# Micro-Slit X-Ray Irradiation Reveals Testicular Tissue-Sparing Effects: An Attempt at High-Precision Radiotherapy for Male Fertility Preservation

The preservation of male fertility during or after radiotherapy has long been desired to improve the quality of life for cancer survivors during their reproductive years. To approach this clinical issue, we focused on the tissue-sparing effect (TSE) in the testes in response to microbeam radiotherapy (MRT). In this study, we used *ex vivo* testicular tissue cultures obtained from *Acr-GFP* transgenic mice and revealed, for the first time, the significant TSE of high-precision MRT for maintaining spermatogenesis using live-tissue fluorescence imaging. This suggests that MRT is a promising approach for preserving male fertility.

The X-ray microbeam technique in the Photon Factory enables the targeting of particular living cells, or even subcellular regions, in a cell population. To date, studies have revealed various important aspects of the biological effects of low-dose irradiation, such as bystander cell-lethal effects or cell cycle modifications of nontargeted cells [1-4]. In 1909, Alban Köhler reported on clinical observations of the tissue-sparing response during grid radiotherapy, in which spatially fractionated radiation is delivered using a grid-like pattern of beams [5]. Since the establishment of the fundamental concept of microbeam radiotherapy (MRT) in the 1990s [6], which is based on the spatial fractionation of synchrotron-generated X-ray microbeams at the microscale level, a notable tissue-sparing effect (TSE) following MRT has been confirmed in various species and tissue types [7]. We have applied this concept to preserve reproductive potential following radiation treatment.

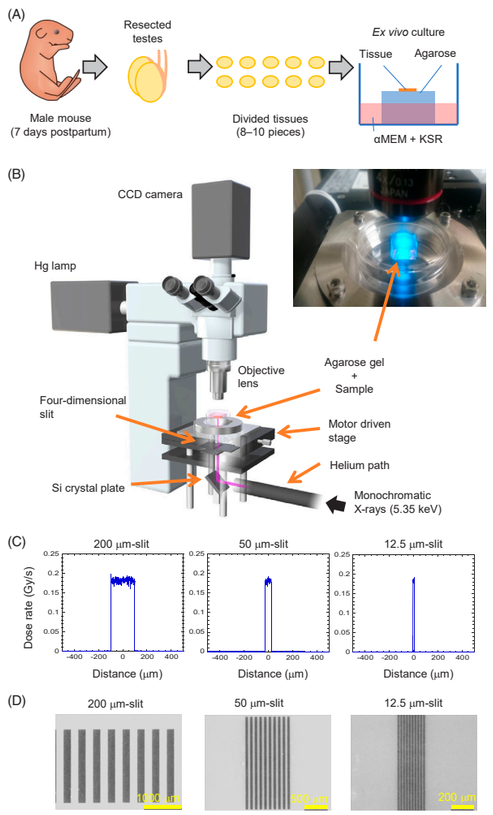


**Figure 1: (A)** Schematic representation of *ex vivo* testicular tissue culture. **(B)** Experimental setup of the micro-slit X-ray irradiation at the BL-27B. **(C)** Dose profiles of the 200, 50, and 12.5 μm-width microbeams, calculated with a Particle and Heavy Ion Transport code System (PHITS). **(D)** Dose profiles of the 200, 50, and 12.5 μm-width microbeams, confirmed using Gafchromic XR-RV3 radiochromic film. Scale bars, 1000, 500, and 200 μm.

Recent advances in organ culture offer the potential for producing samples that mimic our actual bodies for clinical micro-irradiation therapy *ex vivo*. We have intensively investigated the TSE of MRT on cultured testicular tissues obtained from *Acr-GFP* transgenic mice [8, 9]. In this *ex vivo* testes culture system, spermatogenesis can be easily monitored in pieces of tissue by observing the expression of acrosome-green fluorescent protein (Acr-GFP), which is a meiosis-specific biomarker. In this study, testes samples were obtained from 7 days postpartum (dpp) mice, and each sample was cut into 8–10 tissue pieces approximately 1 $mm^3$ in size. Each tissue piece was placed on an agarose gel block immersed in a culture medium [**Fig. 1(A)**]. After removing the medium, the sample dish was reversed and set on the irradiator at the BL-27B. The sample kept tenaciously attaching to the gel block during exposure to the micro-slit X-ray beams. The 5.35-keV monochromatic X-rays were guided in the horizontal direction through a helium path pipe and reflected in the upward direction by Bragg diffraction of a single Si crystal plane (311) [**Fig. 1(B)**]. Approximately 50% of the sample areas were exposed to the micro-slit X-ray beams with widths of 200, 50, and 12.5 μm (center-to-center distances of 400, 100 and 25 μm, respectively) [**Fig. 1(C, D)**]. The dose absorbed by the exposed areas was determined to be 5 Gy from the photon fluence measured by a silicon photodiode. Thus, the total energies deposited in the whole samples, namely, the absorbed doses, were similar for the three beams, namely, an average dose of 2.5 Gy. After exposure, the GFP fluorescence from the samples was observed in real time by a fluorescence microscope for 15 days to investigate the spermatogenesis function of the sample.

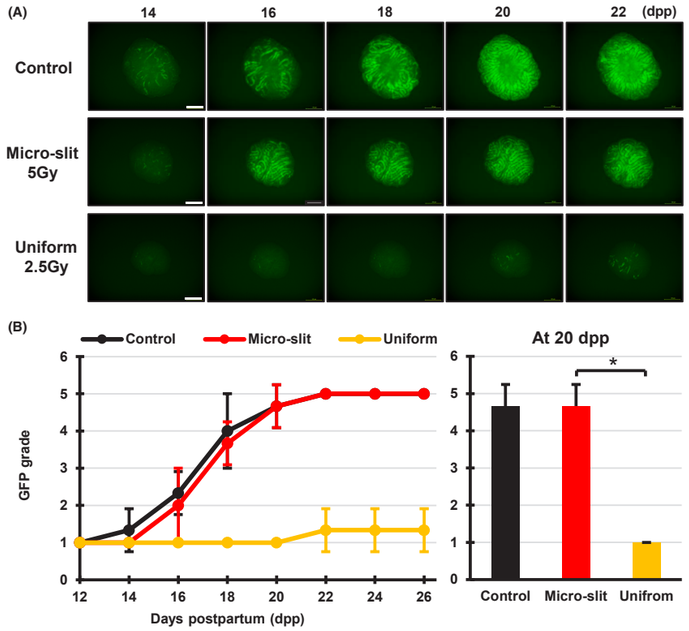


**Figure 2: (A)** Tissue-sparing effect of micro-slit X-rays during spermatogenesis. Representative images show Acr-GFP expression changes in single cultures following 0 Gy (control), 5 Gy micro-slit, and 2.5 Gy uniform X-ray irradiation, from 14 to 22 days postpartum (dpp). Scale bars, 500 μm. **(B)** Chronological changes in Acr-GFP expression after irradiation. A minimum of three tissue samples each from different donor mice were used for each experiment. Data represent the mean GFP expression ± SD. Asterisk indicates P<0.01.

Typical image data obtained are shown in **Fig. 2**. Our live-tissue fluorescence imaging revealed that the dose of 2.5 Gy applied in uniform mode almost completely obliterated the fluorescence signal of Acr-GFP from 14 to 22 dpp, whereas in the case of micro-slit (200 μm width) irradiation, the fluorescence signal remained similar to that of non-irradiated controls, indicating the occurrence of a significant TSE for the preservation of spermatogenesis. This is the first study to visualize the TSE for spermatogenesis in response to non-uniform radiation fields.

Also, we found that the TSE depended on the slit width. The GFP expression significantly differed for the 12.5 μm-slit irradiated tissue when compared with either the 200 or 50 μm-slit irradiated ones. Because the average diameter of spermatogonial cells at 7–8 dpp is approximately 15 μm, some of the spermatogonial cells were unirradiated in the 200 and 50 μm-slit irradiated tissues, whereas almost all the spermatogonial cells were partially or completely irradiated in the 12.5 μm-slit irradiated tissues. This suggests that the effective TSE for spermatogenesis required a non-irradiated spermatogonial cell population. Surviving germ stem cells in the non-irradiated fields may migrate to the irradiated area and colonize it to restore the function, producing the TSE of MRT for maintaining spermatogenesis.

Taken together, our findings showed that the distribution of the irradiation dose in the testes at the microscale level is highly important for preserving male fertility. The technical combination of high-precision MRT and specific *ex vivo* organ models will be useful in further investigations of the radiation-induced acute and late effects on specific physiological functions.

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## BEAMLINE

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