DNA damage on pBR322 plasmid induced by VHEE at ultra-high and conventional dose rate and comparison to IEE and proton beams

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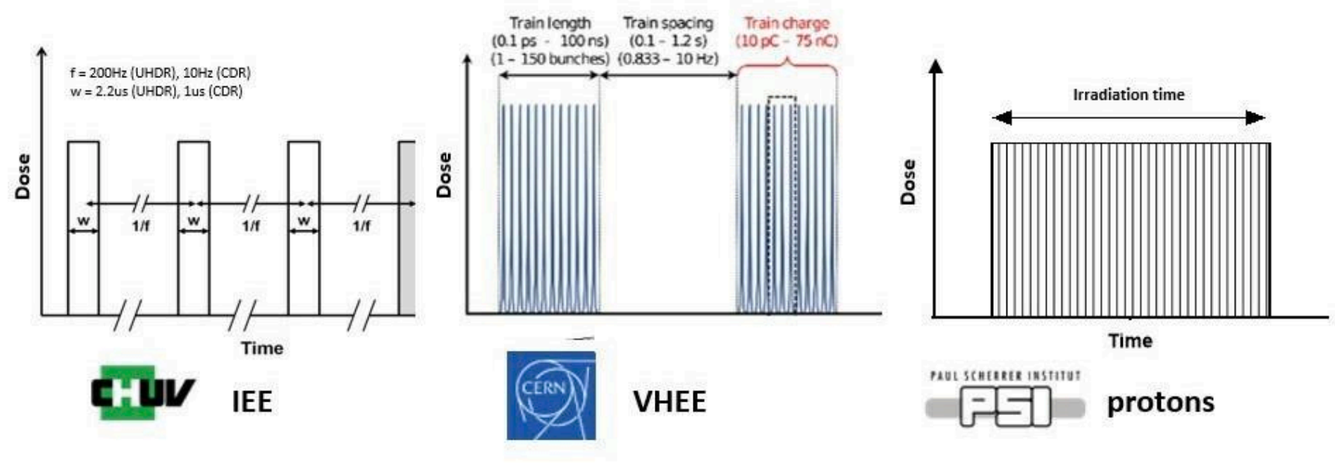
Introduction

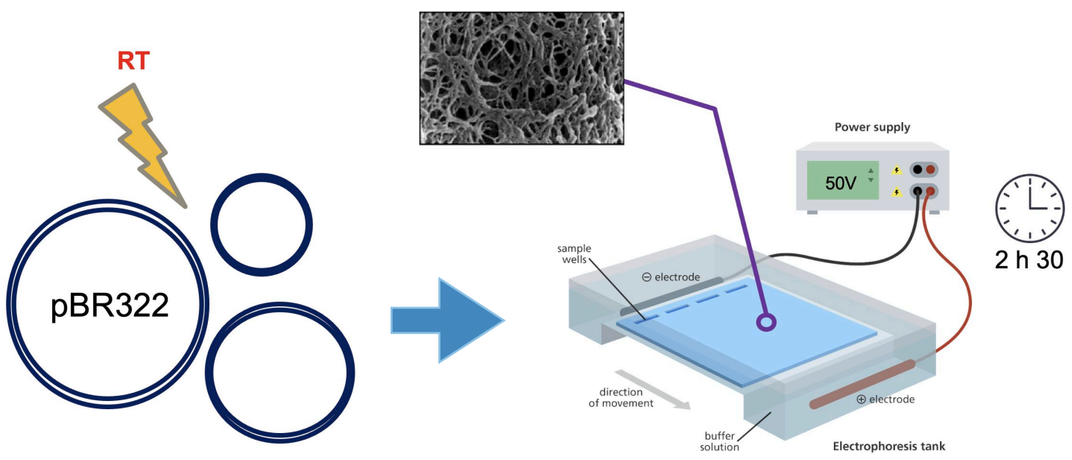
- We investigate the effect of irradiation on plasmid DNA
- We irradiated pBR322 plasmid with the CLEAR VHEE beam line at CERN.
- We compare the effect of the VHEE beam with two other beams able to operate at ultra-high dose rate (UHDR) and conventional dose rate (CONV) who have already been FLASH validated in vivo (Montay-Gruel, 2017; Almeida, 2022)
- We investigate normoxic (21% oxygen) and hypoxic (1% oxygen) conditons as well as the presence of scavengers (DMSO 14mM).

Beams



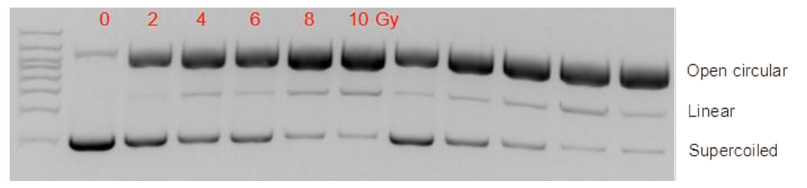
- CLEAR (220MeV, CONV: 0.13 Gy/s, UHDR: 455'000 Gy/s). VHEE, pulsed beam
- eRT6/Oriatron (6 MeV CONV: 0.1 Gy/s, UHDR: >556 Gy/s). IEE, pulsed beam
- Gantry 1/Proton (230MeV, CONV: 1Gy/s, UHDR: 1400 Gy/s). proton, semi-continuous

Methodology

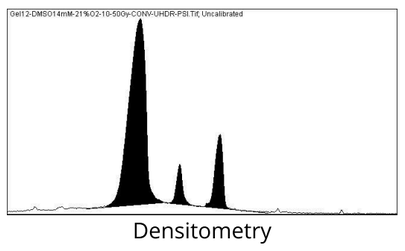


Irradiation in UHDR or CONV of commerical (ThermoFisher) pBR322 plasmid. Purified by membrane dialysis

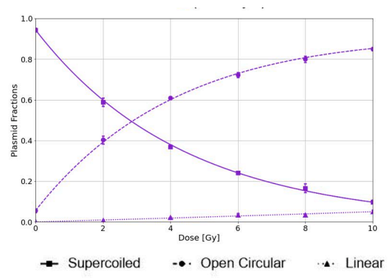
Gel Electrophoresis in a 4% agarose gel



Migration rate depends on plasmid size. Size depends on damage.
Supercoiled = no damage, Open Circular = SSB, Linear = DSB

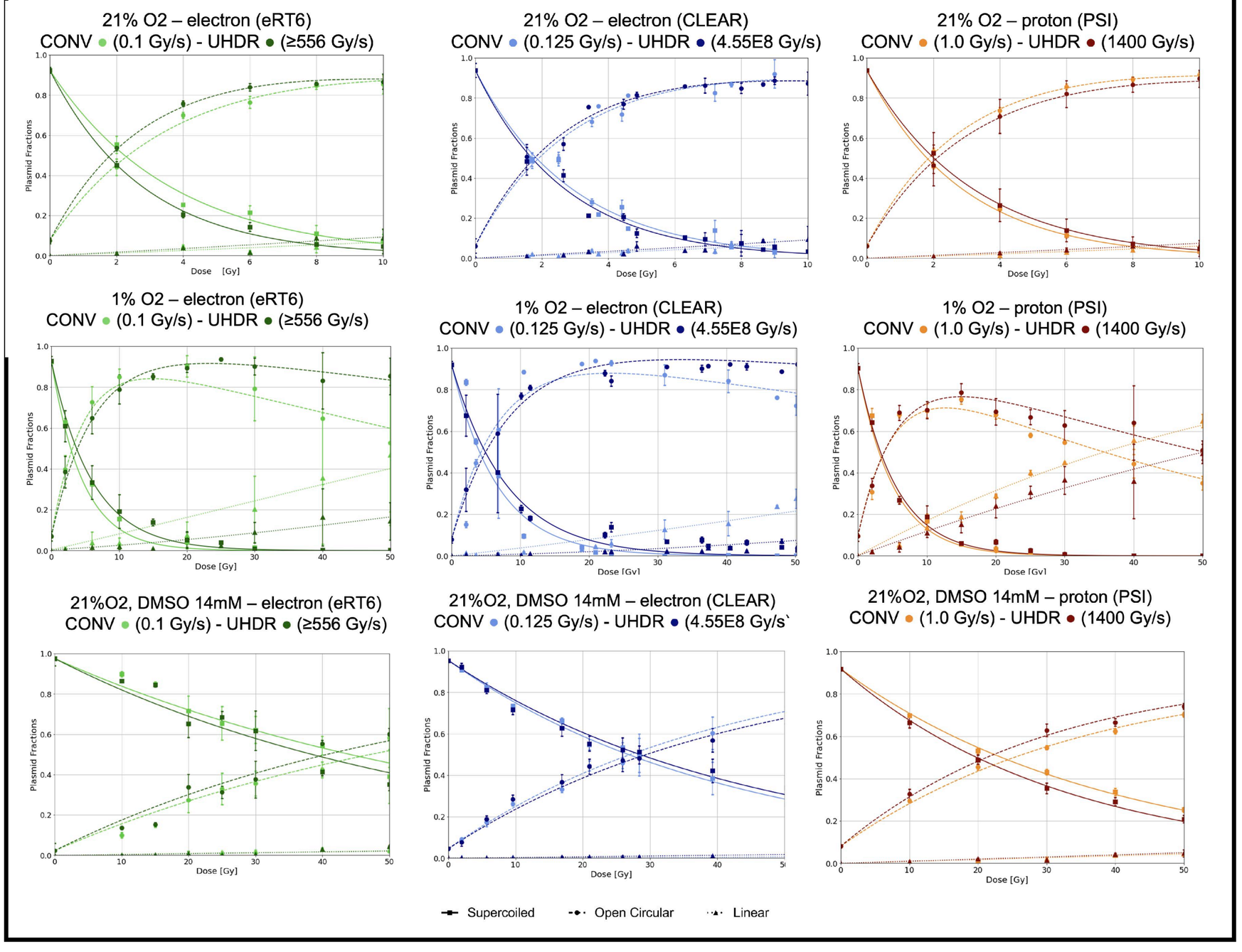


Densitometry



Fitting to McMahon model (McMahon & Curell 2011)

Results



Conclusion

- Under normoxic conditions (21% O2) the pattern of DNA strand-break pattern was similar with all beams, was found to be dose-dependent and dose-rate-independent.
- Under hypoxia (~1% O2) and at higher doses (>20 Gy) less damages were found at UHDR.
- The addition of scavengers modified the damages patterns but did not modify the dose-rate-response.
- **VHEE induced similar DNA damage as IEE, and proton beams on pBR322 plasmid in all observed conditions. Conversely to what has been reported in the recent literature and using FLASH-validated beams and clinically relevant doses (10 Gy), DNA strand breaks on plasmids was found to be dose-rate independent.**

Perspective

The mechanisms causing the sparing at UHDR remain to be investigated.

References

Montay-Gruel P, Petersson K, Jaccard M, Boivin G, Germond JF, Petit B, Doenlen R, Favaudon V, Bochud F, Bailat C, Bourhis J, Vozenin MC. Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100Gy/s. Radiother Oncol. 2017 Sep;124(3):365-369. doi: 10.1016/j.radonc.2017.05.003. Epub 2017 May 22. PMID: 28545957.

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McMahon SJ, Currell FJ. A robust curve-fitting procedure for the analysis of plasmid DNA strand break data from gel electrophoresis. Radiat Res. 2011 Jun;175(6):797-805. doi: 10.1667/RR2514.1. Epub 2011 Apr 5. PMID: 21466384.