Mechanical Characterization of Conventional and FLASH Irradiated Cryogel Scaffolds

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DISCLOSURES: Annika Nikhar (N), Peter Bertone (N), Katherine R. Hixon (N)

INTRODUCTION: Nearly 50% of cancers originating in organs such as the breast, prostate, and lungs spread to the skeleton, making bone a common site for cancer metastasis. Radiation therapy (RT) is a common treatment for metastatic bone disease (MBD); however, conventional external beam radiation therapy (EBRT) often results in adverse effects including soft tissue fibrosis, reduced bone density, and osteonecrosis, leading to an overall weakening of the bone tissue and compromised recovery. Alternatively, FLASH RT involves the delivery of ultrahigh-dose-rate radiation, potentially reducing tissue toxicity commonly associated with conventional EBRT. However, it is unknown whether FLASH offers benefits, compared to conventional radiation, in preserving osteogenic potential in MBD-affected bone. Thus, combined treatments involving bone regeneration in the presence of radiation therapies could revolutionize MBD care by improving healing potential and reducing treatment failures. Biomaterial cryogel scaffolds are macroporous and mechanically durable sponge-like structures that support rich cellular proliferation and integration with surrounding tissue. It is unknown whether irradiation affects the cryogel physical properties and if material degradation occurs over time from radiation exposure. We *hypothesize* that exposure to clinically relevant doses of conventional EBRT or FLASH RT will significantly decrease cryogel mechanical strength and durability.

METHODS: Chitosan-gelatin (4%; 1:4) solution was crosslinked with glutaraldehyde and frozen at -20°C for 18 hours. Scaffolds were then thawed and lyophilized overnight to produce dry, macroporous constructs. These cryogels were irradiated with EBRT (X-Rad 320) or FLASH (Trilogy linac) irradiator systems and exposed to 25, 39, or 48 gray (GY) dose fractions over 1-, 3-, or 6-day intervals, respectively (Fig. 1). Note that these values are based on clinically relevant RT doses. Non-irradiated scaffolds served as controls. Scaffolds were compressed to 50% displacement (Instron 5544) to characterize tensile strength, and resulting Young's moduli were recorded. Additionally, rheology testing can provide a precise characterization of cryogels' deformation under imposed stress; shear and tensile tests define a material's stress limits through moduli G' and G'', which represent the material's elastic and plastic components, respectively. Shear tests were conducted to characterize the scaffold's deformation and viscoelasticity under sweeps of varying frequency and amplitude (Fig. 2). Ongoing work involves a cyclic loading degradation study, where cryogels are subject to 20 oscillations from 5% to 20% displacement over a one-month period. Measurements are taken at 1-, 3-, 7-, 14-, and 28-day intervals to assess for changes in load bearing stress and energy loss over time.

RESULTS: Both hydrated and dry scaffolds subjected to EBRT at all three dose fractions demonstrated no significant differences between compressive moduli, regardless of exposure amount ($p>0.05$). However, an increase in compressive strength trend was observed as irradiation intensity increased for both EBRT and FLASH RT (Fig. 3A). Additionally, dry cryogels appeared to have greater strength compared to their hydrated counterparts. Cryogels subjected to FLASH exhibited significant differences as radiation intensity increased (Fig. 3B); the 1x25 dry group demonstrated significantly decreased compressive strength compared to the 1x25 hydrated group and the 3x13 hydrated group ($p<0.05$). Although non-significant, the 6x8 and 3x13 groups again presented increased load-bearing strength among hydrated scaffolds subject to FLASH RT. Cryogels subjected to FLASH RT also demonstrated overall greater compressive strength compared to their conventionally irradiated counterparts. Preliminary rheology testing revealed a higher G' moduli compared to G'' for non-irradiated, conventional, and FLASH irradiated scaffolds. Cryogels demonstrated greater elastic behavior compared to plastic, indicating their retention of mechanical energy upon deformation. Scaffolds maintained their macroporous and semi-rigid shape following shearing and compression.

DISCUSSION: Characterization of mechanical properties is integral to assessing cryogel functionality. A scaffold's capacity for load bearing stress is vital to its interactions with adjacent bone tissue where durability and flexibility are needed to provide structural support and promote cellular integration. Overall, cryogels demonstrated an increase in compressive strength as RT intensity increased. It is uncertain whether increased RT intensity or decreased total RT exposure contributes to the scaffolds' increased compressive strength. Ambiguity in results may be attributed to small sample sizes and insensitive machine parameters, which likely minimized distinctions among the strengths of varying scaffold types. The application of rheology may allow for characterization of the cryogels' mechanical properties with greater precision. Ongoing work includes shearing tests, involving oscillating sweeps of varying frequency and amplitude. Such tests can quantify the material's elasticity, plasticity, and incompressibility, providing a clearer picture of cryogels' physical behavior. Additionally, ongoing cyclic loading studies aim to characterize changes in compressibility and energy loss properties over time. Scaffolds will undergo compressive oscillations at regular intervals over a one-month period. Both studies include conventional and FLASH irradiated cryogels to assess which method of RT is better suited for tissue regeneration (i.e., minimizing damage of the scaffold mechanical properties).

SIGNIFICANE/CLINICAL RELEVANCE: Combined with existing therapies of MBD, such as RT, cryogels can provide an enhanced treatment option to support tissue regeneration. This study aims to evaluate the mechanical properties of cryogels and their potential to support bone formation in the presence of RT. This is a necessary evaluation so that patients may receive RT following MBD resection to ensure the appropriate amount of MBD tissue is removed, while supporting new bone growth.

REFERENCES: [1] Huang, J.F., et al. Annals of translational medicine (2020). [2] Kraus, E., et al. J. R. Soc. Interface (2022). [3] Tiffany, K., et al. Engineered Regeneration (2020).

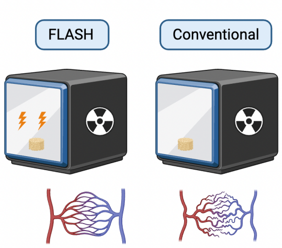

Figure 1. Comparison of FLASH and conventional radiation therapy. Emerging FLASH RT irradiates at ultrahigh doses, potentially reducing tissue toxicity commonly associated with conventional EBRT.

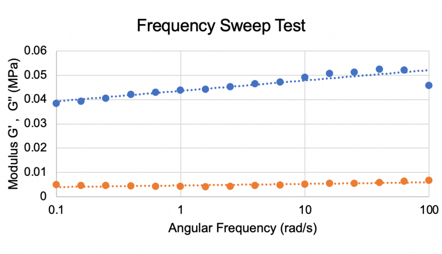

Figure 2. Cryogel frequency sweep test. Non-irradiated cryogel subjected to frequency sweep shearing test. Angular frequency was increased from 0.1 rad/s to 100 rad/s; storage (G'), or elastic, and loss (G''), or plastic, moduli plotted in MPa. Data graphed along a logarithmic scale.

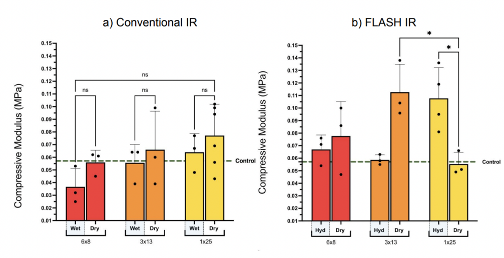

Figure 3. Compression tests of irradiated cryogels. A) Conventionally irradiated cryogel. B) FLASH irradiated cryogel. Dry and hydrated scaffolds were compressed to 50% displacement; resulting Young's moduli was plotted against radiation dosage regime (number of days x Gy per day). Compressive strength increased with RT intensity.