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Radiation injury triggers a cutaneous scarring response, inducing expression of pro-fibrotic genes and increasing collagen thickness in an ex vivo human skin model

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ABSTRACT

Radiation-induced skin fibrosis (RISF) is a devastating outcome of cutaneous injury by irradiation causing diminished quality of life for patients. Though animal models for RISF have been developed, experimental models of evolving RISF in human skin are needed to identify biomarkers that reflect the development, progression, and severity of RISF in patients. Skin from human subjects (n=4) was irradiated with doses from 0-8 Gy, maintained at air-liquid interface, and collected over a 7-day period. Skin viability was assessed every 1-2 days. Pro-fibrotic response was detected via quantitative real-time polymerase chain reaction (qPCR) of four fibrosis-associated genes: actin alpha 2 smooth muscle (ACTA2), collagen alpha-1 chain (COL1A1), connective tissue growth factor (CTGF) and fibronectin 1 (FN1). Total collagen levels were measured by hydroxyproline assay. Global fibrotic response was captured by measurement of dermal collagen bundles using an automated quantitative assessment tool. Radiation injury triggered induction of all four genes: ACTA2, COL1A1, CTGF and FN1. Collagen bundle length significantly increased following irradiation (p=0.0154), though hydroxyproline levels remained stable. Skin viability was preserved throughout the experimental period. These findings indicate that a pro-fibrotic response can be successfully induced in ex vivo human skin after radiation injury. Though total collagen (hydroxyproline) content did not increase, the ex vivo skin remained viable, suggesting that RISF changes could be captured as they evolve beyond the initial experimental period. This new model is well-suited to study the molecular landscape of early RISF, enabling identification of diagnostic and prognostic biomarkers that can pave the way for novel targeted RISF therapies.

BACKGROUND

Radiation-induced skin fibrosis (RISF) is a devastating consequence of radiation exposure and develops as a consequence of aberrant wound healing.

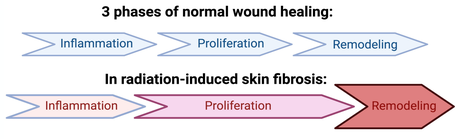
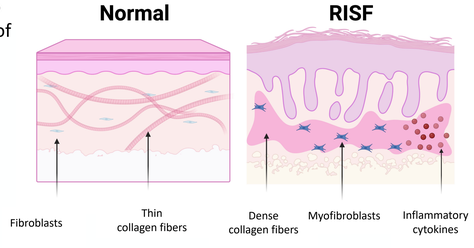


Figure 1. Compared to normal wound healing, in RISF a prolonged proliferation phase is followed by dysfunctional remodeling.

Characteristic changes in the dermis can be seen in RISF.

Figure 2. Histological changes in RISF include differentiation of fibroblasts to myofibroblasts, increased production of extracellular matrix proteins, and upregulated inflammatory signaling.



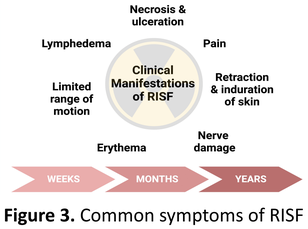


Figure 3. Common symptoms of RISF

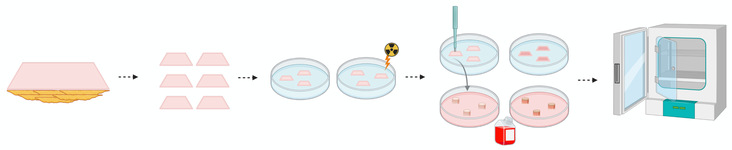
- These histologic changes can manifest as cosmetic and functional impairment in patients, and negatively impact quality of life.

Diagnosis and treatment of RISF remains challenging.

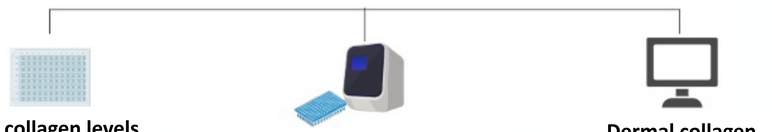
- Diagnosis relies on development of clinical symptoms, which can take years to detect.
- Current available treatments are supportive not curative.

Further studies are needed to investigate the development and progression of RISF to improve diagnosis, treatment and ultimately discover how to prevent RISF. To accomplish this, **experimental models of evolving RISF in human skin are needed**.

METHODS



Skin from human subjects (n=4) was cleaned & sectioned with the subcutaneous fat removed. Half of the tissue was irradiated with a predetermined dose ranging from 0-8 Gy. 8mm punches of non-irradiated and radiated tissue were maintained at air-liquid interface and collected over a 7-day period, respectively. Skin viability was assessed every 1-2 days.



Total collagen levels via Hydroxyproline assay

Expression of pro-fibrotic genes via qPCR

- ✓ Actin alpha 2 smooth muscle (**ACTA2**)
- ✓ Collagen alpha-1 chain (**COL1A1**)
- ✓ Connective tissue growth factor (**CTGF**)
- ✓ Fibronectin 1 (**FN1**)

Dermal collagen bundle thickness via automated quantitative assessment tool

RESULTS

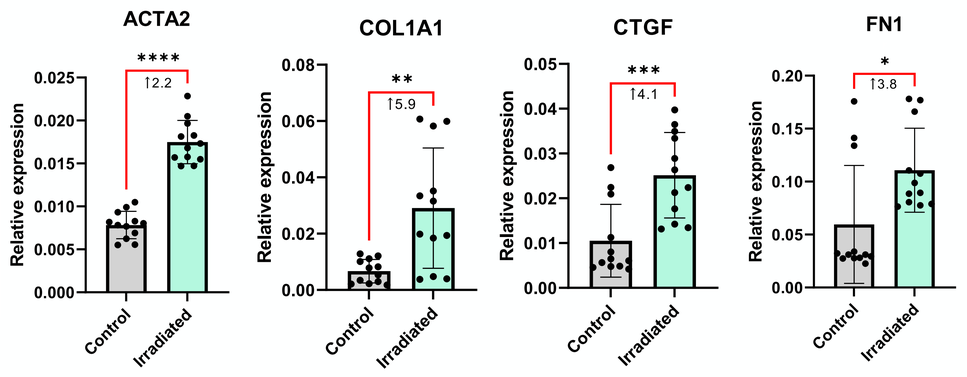


Figure 4. Radiation injury triggered induction of pro-fibrotic genes *ACTA2, COL1A1, CTGF*, and *FN1* in ex vivo skin 7 days post-radiation.

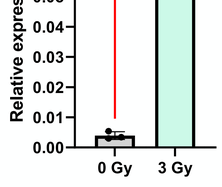


Figure 5. The greatest pro-fibrotic induction was observed in COL1A1 at the 3Gy dose.

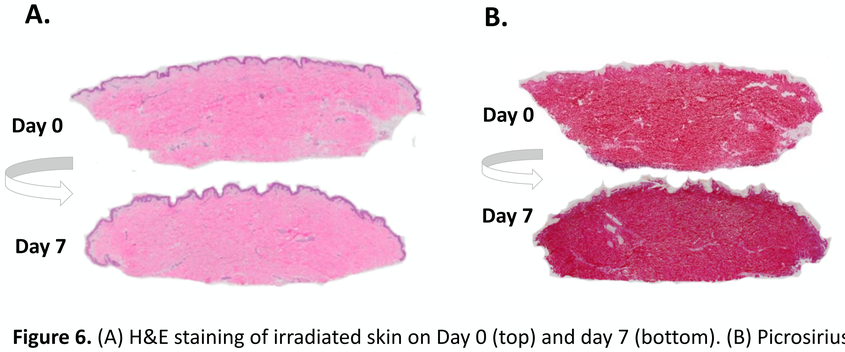


Figure 6. (A) H&E staining of irradiated skin on Day 0 (top) and day 7 (bottom). (B) Picrosirius Red stain highlighting collagen in irradiated skin. (C) Collagen bundle length in non-irradiated and irradiated skin, as assessed by CT-FIRE algorithm (J. S. Bredfeldt, et al. 2014)

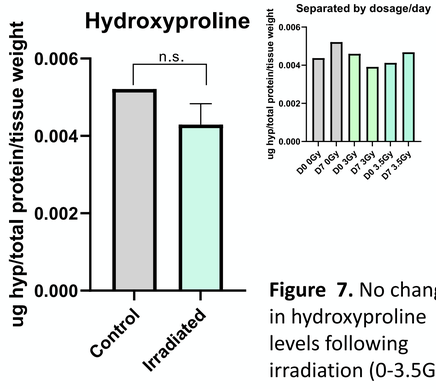


Figure 7. No change in hydroxyproline levels following irradiation (0-3.5Gy).

CONCLUSIONS AND FUTURE DIRECTIONS

Our findings indicate that **a pro-fibrotic response can be successfully induced in ex vivo human skin after radiation injury.** Though total collagen (hydroxyproline) content did not increase, the ex vivo skin remained viable, suggesting that RISF changes could be captured as they evolve beyond the initial experimental period.

This new model is well-suited to **study the molecular landscape of early RISF**, enabling identification of diagnostic and prognostic biomarkers, **paving the way for novel, targeted RISF therapies**.

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