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REVIEW

-Omics potential of in vitro skin models for radiation exposure

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Abstract

There is a growing need to uncover biomarkers of ionizing radiation exposure that leads to a better understanding of how exposures take place, including dose type, rate, and time since exposure. As one of the first organs to be exposed to external sources of ionizing radiation, skin is uniquely positioned in terms of model systems for radiation exposure study. The simultaneous evolution of both MS-based -omics studies, as well as in vitro 3D skin models, has created the ability to develop a far more holistic understanding of how ionizing radiation affects the many interconnected biomolecular processes that occur in human skin. However, there are a limited number of studies describing the biomolecular consequences of low-dose ionizing radiation to the skin. This review will seek to explore the current state-of-the-art technology in terms of in vitro 3D skin models, as well as track the trajectory of MS-based -omics techniques and their application to ionizing radiation research, specifically, the search for biomarkers within the low-dose range.

Keywords 3D tissue model · Skin · Low dose · Ionizing radiation · Multi-omics · Biomarker

Introduction

Understanding skin's response to ionizing radiation has important implications for radiation therapy and radiological protection. The physiological response to ionizing radiation, particularly in the context of medical procedures and UV irradiation has been well documented and reviewed [1–6]. However, the advent of modern -omics pipelines has ushered in a new age of low-dose radiation (LDR) research. This review endeavors to evaluate the suitability of in vitro human skin models for mass spectrometry-based radiation biomarker discovery. Much of what is assumed about LDR exposure is extrapolated from higher-dose studies; there is a growing need to employ modern -omics tools to evaluate biochemical changes in response to LDR.

Ionizing radiation (IR) damage to biological systems largely depends on the type of radiation (α, β, γ, X-ray, n), the energy associated with each type of emission, and the amount of time a cell/tissue/etc. is irradiated. Each type of radioactive particle has an associated penetration depth (Fig. 1) and amount of energy transferred to a material called the linear energy transfer (LET, keV/µm). Cellular damage from ionizing radiation can result from either the direct impact of particles and/or the generation of reactive oxygen/nitrogen species (ROS/RNS) that ultimately alters biomolecules. The field of radiation biology largely uses absorbed dose (Gray, Gy), or the amount of energy (J) absorbed by a unit of biological mass (kg), to describe the amount of radiation a tissue receives. It is generally recognized that the relative biological effectiveness (RBE) is a more representative (though still incomplete) metric that accounts for a multiplicative weighting factor dependent on the LET determined by the radiation type [7, 8]. For example, neutron particles have a high LET and long penetration

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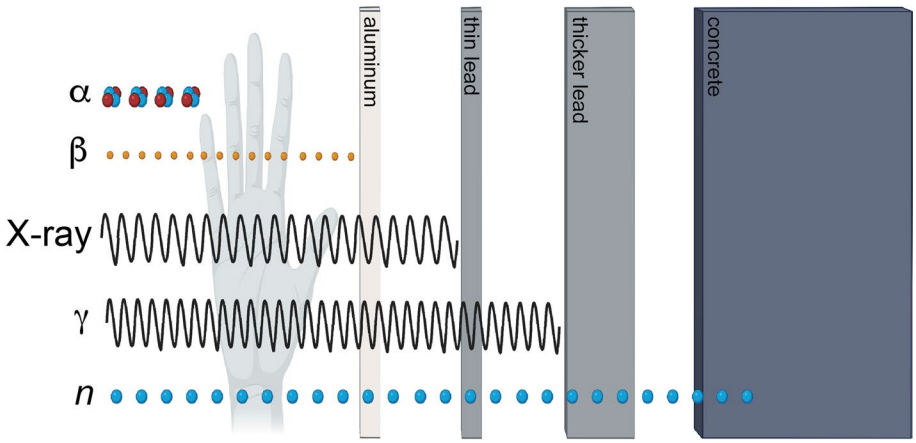

Fig. 1 Common types of ionizing radiation, along with their associated depths of penetration. While alpha-particles have a high LET they are stopped by the first few layers of skin or thin paper. High-energy beta particles can penetrate through several cm of human skin but can be shielded by thin aluminum or plastic. X-ray and gamma-rays have much higher penetration depths being stopped by lead or iron shielding. Neutrons have the highest penetration depth only being attenuated by thick concrete or water

depth increasing the RBE, whereas beta particles have a low LET. Effective dose (Sieverts, Sv) takes into account tissue weighting factors and encompasses the stochastic effects of radiation [9]. These concepts are important with respect to irradiating in vitro skin models for three reasons; (1) the field of radiobiology does not use a unified metric for how much radiation is applied to a sample and for in vitro samples, where there is not an accurate weighting factor, these units are used inappropriately, (2) careful consideration must be made to dosimetry when the geometry of the samples is unique, and (3) in low-dose radiation studies the error associated with the dose each technical replicate receives needs to be defined. To the last point, Monte Carlo N-Particle Transport Code (MCNP) and similar techniques can calculate the dose received by a particular region of tissue in a radiation field and should be employed for in vitro cell/tissue studies [10–12]. For the purposes of this review, we will limit our discussions to absorbed dose (Gy) as a unifying metric, defining LDR as less than 200 mGy.

The integumentary system, specifically the skin, serves many functions, such as protection, regulation, homeostasis, and sensation [13–15]. As one of the largest organs of the human body, skin serves as the body's first line of defense from a variety of environmental onslaughts (*i.e.*, infectious organisms, radiation, etc.). Structurally the skin is quite complex, and its cellular composition is even more diverse (Fig. 2). Briefly, human skin has three main layers: (1) the outermost epidermis, composed of the stratum corneum, granular cell layer and the spinous layer, (2) the dermis, composed of the basal cell layer containing collagen/elastin fibers, sebaceous and sweat glands, nerves, and hair follicles, and vasculature, and (3) the sub-cutaneous tissue or hypodermis made of largely adipose tissue. The stratum corneum and epidermis have six major cell types: Langerhans, keratinocytes, melanocytes, Merkel cells, dendritic epidermal T cells, and CD8 + T cells. The dermis is more complex with macrophages, fibroblasts, plasmacytoid dendritic cells (pDC), dermal dendritic cells, mast cells, neutrophils, skin innate lymphoid cells, and $\gamma\delta$ T cells. The need for a model that best recapitulates this complex biology (including immune cell response) while also preserving longevity and phenotype is paramount for understanding human-relevant drug and insult (e.g., radiation) toxicity and associated mechanisms of action.

Criteria for model selection

Any non-traditional research model will need to be demonstrated as fit for the intended purpose, with an understanding of the benefits and limitations. Thus, the goal in this review is to understand the capabilities of different 3D skin models in meeting the objectives of responding to ionizing radiation in a similar manner to animal or even human models. Determination of this is dependent on multiple factors including specificity, sensitivity, durability, and reproducibility. *Model Specificity* is defined as how interrelated the associated biomarkers are to intact human skin. Does it have the right markers in the right relative amounts? Do signaling cascades proceed as if they were native? *Sensitivity* can be greater or less than human skin, but the level of sensitivity needs to be defined for the model relative to human skin. *Durability* is defined as the ability to noninvasively sample repeatedly, and for commercial models their ability to be shipped without major physiological changes. *Reproducibility* is defined as the inter- and intra-variability between the models and donors (for biopsy models).

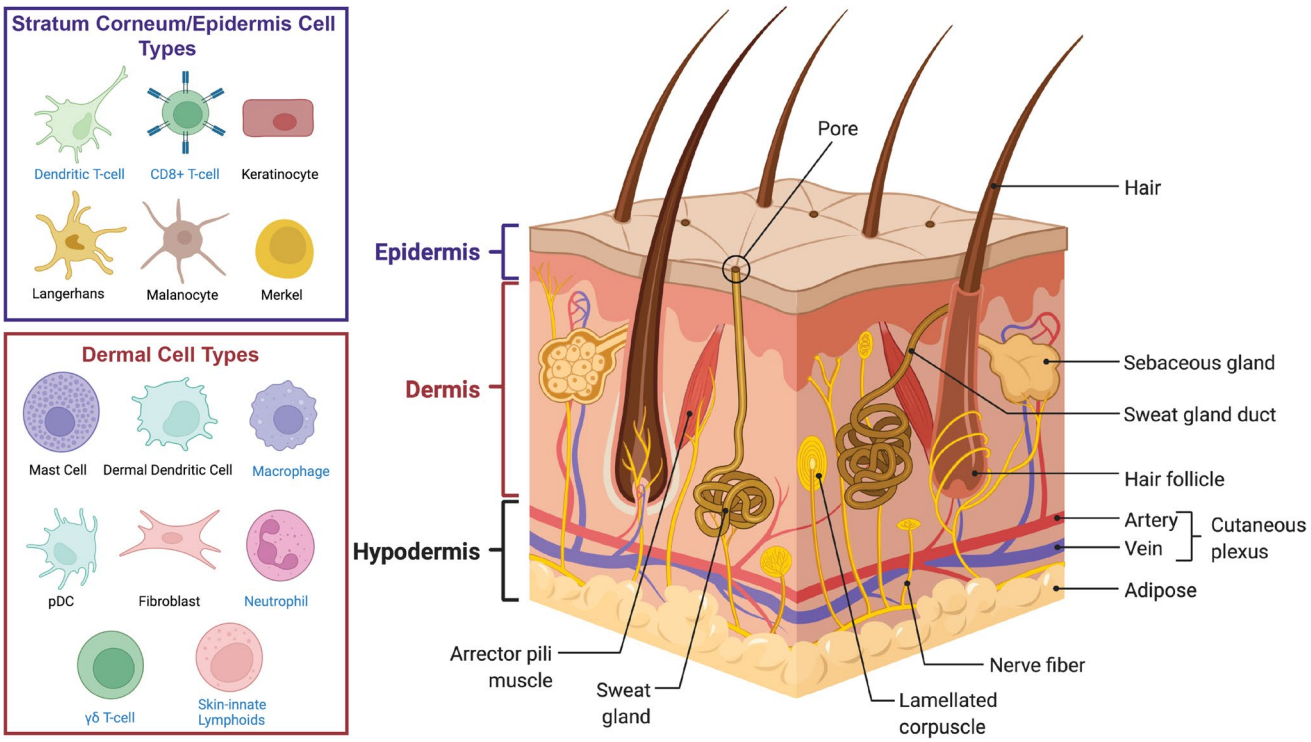

Fig. 2 Simplified 3D model of skin, along with most cell types commonly found in each layer. Immune cells are marked with blue text. The layer of skin immediately exposed to the environment is the stratum corneum with the epidermis underneath. The dermis has the greatest number of different cell types responsible for protecting the body. The hypodermis is largely composed of adipose (fat) tissue

To mimic the complex biology and physiology found in the skin, various companies have employed a variety of different approaches to solve the challenges a realistic in vitro skin model presents [13]. These approaches involve many challenges: the incorporation of the hypodermis, dermis and epidermis, maturated barrier function and an active immune component for more realistic modeling of allergy and other immune-related disease states [16]. Other technical challenges are related to culturing keratinocytes in large quantities and maintaining them in an undifferentiated state [17]. The commercial skin mimetic models discussed here have been validated by various regulatory bodies as alternatives to animal skin for testing corrosion and irritation (Table 1). These models have applications in in vitro testing as opposed to translational medicine (i.e., grafting). One general advantage of reconstructed skin models is in assessing the depth of permeation of compounds and radiation treatments because the layers can be easily separated and individually assessed [18]. The following section offers an overview of the most popular commercial models, a histological comparison of the numbers, and the types of cells included in these models.

Construction of 3D skin models

Dozens of air–liquid interface human skin models exist, generally falling into the following four categories: reconstructed human epidermis (RHE), full-thickness models, RHE cultured on dermis, and skin explant models (Fig. 3, Table 1).

RHE models

The RHE model consists of a stratified keratinocyte epidermis, which can function as either a standalone epidermis or alongside a dermis layer [19]. Many commercial models are standalone stratified epidermis models composed of keratinocytes, and many comparable non-commercial epidermal models have been reported [19, 20].

Full thickness models

When an RHE model is cultured atop a dermal model, the skin mimetic becomes a full-thickness model containing both a dermis and epidermis [19–26]. A commonly used dermal support is composed of fibroblasts embedded in collagen to form a "living dermal equivalent." This model has the advantage over RHE alone in that the interaction of the

Table 1 Summary of commercially available reconstructed skin models. Only models that have been validated by international regulatory bodies are listed

Skin model	Skin layers included	Cell types	Scaffold material	Longevity	Unique features	Advertised applications	Skin corrosion testing approval	Skin irritation testing approval	Other testing approval
EpiSkin	Epidermis only	Keratinocytes	Polycarbonate filter	Not listed	Collagen layer on polycarbonate filter	Skin irritation, skin corrosion, medical devices, UV exposure, DNA damage, bacterial adhesion, omics, permeability	OECD TG 431, ICCVAM, JaCVAM, ECVAM	OECD TG 439, JaCVAM, KoCVAM, ECVAM	N/A
SkinEthic RHE	Epidermis only	Keratinocytes	Polycarbonate filter	Not listed	Collagen layer on porous polycarbonate filter	Skin irritation, skin corrosion, medical devices, UV exposure, DNA damage, bacterial adhesion, omics, permeability	OECD TG 431, ICCVAM, JaCVAM, ECVAM	OECD TG 439, JaCVAM, KoCVAM, ECVAM	N/A
MatTek EpiDerm	Epidermis only	Keratinocytes	Porous polycarbonate membrane	Cultures can be continued for at least 2 weeks (14 days)	Multi layered cell scaffold using human primary keratinocytes on porous polycarbonate filter	Skin physiology and biochemistry, dermatological research, transdermal drug delivery, skin penetration, mode of action, toxicology, UR and IR irradiation, wound healing	OECD TG 431, JaCVAM, ECVAM	OECD TG 439, JaCVAM, KoCVAM, ECVAM	ECVAM approval for phototoxicity testing

Table 1 (continued)

Skin model	Skin layers included	Cell types	Scaffold material	Longevity	Unique features	Advertised applications	Skin corrosion testing approval	Skin irritation testing approval	Other testing approval
Phenion FT (standard and long-life models)	Full thickness model	Fibroblasts and keratinocytes	Porous polycarbonate membrane and a proprietary collagen matrix	Standard model: 7 days Long term model: up to 50 days	Proprietary collagen matrix, options for standard or long-life models depending on application. Custom media requests are also available depending on the desired downstream analysis	Skin physiology and biochemistry, dermatological research, claim substantiation for cosmetic ingredients, transdermal drug delivery studies, skin penetration, mode of action for pharmaceuticals, toxicology, UR and IR irradiation and wound healing	None	None	Under evaluation at ECVAM as an *in vitro* genotoxicity model
Phenion EpiCS	Epidermis only	Keratinocytes	Polycarbonate	Can be cultured for up to 28 days	Multi layered cell scaffold using human primary keratinocytes and a proprietary collagen matrix	Skin corrosion, skin irritation, skin sensitization testing, phototoxicity or genotoxicity studies	OECD TG 431, JaCVAM, ECVAM	OECD TG 439, ECVAM	N/A
LabCyte Epi-Model	Epidermis only	Keratinocytes	Polycarbonate membrane	Documented to survive up to 4 weeks in culture with significant decrease in barrier function	Multi layered cell scaffold using human primary keratinocytes	Skin irritation, skin corrosion, immunohistochemistry, genotoxicity, barrier function assessment	OECD TG 431, JaCVAM	OECD TG 439, KoCVAM, JaCVAM	N/A

OECD Organization for Economic Cooperation and Development, *ICCVAM* Interagency Coordinating Committee on the Validation of Alternative Methods (US-based), *ECVAM* European Centre for the Validation of Alternative Methods, *JaCVAM* Japanese Center for the Validation of Alternative Methods, *KoCVAM* Korean Center for the Validation of Alternative Methods

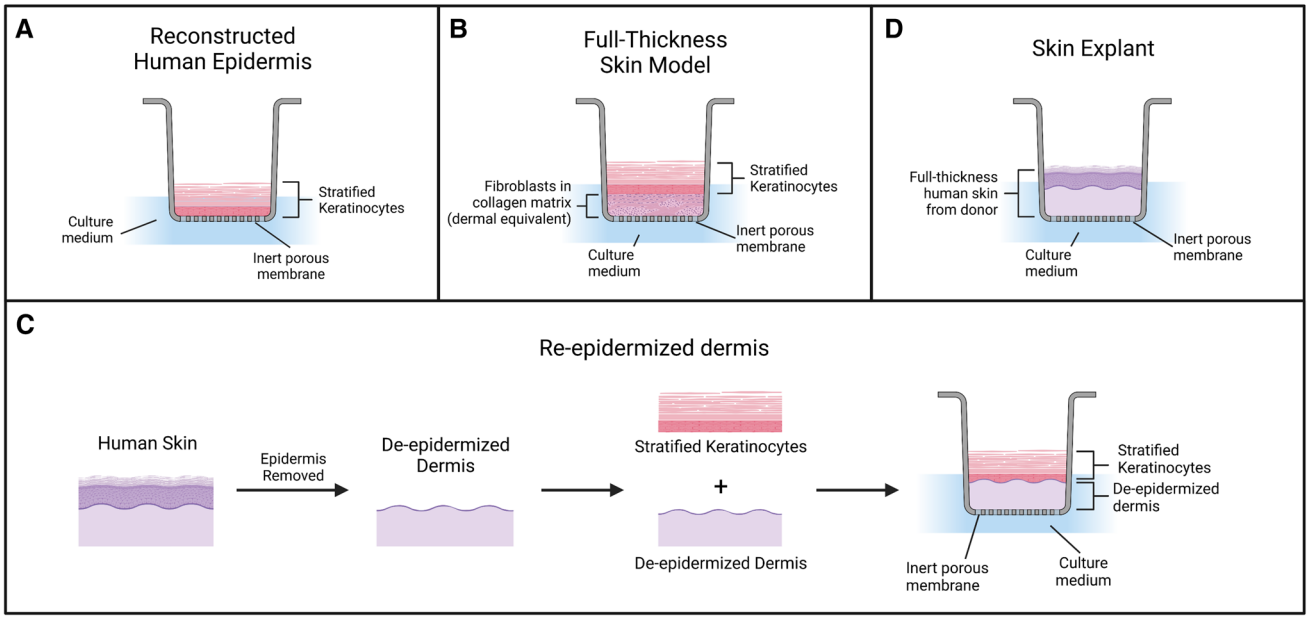

Fig. 3 General categories of skin models cultured at the air–liquid interface. The models discussed in this paper all fall into these general categories with some novel variations. **a** Reconstructed human epidermis (RHE), composed of stratified keratinocytes on an inert porous membrane cultured at the air–liquid interface. **b** A full-thickness model, where a RHE is cultured atop a dermal equivalent composed of fibroblasts embedded in a collagen matrix. **c** Re-epidermized dermis, where the epidermis is removed from human skin leaving a de-epidermized dermis. Then, a RHE is cultured on the de-epidermized dermis to produce a model with both epidermal and dermal components. **d** A skin explant model where human skin is cultured at the air–liquid interface directly from the donor

keratinocytes with fibroblasts allows for more complexity in cellular response and higher integrity of barrier function. When keratinocytes are added atop this dermal equivalent, they proliferate and differentiate to create a stratified epidermis. The resultant living skin equivalent is considered a full-thickness skin model, as it includes both dermal and epidermal mimetics [20, 22, 24, 25, 27].

RHE cultured on dermis

As an alternative to a fibroblast dermal equivalent, de-epidermized human dermis has been used as a culture substrate for stratified keratinocyte-based epithelial mimetics [22, 25]. In these models, the epidermis is removed from human skin and replaced with a lab-cultured RHE, resulting in a model that contains both dermis and epidermis [22, 25].

Although some reconstructed skin models have been validated by regulatory bodies as replacements for standard animal-based irritation and corrosion tests, the Organization for Economic Cooperation and Development (OECD) acknowledges that skin mimetics may not be an appropriate method for all chemicals and recommends their use as an initial method to be followed by in vivo studies [28]. While these models can be used to evaluate toxin response, they lack the barrier function and complexity required to accurately predict the effects of those toxins. Additionally, there is no reproducible and agreed-upon method to incorporate immune cells into skin mimetics [21]. Thus, their limitations need to be taken into consideration when applying these models to study radiation biomarkers with multi-omics methods.

Skin explant models

Histocultures, a type of skin explant model, are derived from surgical excess and contain all the cell types present within in vivo skin, and thus provide a highly accurate model of the in vivo response to toxins. The inclusion of immune cells, fatty layers, and microvasculature naturally present in human skin creates a complexity that can both contribute to the model's in vivo relevance and present an added challenge since it cannot be experimentally controlled [21]. Additionally, skin can vary significantly between donors based on age and genetics, and the amount of tissue available from each donor is limited. Explants are typically cultured at the ALI to maintain skin polarity and can retain keratinocyte viability for 75 days or more [29]. However, changes in metabolic activity, a decrease in barrier function, spongiosis, necrosis, parakeratosis and epidermal/dermal separation begin to occur after 9 days [30]. It is generally assumed that the degradation occurs due to removal from the host environment, immune cell activation, and lack of specific, renewable nutrient,

mechanical, or environmental cues. However, no study has yet to date defined what the specific missing cues are to prolong the lifespan of these cultures. Nonetheless, there are no skin mimetics that can fully recapitulate the barrier and metabolic functions of explanted human skin. Skin explant models should be considered as potentially suitable for -omics studies, along with consideration of their limitations and rapid degradation potential of the cultures.

Commercial models

MatTek

The MatTek corporation (Ashland, MA, USA) offers two models: a RHE full-thickness skin model (EpiDermFT™) and an epidermal model (EpiDerm™) [31]. The lifespan of the full-thickness model is 14 days post delivery, whereas the epidermal model is expected to have up to 21 days of lifespan. The epidermal model has been utilized for genotoxicity studies including the micronucleus assay [32, 33]. Micronuclei are small extranuclear bodies resulting from chromosome damage or even whole chromosomes lagging behind during anaphase. This is also a standard assay performed in radiation exposure studies and the absorbed dose can be quantified by fitting to known curves for low and high LET types of radiation [34]. Whereas this assay is performed on lymphocytes for radiation studies, similar effects and curves might also be feasible for skin. The epidermal model has been approved and validated by the European Center for the Validation of Alternative Methods (ECVAM) and approved by the Organization for Economic Cooperation and Development (OECD) to test for corrosion, irritation, and photo-toxicity [19, 31, 35]. The full-thickness model has been utilized in non-ionizing radiation studies inducing phosphorylation of Histone 2AX (H2AX) [36].

Phenion® Full Thickness (FT), FT LONG-LIFE, and epiCS reconstructed skin models

With advancements in biocompatible materials and cell culture techniques, in 2007, a novel skin model was developed by Henkel AG & Co. (Düsseldorf, DE) that was later branded under the name Phenion®. The Phenion® FT Skin Model, named for containing both a dermis and epidermis, employs the use of keratinocytes and fibroblasts that are isolated from the same human donor. These are then seeded into a rigid collagen matrix to help provide a suitable substrate for the cells to attach and interact with one another in a 3D environment [37]. The Phenion® FT Skin Model features a fully stratified epidermis, a multi-layered stratum corneum as well as a dermis layer. Once the cells have matured to a satisfactory proprietary standard, the multi-cell co-culture is airlifted to provide further physical and environmental cues for further differentiation of the tissue. The use of a rigid, proprietary collagen sponge into which the cells take residence provides the culture with a degree of tensile strength that is needed for chemical and mechanical interrogation studies. The Phenion® FT Skin Model is available in various sizes (e.g., 1.4 cm or 3.1 cm) and formats (e.g., standard or tissue insert) depending on the application and customer needs [38]. The lifespan of these models range from 7 days for the short-term FTM to 50 days in culture for the "LONG-LIFE" variant. One of the drawbacks to the Phenion FTM is that because it only utilizes keratinocytes and fibroblasts, it does not have an active immune component. Another potential drawback is the relative maturity of the dermo-epithelial barrier.

The FT LONG-LIFE model is better suited for chronic skin exposure studies as well as recovery or tissue regeneration studies. Tissues can be cultured in a "raft" format with media underneath or in a suspended permeable support device for more individualized testing conditions. The medium used to culture these tissues is proprietary and is amenable to customer requests (i.e., no phenol red, low serum, etc.). Because this model uses human-derived cells isolated from a single donor for a given batch and allows the cells to differentiate into their respective skin cells, its application for -omics research to capture a native human skin response is very promising.

The Phenion® epiCS model is composed of stratified keratinocytes on a porous polycarbonate substrate in an air–liquid interface (ALI) and is specifically tailored for assaying skin corrosives, irritants, and sensitizers [39]. The composition of this model resembles the human epidermis with developed layers, including the stratum basale, stratum spinosum, stratum granulosum and stratum corneum, and like human skin, the model also provides barrier function for permeability studies. The epiCS model has been used in assays that monitor IL-18 release as a measure of skin sensitization in response to compound exposure with promising performance [40–42]. Although epiCS has not been used for many peer-reviewed studies, the manufacturer claims it has applications in testing skin corrosion, irritation, sensitization, photo-toxicity, and genotoxicity studies. The epiCS model is approved by ECVAM for testing skin irritation and corrosion.

Episkin

L'Oreal S.A. (Clichy, Hauts-de-Seine, FR) acquired EpiSkin S.A. (Lyon, FR) in 1997 to overcome challenges with animal testing and it has grown into one of the most popular RHE models. EpiSkin is an RHE comprised human keratinocytes cultured on a collagen matrix at the ALI. According to the manufacturer, the addition of collagen prevents shrinking

of cell layers, thereby keeping the layers tighter together. The RHE contains the same layers as the native human epidermis, but with a thickened stratum corneum, and major differences in cell morphology compared to human skin [20, 43]. Like most other RHE models, EpiSkin is significantly more permeable to compounds than human skin and retains a greater proportion of compounds that have a high protein binding capacity as a result of the collagen layer. Significant variability has been noted between batches [43, 44]. EpiSkin has proven effective in photo-toxicity and genotoxicity studies and is one of the models approved for use by the ECVAM to test skin irritation and corrosion; however, like most RHE-based cultures, studies are generally performed within three days of receiving the samples and follow-on studies with increased relevance to human physiology are recommended [19, 45–49].

SkinEthic™

SkinEthic™ RHE, acquired by Episkin in 2006 (under the L'Oreal product offerings), is composed of human keratinocytes on an inert polycarbonate filter cultured at an ALI [43]. The model is cultured for 17 days prior to use, and contains some of the major layers of the native human epidermis, namely the stratum corneum, stratum granulosum, and stratum spinosum. For this model, the stratum corneum is significantly thicker than native human skin [20, 43, 50]. Although the lipid composition of the model is generally similar to that of native human skin, differences in the distribution of lipids throughout the tissue have been observed [20, 43]. SkinEthic™ RHE has shown utility in photo-toxicity, irritation, and corrosion studies. However, like most reconstructed human epidermis models, its barrier function and performance in permeability and absorption studies are significantly different from the performance of human full-thickness skin or human epidermis, with studies reporting a sevenfold increase in permeability as compared to native human skin [20, 43, 51–53]. This increased permeability is typical for RHE models [54]. Reproducibility of results is a persistent issue with SkinEthic RHE; the model can correctly rank compounds in order of irritation potential, but with varying reports of reproducibility [44, 55]. Similarly, the model's enzymatic activity is comparable to normal human epidermis but is highly variable [43, 56].

LabCyte EPI-model

LabCyte (San Jose, CA, USA), a subsidiary of Beckman Coulter Inc., offers the LabCyte EPI-MODEL (produced by Japan Tissue Engineering Co., Ltd., Aichi, Japan), which is a RHE composed of human keratinocytes on a porous, inert polyethylene terephthalate (PET) membrane [54, 57]. The structure of the model is similar to the native human epidermis and includes the basal layer, stratum spinosum, granular layer, and the stratum corneum. Esterases, which play a major role in skin metabolism, are similar in localization and activity as seen in the native human epidermis [58]. However, there are large variations in the RHE data and between human skin donors, making comparison difficult [58]. The EPI-MODEL demonstrates around tenfold higher permeability to compounds than native human epidermis. It has shown success in a genotoxicity study, although it is more commonly used for skin irritation and corrosion studies [57, 59]. Its use is encompassed under OECD testing guidelines as an alternative to animal testing for skin corrosion and irritation, and has been approved by the Japanese Center for the Validation of Alternative Methods (JaCVAM) to test for corrosion and irritation [35, 57].

Multiomics in skin radiation research

From the success of the human genome project, the landscape of MS-based -omics techniques has undergone a gradual shift within the last twenty years. The emergence of enabling technologies has shifted how biomarker discovery and disease/injury characterization is performed [60, 61]. Gel-based techniques such as two-dimensional difference gel electrophoresis (2D-DIGE) have slowly given way to faster, more comprehensive, and less labor-intensive workflows utilizing ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS/MS) [62, 63]. As this shift has occurred and MS-based technology and bioinformatic pipelines have drastically improved, there has been a subsequent drastic improvement in our holistic understanding of biological processes affected by IR, specifically LDR. Quantifying IR exposure has long been reliant on cytogenetic techniques such as the labor-intensive dicentric chromosome assay (DCA), which has been the gold standard of radiation exposure detection for decades [64]. However, the rise of MS-based -omics techniques has reignited a desire to seek out new biomarkers for radiation exposure that can provide enhanced exposure information (e.g., dose rate/type, time since exposure) [65]. Utilizing these techniques has, thus far, provided unparalleled ability to investigate many different tissue and cell types susceptible to IR exposure, and shows great promise for expanding upon our ability to investigate exposure in real-world scenarios and for improving personalized medicine [66, 67].

The skin is one of the most complex sample types to investigate for IR exposure from an -omics perspective, owing to its diversity of cell types and the biochemical functions it serves (Fig. 2). The many critical functions of the skin, such as thermoregulation, sensation, and excretion, create a delicate interplay between many different metabolic processes [68]. Skin has been shown to exhibit responses to

ionizing radiation both on short (hours) and long (> 7 days) time scales, and these changes can be dose-dependent [69]. Many studies utilize standard 2D cell cultures for -omics investigations of radiation exposure, including proteomics, metabolomics, and others [70–76]. However, there are significant drawbacks associated with utilizing 2D rather than 3D or in vivo skin models [77, 78]. Studies of IR penetration are not possible with a 2D model, and cell signaling, cell differentiation, and protein expression have all been shown to be less accurate in 2D models (particularly with the absence of immune cells). Additionally, 3D skin models can be engineered with genetic alterations and avoid key morphological differences with commonly used laboratory animal (*e.g.,* murine) vs. human skin models [68]. Currently, some IR studies of in vitro 3D skin models have been performed for genomics [79], transcriptomics [80], proteomics [81–83], and metabolomics [84] with commercially available skin models. However, the limited body of literature of in vitro 3D human skin models used for MS-based -omics in radiation exposure indicates this field is still relatively unexplored.

One of the unique challenges associated with 3D skin model sampling/preparation is that homogenization of tissue often includes several strata of skin, which can in turn complicate analysis; high-abundance structural proteins, such as fibroblasts and keratinocytes, often overwhelm important but lower-abundance proteins found in each layer. Consideration must be made to the stratum/strata of skin that is to be investigated, as well as the cell types present within; this factors into the sampling technique (punch biopsy, suction blister, tape stripping, etc.) to be employed [85]. However, sample preparation for 3D skin models is in principle the same as for skin biopsies; for most MS-based -omics applications, tissue/cell samples are homogenized and lysed, followed by in-house procedures for each application. One unique sample preparation method from Dyring-Andersen et al. utilized curettage and individual punch biopsies, along with cell cultures, to carefully map the entire proteome of each individual section (outer epidermis, inner epidermis, dermis, and sub-cutis) of complete human skin biopsies [86]. By creating their own data-dependent acquisition (DDA)-based approach to create an in-house proteome library, they were able to identify an astonishing 10,701 proteins across these four sub-layers of skin; a majority of these proteins (56.3%) were expressed in all four sub-layers but in substantially different (six orders of magnitude) abundances. These findings only further showcase the unique proteomic profiles within each subsection of skin tissue. To the best of our knowledge, there is only one instance in the literature of MS-based multi-omics workflows described for 3D skin models exposed to LDR [81]; more in-depth investigation is sorely lacking.

As MS-based -omics workflows have become exponentially more complicated (Fig. 4), manual data interpretation has become impossible and bioinformatics workflows are now indispensable [87]. These workflows, including comparisons of the online databases and search tools used to interpret data, have been reviewed previously for MS-based -omics [88–90]. The inherent complexity of MS-based proteomics analysis does, however, require special considerations for how data are generated. Analysis of peptide sequences via MS generally falls into two broad categories: data-dependent acquisition (DDA) or data-independent acquisition (DIA). DDA selects a subset of precursor MS1 peaks, typically the most abundant, to be fragmented into MS2, whereas DIA acquires a MS1 spectrum followed by MS2 spectra for a certain limited mass range, which is then repeated across the entire mass range. Although DDA has historically been used very successfully to generate proteome coverage, its stochastic nature can inherently limit its sensitivity for low-abundance peptides and leads to undersampling in many cases [91, 92]. Alternatively, one of the main limitations for DIA currently is the complexity and computational burden of the data that are produced. It has recently been shown that both DDA and DIA have better protein quantification reproducibility using local library searching *vs*. online database queries; for larger datasets, this library building can therefore enhance the quality of the data that are produced [93].

Biomarkers of low dose radiation exposure

There are many biomarkers for LDR exposure that have been discovered based upon years of multi-omics experiments that can be broadly characterized by DNA/RNA repair mechanisms, lipid oxidation, and metabolic changes [94–96]. However, many of the most promising biomarkers for LDR exposure, such as serum amylase and diamine oxidase, arise from system-level dysregulation and are not skin-specific [97]. Some, such as γ-H2AX (Ser-139 phosphorylation of H2AX), which is responsible for early DNA repair and largely studied as a marker for γ/*X*-ray-radiation-induced damage, have received considerable attention over the years [98, 99]. γ-H2AX is of note because this change is induced at radiation doses as low as 1 mGy and has been studied in cultured skin biopsy models [100–102].

Exposure to ionizing radiation can promote a general stress response in cells, tissues, and whole organs through a multitude of different pathways. This poses a significant hurdle for LDR biomarker research for several reasons: these stress responses are often not unique to IR exposure, they can be highly dose- or individual-dependent, they are often not indicative of the type of radiation encountered, and in some cases can be quite transient [103, 104]. There

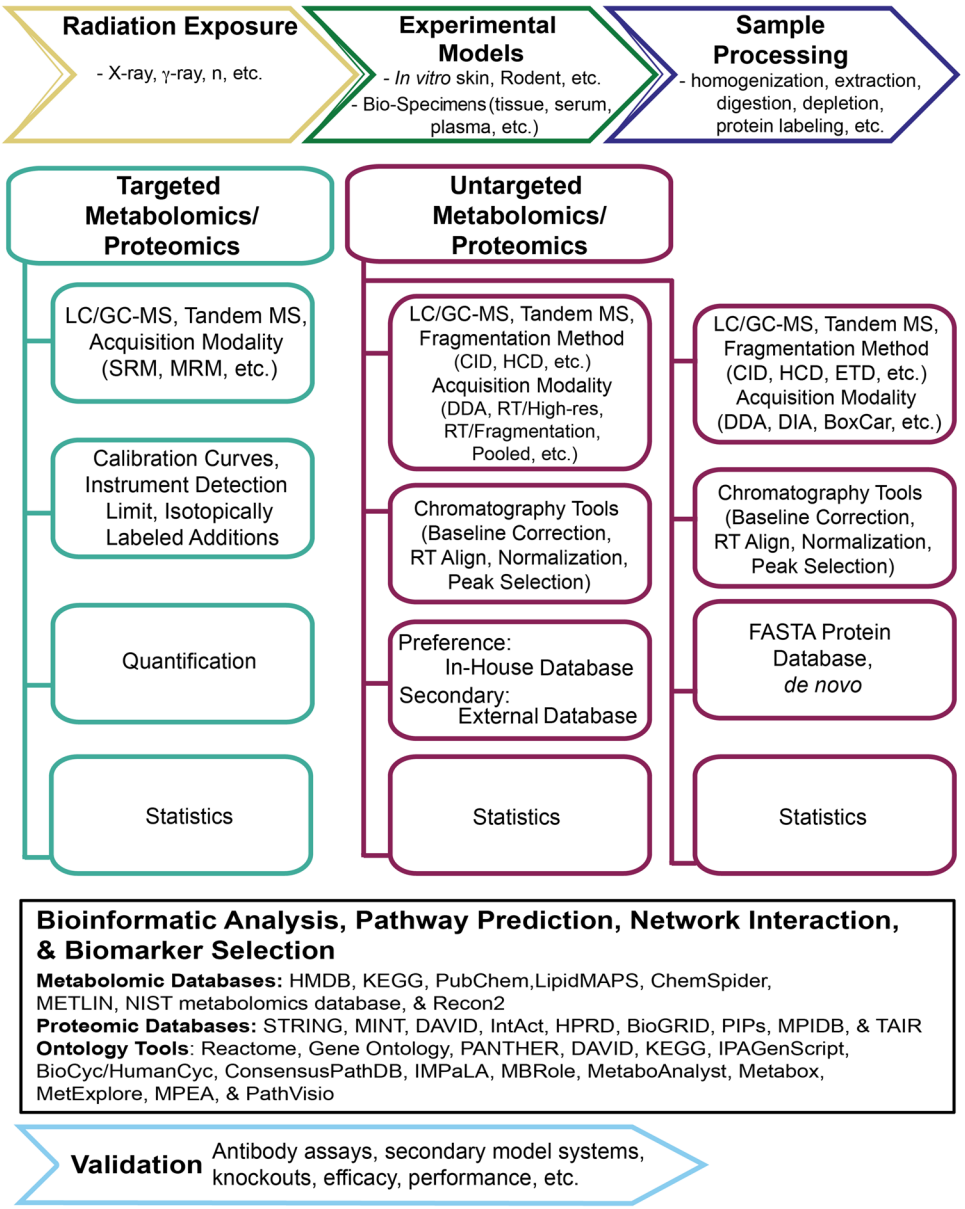

Fig. 4 Sample workflow for MS-based -omics analysis. Beginning with sample radiation exposure, this workflow includes both targeted (analyte(s) are known) and untargeted (analyte(s) are unknown) mass spectrometry and highlights the components involved in each step. The ontological tools listed here are not exhaustive but represent the bulk of available options

is even a growing body of evidence that challenges the more traditional linear no-threshold (LNT) model at low doses; hormetic effects have been observed in many instances of low-dose exposure [105].

As previously stated, there is a relative dearth of published information on in vitro 3D skin models interrogated by MS-based -omics; this holds true for clinical low-dose radiation studies, as well. However, there is still much relevant information to be gathered from other in vivo and ex vivo skin-related studies that can provide useful comparisons for evaluating the suitability of a given in vitro skin model. Radiotherapy (RT)-induced damage to the skin can activate physiological responses in a similar method to other sources of low-dose ionizing radiation [106]. This response is usually dose- or individual-dependent, and can result from either direct exposure or via non-targeted (bystander) effects [107]. In particular, bystander effects are of interest clinically due to the need to localize ionizing radiation effects to tumors or malignant cells only. These experiments have thus far been performed in murine models, human cell cultures, and some 3D skin models. Guipaud et al. followed a time-course analysis for gamma-irradiated (0–80 Gy) mice utilizing skin biopsies, as well as proteomic serum analysis [108]. Histological and 2D-DIGE-MS results were compared and several acute-phase proteins (APP), as well as proteins from the coagulation system were found to be significantly changed in the irradiated mice. Lacombe et al. exposed T lymphocytes cultured from patient blood with/without sub-cutaneous radiation-induced breast fibrosis to sham and 8 Gy radiation doses ex vivo [109]. Forty-eight

hours after irradiation, samples were subjected to isobaric tags for relative and absolute quantitation via iTRAQ (an isobaric labeling technique that enables protein quantitation) labeling and processed for proteomic analysis [110]. Four of the 23 proteins that matched pre-set criteria were involved in adenylate kinase 2 (AK2) overexpression and oxidative stress regulation. An increase was also seen after 48 h in both ROS as well as NADPH oxidases (NOXs).

MS-based -omics approaches have been used to study IR effects in many different tissues and cell types to better understand possible biomarkers for exposure. Chaze et al. investigated the serum proteome by 2D-DIGE and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF–MS) after relatively high (20–80 Gy) doses of IR to murine skin models, and found a panel of 14 biomarkers, mostly apolipoproteins and proteins of the complement system (Complement factor I, Complement factor H, etc.), that allowed them to discriminate between non-irradiated and irradiated animals [111]. A follow-on enzyme-linked immunosorbent assay (ELISA) study indicated that eight of these proteins were able to discriminate between doses of < 2 Gy and > 10 Gy [112]. Skiöld et al. used isotope-coded protein labeling (ICPL) analysis on blood samples gathered in vitro and irradiated from 1 to 150 mGy and found differences in protein expression between radiation-sensitive and normo-sensitive breast cancer patients at both levels [112]. Levels of PRDX2 and BLVRB, both associated with oxidative stress, were found up-regulated in the radiation-sensitive patients but remained unchanged in the normo-sensitive patients. However, protein expression levels are not the only changes that can indicate possible biomarkers for IR exposure. Post-translational modifications (PTM) can bring added complexity but also granularity to possible IR exposure biomarkers. Beli et al. were able to show that protein phosphorylation associated with DNA damage response (DDR), a pathway commonly associated with IR exposure, either increased (PPM1G) or decreased (THRAP3) their presence at the site of DNA damage [113]. Schettino et al. utilized a micronuclei assay to determine DNA damage in the EPI-200 (MatTek) in vitro human skin model to investigate bystander effects for low-dose ionizing radiation [114]. A 3.5 MeV proton source (0.1–1.0 Gy) was used to irradiate the tissue, which was subsequently sectioned by microtome and cells from these slices (~ 200 µm thick) were isolated. Micronuclei frequency were found to be most elevated closest to the radiation source, with decreasing frequency farther away. The relatively small amount of micro-nucleation at large (several mm) distances from the source indicate that bystander effects did not coincide with significant cellular damage. Although this approach did not utilize MS-based analysis, it is one of the few studies done on an in vitro 3D skin model and points toward the importance of considering bystander effects with these models. At least one report of metabolomics changes in multiple cell types support the idea that LDR exposure can be difficult to track at this level [115]. However, the relative ease of sampling associated with metabolomics, as well as its ability to track transient (~ hours) changes in the cellular metabolome after IR exposure warrant further investigation by this technique.

Although there has been significant interest in the effects of IR on various tissues and cell cultures in regard to radiotherapy and clinical applications, there once again remains a lack of MS-based -omics investigations. Nearly all of the associated research has been focused on genomics and transcriptomics of ionizing radiation, and often at radiation doses (10 s of Gy) that far exceed what could be considered low dose [116, 117]. There are also many logistical and reporting hurdles to overcome. It can be difficult to directly compare studies, even with the same matrix (e.g., skin, blood, serum), without detailed descriptions of the IR source and dosing conditions, which is not always made available. Sample collection and preparation is a crucial first step after IR exposure, but these procedures are also often not descriptive enough to allow for methodologies to be transferable from one laboratory to another. Nevertheless, the significant advantages in terms of data granularity and complexity for modern MS-based -omics applications suggests that further investigations are warranted [118].

Conclusion

As more information is gathered about the effects of LDR exposure, it is becoming clear that MS-based -omics will play a critical role in future [119]. The speed, sensitivity, and granularity of which these techniques are capable will provide a far more holistic picture of the complex responses of cells, tissues, and organs to LDR. This information will be essential in applying LDR research to personalized medicine, where phenotype and radiation sensitivity must be considered. Concurrently, the continued development of in vitro 3D skin models promises to provide excellent spatiotemporal results as these models are improved. These spatiotemporal results can also be further improved upon with the addition of imaging mass spectrometry (IMS) techniques [120, 121]. The ability to image biomarkers within different strata of skin could serve as a potential solution to issues with whole tissue homogenization, and should be seen as a natural progression beyond immuno-histochemical staining for visualization of biomarkers in tissue. As more MS-based studies are conducted, the advantages of IMS should be included wherever possible, for both larger (proteins) and smaller (metabolites) biomarkers. The ability to move beyond animal-based models has significant advantages in terms of throughput and cost, but there are still limitations

to how well these 3D models are able to mimic their in vivo counterparts. Indeed, the complexity of both the analysis technique as well as the in vitro model will necessitate advances in bioinformatics, as well. By leveraging all these technologies and capabilities in unison, it will become possible to probe the most difficult of questions related to LDR, namely the ability to determine dose type/rate and level of exposure in both the short and long term.

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