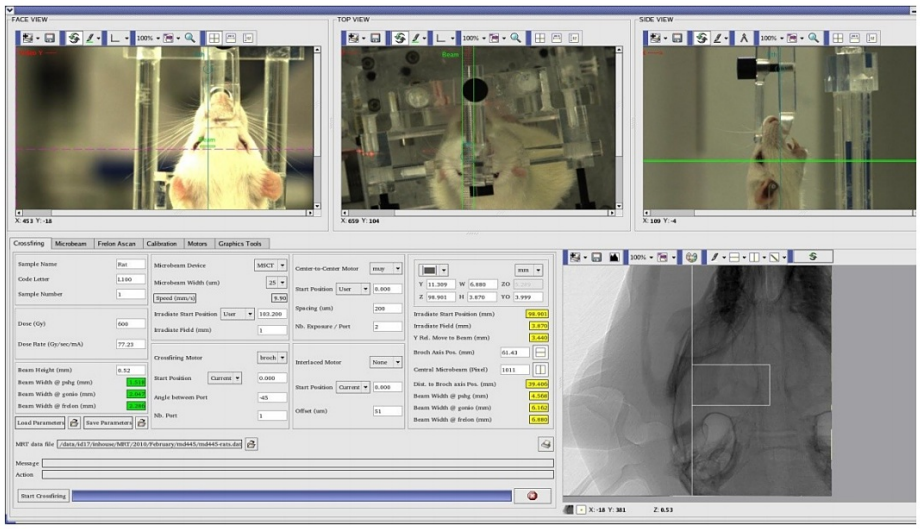

FIGURE 5: Stereotactic set-up used for rat brain irradiation.

For the prepositioning of the target,based on external markers, 3 high resolution video-cameras are installed. Bottom right: the X-ray radiography, taken just before the irradiation, allows for the submillimetric identification of the target.

Conclusions

The irradiation of normal brain and spinal cord with microbeam arrays is characterized by a distinct tissue-sparing effect. Peak doses between 300 and 600 Gy are in most cases well tolerated by the CNS with little or

no histological evidence of brain damage. Fast sprouting and recovery of the microvascular bed has been observed in normal CNS tissue while the inability of neoplastic vascular network to repair itself exposes neoplastic tissue to an enhanced tumoricidal effect. Microbeam radiosurgery is able to induce non-invasively microradiosurgical lesions in the rat brain, thus offering a new way to develop experimental models to study PD, Huntington's disease and many other CNS disorders. A recent development of microbeam research is the use of arrays of microbeams to induce cortical transections aiming to modulate cortical function through the selective cutting of horizontal axons connecting adjacent columns.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

Authors sincerely thank Dr. H. Requardt, and Dr. C. Nemoz for their invaluable support for performing the preclinical trials at the ESRF, Dr. M. Renier, T. Brochard, and G. Berruyer for the mechanical and software developments, Dr. G. Le Duc and D. Dallery for their precious help in the biological experiments and animal care.

References

1. Brauer-Krisch E, Requardt H, Brochard T, Berruyer G, Renier M, Laissue JA, Bravin A: New technology enables high precision multislit collimators for microbeam radiation therapy. Rev Sci Instrum. 2009, 80:10.1063/1.3170035
2. Prezado Y, Thengumpallil S, Renier M, Bravin A: X-ray energy optimization in minibeam radiation therapy. Med Phys. 2009, 36:4897-4902.
3. Romanelli P, Bravin A: Synchrotron-generated microbeam radiosurgery: a new experimental approach to modulate brain function. Neurol Res. 10.1371/journal.pone.0053549
4. Siegbahn EA, Brauer-Krisch E, Stepanek J, Blattmann H, Laissue JA, Bravin A: Determination of dosimetrical quantities used in microbeam radiation therapy (MRT) with Monte Carlo simulations. Med Phys. 2006, 33:3248-3259.
5. Martinez-Rovira I, Sempau J, Fernandez-Varea JM, Bravin A, Prezado Y: Monte Carlo dosimetry for forthcoming clinical trials in x-ray microbeam radiation therapy. Med Biol. 2010, 55:4375-88. 10.1088/0031-9155/55/15/012
6. Zeman W, Curtis H, Baker CP: Histopathologic effect of high-energy-particle microbeams on the visual cortex of the mouse brain. Radiat Res. 1961, 15:496-514.
7. Curtis HJ: The interpretation of microbeam experiments for manned space flight . Radiat Res Suppl. 1967, 7:258-264. 10.1118/1.3049786
8. Curtis HJ: The use of deuteron microbeam for simulating the biological effects of heavy cosmic- ray particles. Radiat Res Suppl. 1967, 7:250-257. 10.1118/1.3049786
9. Slatkin DN, Spanne P, Dilmanian FA, Sandborg M: Microbeam radiation therapy. Med Phys. 1992, 19:1395-1400.
10. Slatkin DN, Spanne P, Dilmanian FA, Gebbers JO, Laissue JA: Subacute neuropathological effects of microplanar beams of x-rays from a synchrotron wiggler. Proc Natl Acad Sci U S A. 1995, 92:8783-8787. 10.1073/pnas.92.19.8783
11. Laissue JA, Geiser G, Spanne PO, Dilmanian FA, Gebbers JO, Geiser M, Wu XY, Makar MS, Micca PL, Nawrocky MM, Joel DD, Slatkin DN: Neuropathology of ablation of rat gliosarcomas and contiguous brain tissues using a microplanar beam of synchrotron-wiggler-generated X rays. Int J Cancer. 1998, 78:654-660.
12. Dilmanian FA, Zhong Z, Bacarian T, Benveniste H, Romanelli P, Wang R, Welwart J, Yuasa T, Rosen EM, Anschel DJ: Interlaced x-ray microplanar beams: a radiosurgery approach with clinical potential . Proc Natl Acad Sci U S A. 2006, 103:9709-9714. 10.1073/pnas.0603567103
13. Slatkin DN, Spanne P, Dilmanian FA, Gebbers JO, Laissue JA: Subacute neuropathological effects of microplanar beams of x-rays from a synchrotron wiggler. Proc Natl Acad Sci U S A. 1995, 92:8783-8787.
14. Dilmanian FA, Button TM, Le Duc G, Zhong N, Peria LA, Smith JA, Martinez SR, Bacarian T, Tammam J, Ren B, Farmer PM, Kalef-Ezra J, Micca PL, Nawrocky MM, Niederer JA, Recksiek FP, Fuchs A, Rosen EM: Response of rat intracranial 9L gliosarcoma to microbeam radiation therapy . Neuro Oncol. 2002, 4:26-38. 10.1093/neuonc/4.1.26
15. Dilmanian FA, Qu Y, Liu S, Cool CD, Gilbert J, Hainfeld JF, Kruse CA, Laterra J, Lenihan D, Nawrocky MM, Pappas G, Sze CI, Yuasa T, Zhong N, Zhong Z, McDonald JVV: X-ray microbeams: Tumor therapy and central nervous system research. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment. 2005, 548:30-37. 10.1016/j.nima.2005.03.062
16. Laissue JA, Lyubimova N, Wagner HP, Archer DW, Slatkin DN, Di Michiel M, Nemoz C, Renier M, Brauer E, Spanne PO, Gebbers J-0, Dixon K, Blattmann H: Microbeam radiation therapy, in medical applications of penetrating radiation. Proceedings of SPIE. Bradford Barber H, Roehrig H (ed): 1999. 3770:38-45.
17. Laissue JA, Blattmann H, Di Michiel M, Slatkin DN, Lyubimova N, Guzman R, Zimmermann W, Birrer S, Bley

T, Kircher P, et al.: Penetrating Radiation Systems and Applications III . SPIE Conference Proceeding. Barber HB, Roehrig H, Doty FP, Schirato RC, Morton EJ (ed): Bellingham, WA; 2001. 4508:65-73. 10.1117/12.450782

18. Dilmanian FA, Morris GM, Le Duc G, Huang X, Ren B, Bacarian T, Allen JC, Kalef-Ezra J, Orion I, Rosen EM, Sandhu T, Sathe P, Wu XY, Zhong Z, Shivaprasad HL: Response of avian embryonic brain to spatially segmented x-ray microbeams. Cell Mol Biol. 2001, 47:485-493.
19. Dilmanian FA, Morris GM, Zhong N, Bacarian T, Hainfeld JF, Kalef-Ezra J, Brewington LJ, Tammam J, Rosen EM: Murine EMT-6 carcinoma high therapeutic efficacy of microbeam radiation therapy . Radiat Res. 2003, 159:632-641.
20. Miura M, Blattmann H, Brauer-Krisch E, Bravin A, Hanson AL, Nawrocky MM, Micca PL, Slatkin DN, Laissue JA: Radiosurgical palliation of aggressive murine SCCVII squamous cell carcinomas using synchrotron-generated X-ray microbeams. Br J Radiol. 2006, 79:71-75. 10.1259/bjr/50464795
21. Zhong N, Morris GM, Bacarian T, Rosen EM, Dilmanian FA: Response of rat skin to high-dose unidirectional x-ray microbeams: a histological study. Radiat Res. 2003, 160:133-142. 10.1667/3033
22. Laissue JA, Blattmann H, Wagner HP, Grotzer MA, Slatkin DN: Prospects for microbeam radiation therapy of brain tumours in children to reduce neurological sequelae. Dev Med Child Neurol. 2007, 49:577-581. 10.1111/j.1469-8749.2007.00577.x
23. Dilmanian FA, Qu Y, Feinendegen LE, Peria LA, Bacarian T, Henn FA, Kalef-Ezra J, Liu S, Zhong Z, McDonald JVV: Tissue-sparing effect of x-ray microplanar beams particularly in the CNS: is a bystander effect involved?. Exp Hematol. 2007, 35:69-77. 10.1016/j.exphem.2007.01.014
24. Blattmann H, Gebbers J-0, Brauer-Krisch E, Bravin A, Le Duc G, Burkard W, Di Michiel M, Djonov V. Slatkin DN, Stepanek J, Laissue JA: Applications of synchrotron X-rays to radiotherapy . Nucl Instr Meth Physics Res A. 2005, 548:17-22. 10.1016/j.nima.2005.03.060
25. Gabbiani G, Gabbiani F, Heimark RL, Schwartz SM: Organization of actin cytoskeleton during early endothelial regeneration in vitro. J Cell Sci. 1984, 64:39-50.
26. Serduc R, Christen T, Laissue J, et al.: Brain tumor vessel response to synchrotron microbeam radiation therapy: a short-term in vivo study. Phys Med Biol. 2008, 53:3609-3622. 10.1088/0031-9155/53/13/015
27. Serduc R, Verant P, Vial JC, Farion R, Rocas L, Remy C, Fadlallah T, Brauer E,Bravin A, Laissue J, Blattmann H, van der Sanden B: In vivo two-photon microscopy study of short-term effects of microbeam irradiation on normal mouse brain microvasculature. Int J Radiat Oncol Biol Phys. 2006, 64:1519-27. 10.1016/j.ijrobp.2005.11.047
28. Smilowitz HM, Blattmann H, Brauer-Krisch E, Bravin A, Di Michiel M, Gebbers J-0, Hanson AL, Lyubimova N, Slatkin DN, Stepanek J, Laissue JA: Synergy of gene-mediated immunoprophylaxis and microbeam radiation therapy (MRT) for advanced intracerebral rat 9L gliosarcomas. J Neurooncol. 2006, 78:135-143.
29. Spiga J, Siegbahn EA, Brauer-Krisch E, Randaccio P, Bravin A: The GEANT4 toolkit for microdosimetry calculations: application to microbeam radiation therapy (MRT). Med Phys. 2007, 34:4322-30. 10.1118/1.2794170
30. Regnard P, Le Duc G, Brauer-Krisch E, et al.: Irradiation of intracerebral 9L gliosarcoma by a single array of microplanar x-ray beams from a synchrotron: balance between curing and sparing. Phys Med Biol. 2008, 53:861-878. 10.1088/0031-9155/53/4/003
31. Brauer-Krisch E, Requardt H, Regnard P, Corde S, Siegbahn E, LeDuc G, Brochard T, Blattmann H, Laissue J, Bravin A: Exploiting geometrical irradiation possibilities in MRT application . Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment. Menk RH, Arfelli F, Bertocchi L, Castelli E, Longo R, Tromba G (ed): Elsevier B.V., 2005. 548:69-71.
32. Morrell F, Whisler WW, Bleck TP: Multiple subpial transection: a new approach to the surgical treatment of focal epilepsy. J Neurosurg. 1989, 70:231-239. 10.3171/jns.1989.70.2.0231
33. Orbach D, Romanelli P, Devinsky 0, Doyle W: Late seizure recurrence after multiple subpial transections . Epilepsia. 2001, 42:1130-3. 10.1046/j.1528-1157.2001.45300.x