Non-invasive biodosimetry: space applications of skin swabs-based omics

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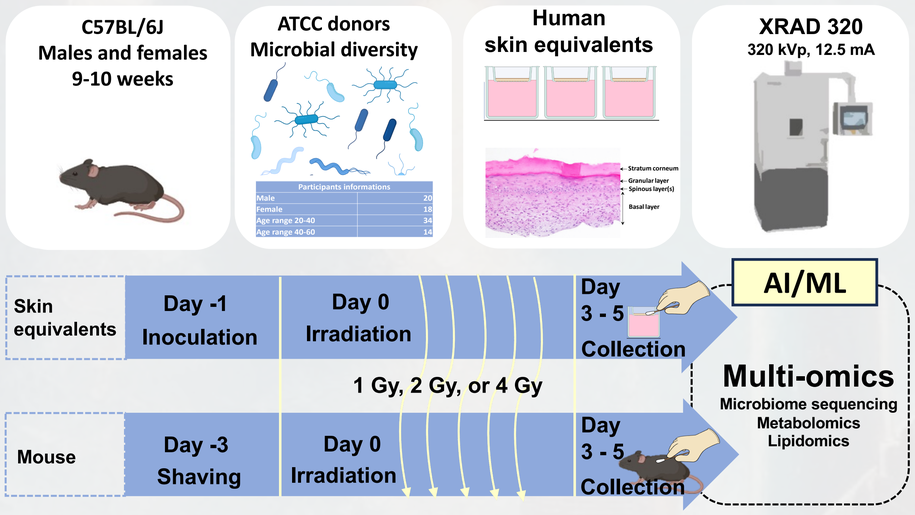
ABSTRACT

Rationale: Acute Radiation Syndrome (ARS) and long-term effects of radiation exposure such as cancer and metabolic diseases development are major risks for long duration and deep space missions. Current biodosimetry methods include invasive sampling and complex time-consuming molecular biology while having poor prognostic power. Space radiobiology research is complicated by experimental constraints and limited human subjects. Radiation exposure induces metabolic changes in various biological tissues. The skin with its microbiome is the first exposed layer to radiation and is easily accessible. **Objectives:** To develop a non-invasive method for rapid diagnostic and routine monitoring of metabolic and microbiome changes on skin for both astronauts and civilians exposed to radiation. **Method:** Our approach combines integrated multi-omic analysis and Artificial Intelligence/Machine Learning (AI/ML) to predict radiation dose level and type as well as date of exposure through the screen of metabolomic and microbiome signatures on the skin. Our project's research includes 1) an exploration phase in mice and bioengineered human skin equivalents exposed to low-dose radiation (<1Gy) and up to 4Gy, sampled (skin swabs) at multiple timepoints after exposure, and 2) a blinded test validation on mice skin swabs samples provided by the US government. **Our preliminary results** show that both unsupervised and supervised machine learning techniques can decipher dose and time since exposure from skin swabs samples.

Methods: Mouse and Human skin models



Human skin equivalents recapitulate *in vivo* skin molecular responses to irradiation

Here we present the data observed at 1 Gy, 2 Gy, and 4 Gy in mice and human skin equivalents 3 days after x-rays exposures. Both model results converge toward a general signature of radiation exposure on the skin, while specific metabolites decipher between the 3 doses.

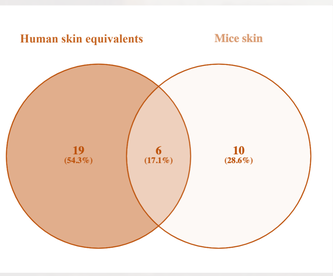


Figure 1. Venn diagram of Metabolite set enrichment analysis performed on MetaboAnalyst 5.0 online software of compounds with a VIP score > 1 found in common at all exposure doses (1, 2, and 4 Gy) in Human skin equivalents and Mouse skin (partial least square differential analysis, PLS-DA).

Metabolomic studies have never been run on irradiated bioengineered human skin colonized by human microbiome. This Venn diagram shows that bioengineered human skin equivalents and mouse skin share common biological pathways after irradiation. Our bioengineered human skin equivalent model recapitulates *in vivo* skin molecular responses to radiation.

Skin metabolome features differ between doses

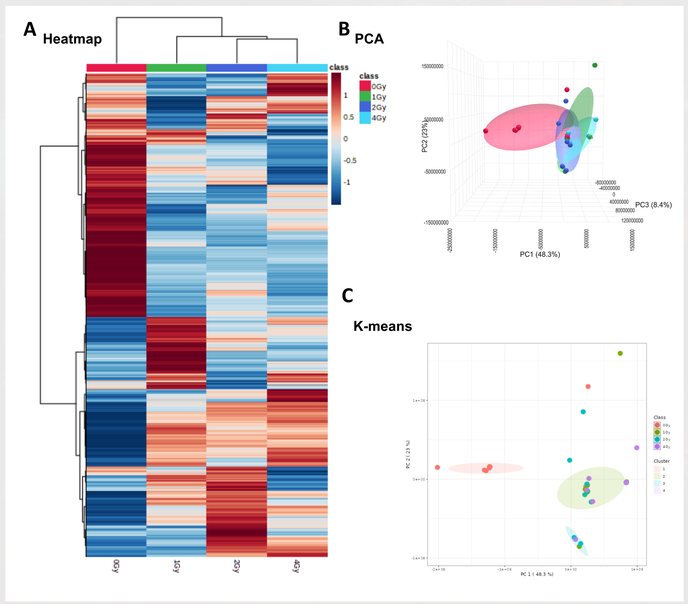


*Figure 2. Human skin equivalent **A)** Heatmap representation of average abundances of the top spectral features (ANOVA / P-value, method), **B)** Principal Component Analysis (PCA), and **C)** K-means clustering of Sham, 1 Gy, 2 Gy, and 4 Gy exposed groups.*

Skin metabolome biomarkers candidates can decipher between doses

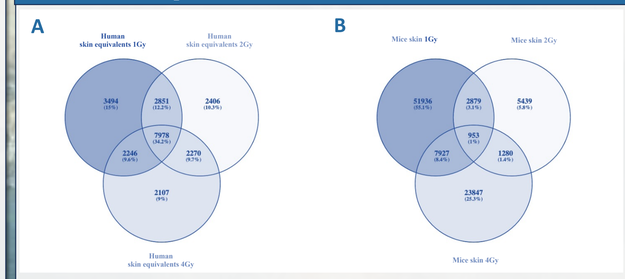


*Figure 3. Venn diagram of spectral features with a VIP scored > 1 at 1, 2, and 4Gy) in **A)** Human skin equivalents and **B)** Mice skin.*

In both mouse skin and human skin equivalents, compounds with PLS-DA VIP score > 1 are found exclusively at either 1 Gy, 2 Gy, or 4 Gy. These results suggest that skin metabolomic signatures depends on dose levels, and that particular metabolomic features can decipher between doses.

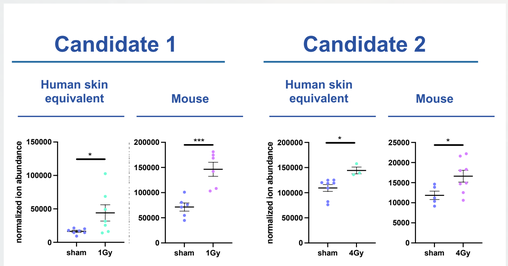


Figure 4. Abundance levels of select candidate biomarkers in both human skin equivalents and mice skin for biomarker candidates (biologically relevant) for 1 Gy (left) and 4 Gy (right)

Candidate skin metabolic biomarkers candidates can deciphers between doses

Skin microbiome features differ between doses

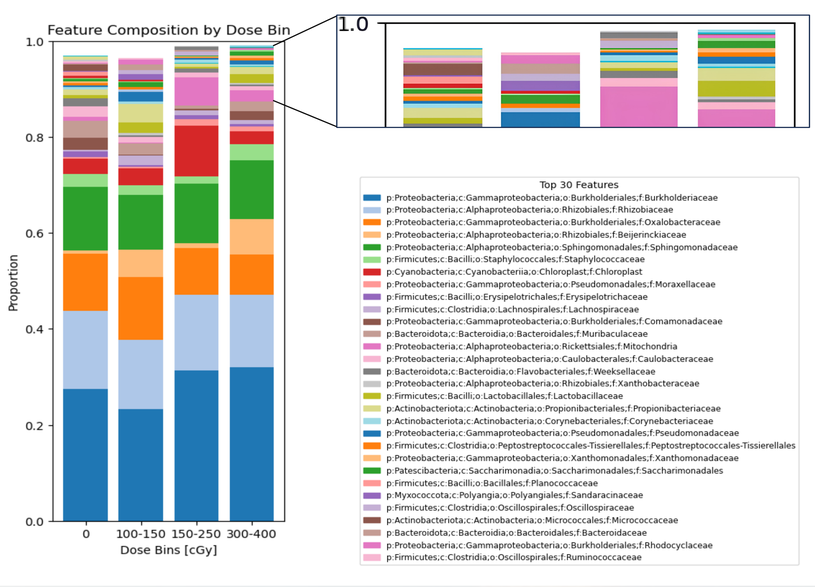
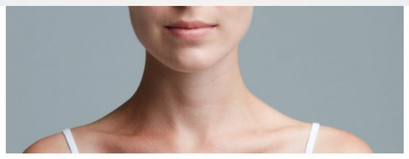
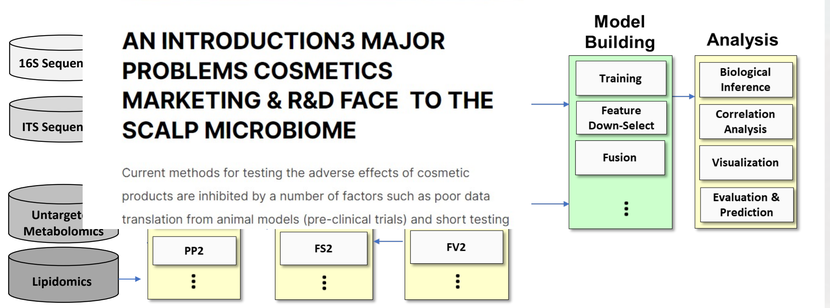


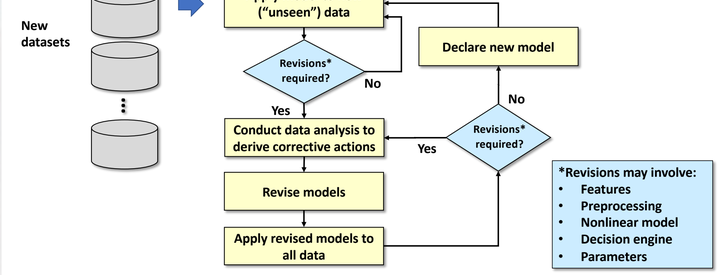
Figure 5. Taxonomic Data From 16S Sequencing. QIIME2 is used to analyze and predict taxonomic assignments of ASVs mouse skin swab. .

Microbiome profile differs between doses and could serve as direct input into predictive models through BIOMON (developed for DARPA and IARPA).

Artific learning prediction model construction







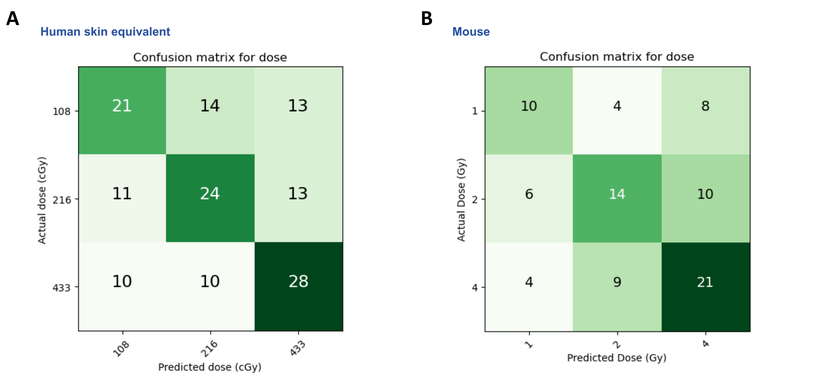


Figure 6. Dose prediction confusion matrix showing predicted dose vs actual dose using a machine learned classifier in A) skin mouse metabolome, and B) human skin equivalent metabolome.

The classifier shows a high number of points along the diagonal, indicating a correct classification. In the human skin model, the classifier demonstrates a bias towards higher predicted doses due to an imbalance of training samples, which will likely be remedied by the introduction of more samples.

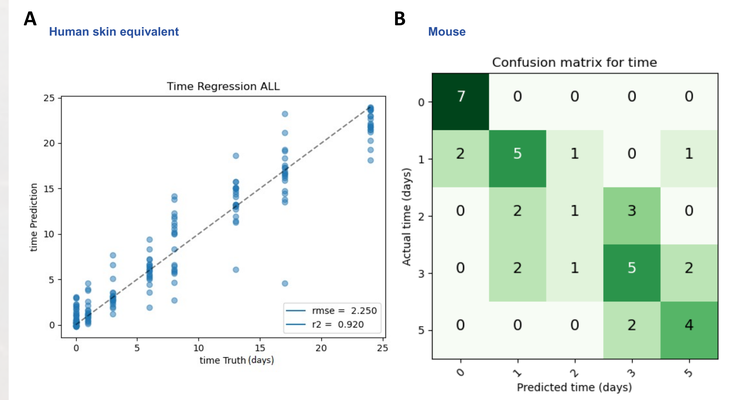


Figure 7. A) Actual time since irradiation event in days (x-axis) vs predicted time since irradiation (y-axis) for 180 unique mouse swabs. Predictions were made using a machine-learned regressor on untargeted LC-MS data. B) Confusion matrix showing the actual time in days vs the time predicted by a machine-learned classifier in human skin equivalent metabolome.

The classifier demonstrates low amounts of confusion from the pre-irradiated samples, and fairly low levels of confusion for the irradiated samples. This already promising predictive power will be increased by the introduction of further samples.

Conclusion and perspectives

Skin swab-based omics is a promising biodosimetry method to support human performance both on Earth and beyond while expanding our understanding of radiobiology. The next phase of our project will investigate low doses (< 1Gy) exposure signatures trackable on skin which will be translatable to space biology. Artificial skin experiments have never been conducted in space. Requiring few and simple handling procedures, bioengineered skin equivalents represent a valuable and bioethic tool for space biology research and support the future of humanity in the universe.

Acknowledgments

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