Proceedings of ICBioMed 2025 Symposium: AI for Healthcare: Advanced Medical Data Analytics and Smart Rehabilitation
DOI: 10.54254/2753-8818/2025.AU38993

Comparative Analysis in Radiation-Induced DNA Repairing in Primed and Naïve Human Pluripotent Stem Cells

Yuliang Ma

Xi'an New Channel-Northwestern Polytechnical University, Xi'an, China
Mayuliang2026@163.com

Abstract. Stem cell therapy shows great potential for regenerative medicine but faces significant challenges in the post-radiotherapy tumor microenvironment, since there exit persistent DNA damage and reduced cell viability. Although pluripotent stem cells of human are largely used in stem cell therapies, the potential impact of their distinct pluripotency states, naïve (pre-implantation) and primed (post-implantation), on radiation response remains largely unexplored. This study proposes a comparative analysis of human primed and naïve pluripotent stem cells (hPSCs) to test differential resistance to ionizing radiation. The experimental design involves generating naïve and primed iPSCs through mRNA-based reprogramming from human dermal fibroblasts to ensure a consistent genetic background, followed by exposed to a gradient of γ-radiation doses. Cell viability will be measured quantitively using the CCK-8 assay, and the DNA double-strand break (DSB) repairing rate will be evaluated through long-amplicon quantitative PCR assay (QPCR) to calculate lesion frequency. I hypothesize that one pluripotent state will demonstrate superior damage response, shown through either a higher cell survival percentage or an accelerated DNA repair rate. Identifying which state possesses greater inherent resistance could provide critical evidence of selecting or engineering more resilient stem cells, thereby improving the efficiency and applicability of stem cell therapies following a radiotherapy.

Keywords: Naive Pluripotency, Primed Pluripotency, Human Induced Pluripotent Stem Cells (hiPSCs), DNA Damage Repair, Ionizing Radiation, Radio-resistance, Stem Cell Therapy

1. Introduction

1.1. Introduction to stem cells

Stem cells are recognized as a specific cell type with self-renewal capability and differentiating potential into other cell types at the appropriate environment and under certain signaling stimulation [1, 2].Stem cells can be grouped into four kinds: totipotent stem cells (TSCs, able to differentiate to all cells types that build up a human individual.); pluripotent stem cells (PSCs, similar to the previous one, but unable to form a complete individual, which can be either gained from embryonic stem cells or reprogrammed from somatic cell, called iPSCs as shown in the Figure 1 [3, 4]); multipotent stem cells (MSCs, they can develop to become most of cell kinds but not all.) and unipotent stem cells (USCs, only able to develop into a certain cell kind.) [5]

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2025.AU38993

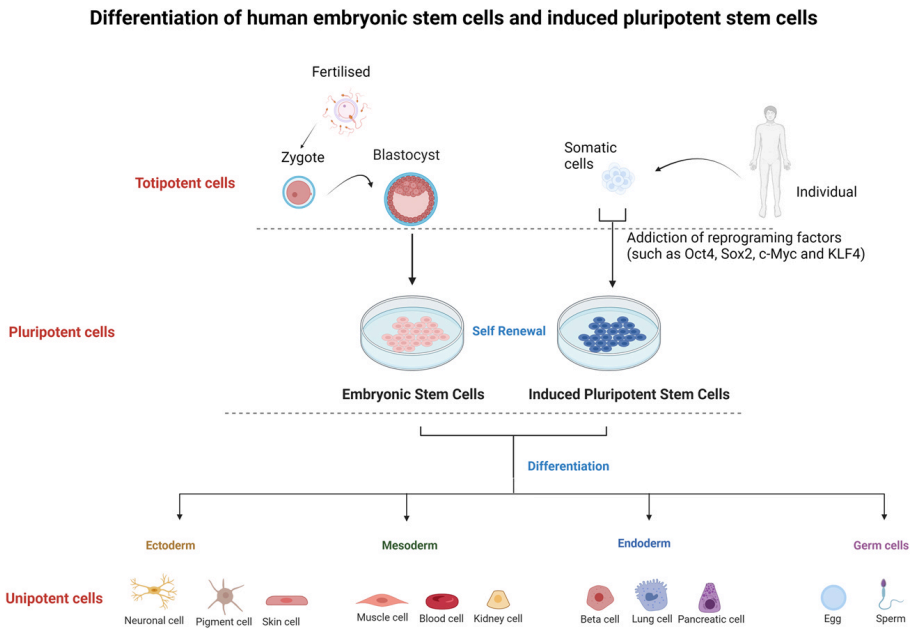


Figure 1. The generation process and differentiation steps of embryonic and induced pluripotent stem cell gaining from human [5]

1.2. Introduction to primed and naïve stem cells

In this article, I am going to concentrated on human primed (hpPSCs) and naïve stem cells (hnPSCs), which recognized as different states in the category of pluripotent stage. Naïve stage, called in 'ground state' of pluripotency, are originate in the blastocyst's pre-implantation epiblast. Naïve state possesses much higher developmental potential, enabling them to emerge into all kind of the fetus's cell kinds, including reproduction cells. In contrast, the primed state exists in the epiblast after the implantation, during which, cell begins to respond to extrinsic signals from the surrounding tissues, preparing them for following lineage specification [6]. Recently, it is well-established in the scientific community that stem cells in different pluripotent states exhibit significant differences in gene expression, metabolism, as well as differentiation propensity [7, 8]. However, how they differ in their response mechanisms to lethal stress, especially ionizing-radiation, remains largely unexplored.

1.3. Problem related with stem cell therapy

With the increasingly comprehensive understanding in self-regenerative ability as well as pluripotency in stem cells [9], stem cell therapy (SCT) has caught great attention lately. SCT is known as a regenerative medical approach by transplanting human stem cells to a specific hurt position that requires re-establish or restore normal tissues [10]. However, stem cell therapy for cancer is confronted with critical challenges after radiotherapy [11]. Even though residual radioactive substances remain negligible post-radiotherapy, the therapy is largely hindered by the radiation-induced hostile micro-environment, which characterized by sustained oxidative stress [12], inflammatory cytokine accumulation [13], hypoxia [14], and impaired stromal support [15]. This lethal environment might inhibit the induced stem cells' self-renewal capacity and proliferative potential [16]. Therefore, this proposal is going to focus on the radiation response on two special

DOI: 10.54254/2753-8818/2025.AU38993

kinds of pluripotent state: naïve and primed state, aiming to test their potential of dealing with the harmful post-radiotherapy micro-environment.

1.4. Why primed as well as naïve stem cells are chosen

Reasons why hnPSCs and hpPSCs are chosen to make a comparison in radiation-triggered DNA damage are due to the following aspects. Most studies investigate pluripotent stem cells of human (hPSCs) as one whole part at most of the time, such as deriving the hippocampal lineage from hPSCs [17], developing pluripotent stem cell-based human tenocytes [18], Acquire organoids to cure damages by inducing pluripotent cells of human [19], and so on. However, a current research gap becomes unveiled, researchers overlook the internal differences in PSCs arising from their pluripotent states. That's the reason why comparison between naïve state and primed state of pluripotency of human is specially highlighted in this article. Moreover, limitations lie in comparison between other stem cell types, too. To be more specific, if we investigate the reaction differences of radiation from two distinguished stem cell categories, variables are not controlled. For instance, studying unipotent and adult stem cells fails to control the following three factors: They belong to distinct genetic backgrounds [20]; They reside in different tissue micro-environments [21]; They have divergent differentiation fates [22]. If differences in their responses to radiation are observed, it remains hard to decide whether the variation comes from their environmental conditions or their intrinsic pluripotency stages. In contrast, primed and naive stem cells both belong to the pluripotent stem cell category, with similar genetic backgrounds, making variable control much more feasible [23].

1.5. Research aim

This article focuses to investigate the divergent responses of naive and primed stem cells to radiation-induced DNA damage and also their repairing dynamics. The purpose of this proposal is to establish a basic understanding the behind mechanics of how naïve as well as primed state of PSCs dealing with deadly radiation pressure, and thus selecting a kind of pluripotent stem cells that are more resilient to radiation-induced DNA damage, providing a basis for selecting or further engineering stem cells that can thrive in the damaged post-radiotherapy microenvironment, which has the promise of improving SCT further. The experimental methods utilize the radiation of certain dose on primed and naïve stem cells, to test any difference in DNA response and repairing efficiency of these two, the DNA repairing process is evaluated from two perspective: stem cell viability and DNA repairing rate.

2. Experimental design

2.1. Cell gaining

2.1.1. Acquire sources in naïve state

Human somatic cells are used to be induced back to naïve state of stem cell. Here is the specific method: Primary human dermal fibroblasts are cultured to the logarithmic growth phase to serve as the source of somatic cells. A non-integrating vector carrying the OSK genes (which includes encoding OCT4, SOX2, and KLF4 factors) is introduced into approximately 1×10^6 human fibroblasts through the process of nucleofection. Twenty-four hours after transfection, the cells are plated on mitotically inactivated embryonic fibroblasts of human (HEFs) feeder plate. At the same

DOI: 10.54254/2753-8818/2025.AU38993

time, the culture medium should be immediately replaced with the naive reprogramming medium, the content shown as follow:

• base medium: consisted of DMEM/F12 containing Knockout Serum Replacement (KSR) of 20%

• additives: bFGF of 4 ng/mL and hLIF of 10 ng/mL

• small molecule inhibitor cocktail: 3 μM CHIR99021, 0.5 μM PD0325901, 10 μM SP600125, also 10 μM SB203580.

Culturing medium is renewed entirely every single day. After approximately 3 or 4 weeks of culture, typical dome-shaped colonies will emerge. Individual colonies are manually picked, harvested as single cells by using Accutase and re-plated back on fresh HEF feeder layers for continued expansion. For long-term culture, the cells are maintained in a naive maintenance medium and passaged for every 4 to 5 days, then dissociated into single cells using Accutase. The acquired naive stem cells should have the following characteristics: a dome-shaped, compact colony morphology; positive expression of NANOG, OCT4, TRA-1-60 and markers likewise; and high expression of naive-state genes including TBX3 and KLF5 [24-25].

2.1.2. Acquire primed pluripotent cell

hpPSCs can be directly set apart from blastocysts of human. Obtaining legal, early human blastocysts under ethical regulations (typically at days 5-7 post-fertilization). Isolate the inner cell mass (ICM) by removing the trophectoderm through immune-surgery (using antibodies and complement to eliminate the trophectoderm) or mechanical dissection. Seed the ICM onto a feeder layer of irradiated or mitomycin C-treated human embryonic fibroblasts (HEFs) for initial culture. More importantly, culture the cells using a Primed-state-specific medium. The core components of the medium should include:

• Base Medium: DMEM/F12

• Key Additives:

• Bfgf (FGF2): This is the most critical cytokine for maintaining the Primed state, usually used at a concentration of 4-20 ng/mL.

• The TGF-β/Activin A signaling acts as a key regulator in maintaining the human primed state.

• Other Components: Such as L-Glutamine, KSR (typically 20%), and so on.

After several days of culture, the ICM cells will adhere and give rise to colonies exhibiting the typical Primed morphology: flat, loose, and with vague boundaries. These colonies are then picked, dissociated (usually into small clumps using collagenase or disease, rather than single cells), and subsequently passaged and expanded to eventually establish a stable Primed hESC cell group (Figure 2) [26-27].

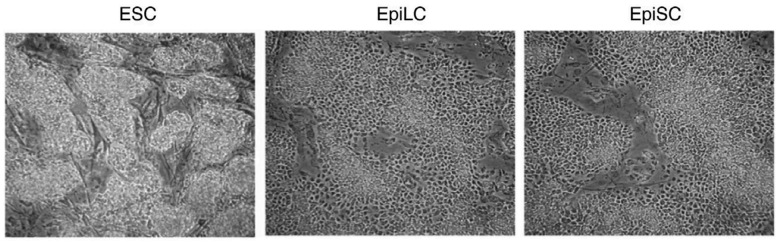


Figure 2. Micrograph showing ESCs, EpilLCs, and EpiSCs., the difference in cellular feature and appearance can be observed to understand what shape of the cell is wanted in the experiment [28]

DOI: 10.54254/2753-8818/2025.AU38993

2.2. Experimental group design and radiation treatment

To investigate the radiation sensitivity and DNA damage response of naive and primed hPSCs, the following groups are established:

2.2.1. Independent variables

Cell State: Naive hPSCs and Primed hPSCs.

Various Radiation Treatment

2.2.2. Radiation treatment groups

In order to control time of exposure and radiation dose, the cells are divided into four groups.

Negative Base Control: experimental sources not exposed to radiation.

Positive Base Control: experimental sources exposed to a benchmark radiation dose (2 Gy of γ-radiation, delivered by a ^{60}Co irradiator for a fixed duration of 24 h).

Dose Gradient: Cells were irradiated with γ-rays at doses ranging from 0.5 to 4 Gy, adding 0.5 Gy each time, with all groups assessed at the same post-irradiation time point (24 h).

Time Gradient: test sources irradiated with a fixed dose of radiation (2 Gy) and assessed at 0h, 4h, 8h, 12h, 16h, 20h, 24h post-irradiation [29].

2.3. Assessment of cell viability post-radiation

2.3.1. Use cell counting Kit-8 measure cell viability

Measurements of cell viability and proliferation is commonly performed using the Cell Counting Kit-8 (CCK-8) assay. It employs a tetrazolium salt called WST-8, that is reduced using dehydrogenase enzymes into a orange formazan that can dissolve in water in metabolically active cells. The concentration of this formazan, can be quantified through colorimetric devices. The intense of the light observed is proportional to the survived cell number [30]. For each post-irradiation groups mentioned above, Each well received an addition of CCK-8 reagent. (10 μL reagent per 100 μL of medium) and cultured for 2 hours at the temperature of 37℃. Absorbance should be recorded at 450 nm within one microplate reader [31].

2.3.2. Data analysis

Plot a line graph showing Cell Viability (%) over Groups for both primed as well as naïve state [32] Rate at which the viability line drops and whether it recovers visually represents how quickly the cells are being killed by the radiation. Cell viability can be calculated by the equation shown below, where the absorbance of experimental group indicates test groups that receive radiation treatment. The blank group means the group without any experimental sources, in order to eliminated any background effects. The group of control represents group of positive control mentioned above, cells without any radiation treatment [33].

$$CellSurvival(\%) = \frac{[ExperimentalGroupAbsorbance - BlankGroupAbsorbance]}{[ControlGroupAbsorbance - BlankGroupAbsorbance]} * 100\%$$

DOI: 10.54254/2753-8818/2025.AU38993

2.4. Evaluation of DNA repair efficiency

DNA repair is measured by quantifying the rate of double strands of DNA break, long-amplicon quantitative PCR (QPCR) assay is utilized [34]. Mechanism behind is that DNA polymerase employed in PCR can only extend DNA strands from undamaged primers and replicate undamaged templates. When a DNA template contains a lesion (such as a strand break, oxidative damage and etc.) within the region that need to be amplified, it will significantly hinder or completely block the polymerase's progress, therefore, DNA damage can be quantified in this way [35].

2.4.1. DNA damage induction and sample collection

After being exposed to a specific dose or time of ionizing radiation, stem cells are continued to be cultured in a 37°C incubator for repair. Cells (about 1×10^6 cells per sample) are collected immediately (0h) and then ranging from 6h to 48h, increasing 6 hours each time in order to observe the dynamic process of DNA repairing. The cell pellets can be obtained by centrifugation and should be kept at -80°C until the extraction of DNA.

2.4.2. Isolation of high-molecular-weight genomic DNA

DNA should be purified from cell pellets for genomic analysis using QIAGEN Genomic-tip 20/G columns or the DNeasy Blood & Tissue Kit. A key point is to avoid the use of phenolic reagents in order to ensure high-quality, high-molecular-weight DNA templates, which are crucial for long-fragment amplification. The extracted DNA is dissolved in buffer solution (pH 8.0).

2.4.3. Accurate DNA templates quantification

The concentration of the extracted DNA was determined precisely using PicoGreen quantification reagent for double-stranded DNA. After dilution, DNA samples were analyzed with a known concentration DNA standard curve in a dark well plate with 96 holes. Fluorescence intensity can be determined using a fluorescence microplate reader to ensure consistent starting template amounts for all QPCR reactions.

2.4.4. Mitochondrial DNA linearizing

If extraction methods (like QIAcube) are used, the extracted mitochondrial DNA (mtDNA) is mostly in a supercoiled structure, which can affect amplification efficiency. Therefore, prior to QPCR, mtDNA samples require restriction enzyme digestion to eliminate supercoil. DNA digestion requires the appropriate restriction enzyme (e.g. PvuII for human samples) at 37°C for 1–2 hours to linearize the mtDNA outside the target amplification region of the mitochondrial genome, thereby ensuring amplification efficiency.

2.4.5. Long-fragment and short-fragment QPCR amplification

To measure DNA damage, two rounds of QPCR amplification are performed for each sample:

• Long fragment (~10–15 kb): Used to detect DNA damage. Damage blocks DNA polymerase, resulting in reduced amplification products.

• Short fragment (~200 bp): Used for normalization. Due to its short length, it is less affected by damage, and amplification efficiency remains relatively constant.

DOI: 10.54254/2753-8818/2025.AU38993

2.4.6. Quantification of PCR products

After QPCR, a portion of the product is taken and quantified again using the PicoGreen method (with a no-template control as blank). The damage frequency can be calculated by comparing the amplification yield of damaged samples with that of undamaged control samples.

2.4.7. Data analysis of DNA repair efficiency

Lesion frequency is used to describe the average number of damage sites present on a DNA segment of a specific length. Mean value as well as standard deviation (SD) of the lesion frequency (Lesions/10 kb) for each time point (0ht to 48h as mentioned before) should be determined. Use the equation below to calculate Lesion Frequency, where 'A' represents the amount of PCR product generated. 'Control' means the DNA amplified from a non-radiated sample. 'Damaged' means the amount of DNA amplified from a treated sample.

$$lesion\ Frequency = \ -\frac{\ln\left(A\ damaged\right)}{\ln\left(A\ control\right)}$$

Plot a line graph of Lesion Frequency against Times. Represent individual point using the mean data and error bars to show SD. As a result, this graph can visually display a decreasing trend of damage over time, which represents repairing process and make a preliminary comparison of the repairing rate [36].

3. Predicted results

There are 3 possible situations where the experiments above might discover:

Scenario 1: There is no significant difference in either cell survival rate or DNA repairing rate, indicating that there is almost no difference in the DNA repairing due to a radiation damage between hpPSCs and hnPSCs.

Scenario 2: There is either a quicker DNA repairing rate or higher cell viability percentage for primed stem cells, indicating that primed stem cells have greater resistance in facing radiation damage, showing greater potential of being used in improving stem cell therapy.

Scenario 3: These is either accelerated DNA repair process or higher cell survival for naïve stem cells, which shows its superior resistance to radiation damage. This enhanced resilience unveils its potential for improving stem cell therapy.

4. Discussion

The experimental approach outlined above will be performed at a single-cell level to compare the characteristics of primed and naïve stem cells. While this simplified strategy provides essential insights into their intrinsic properties, the translational application of these findings in stem cell therapy is often constrained by the intricate and dynamic microenvironment of human organs, which single-cell cultures cannot fully replicate. To overcome this critical gap between simplistic in vitro models and the complex internal physiology, organoid technology acts a powerful intermediate. Organoids are defined as miniaturized and simplified versions of organs generated in vitro in three dimensions; they mimic the key function, structure, and biological complexity of their in vivo

DOI: 10.54254/2753-8818/2025.AU38993

counterparts [37]. This model system can be strategically employed to further validate the differential radiation responses of naïve and primed hPSCs by providing a more real living environment, as illustrated in Figure 3. For instance, a proposed methodology would involve exposing the organoids to a defined dose of radiation to simulate tissue injury. One could implant merely hpPSCs, only hnPSCs, or co-culture with both populations into these irradiated organoids. The subsequent regenerative process could then be monitored by live-cell imaging, immunohistochemistry, or functional assays as mentioned previously. Key standards of evaluation would not only include the comparative survival rates of the implanted stem cell populations but also the repairing efficiency in a 3D structure as a whole. The repairing process in 3D structure requires much more than in 2D structure, such as migration power of stem cells towards damage sites, differentiating into appropriate organ-specific cell lineages, or restoring the damaged function or tissue activity. This organoid-based plan moves the investigation beyond the single-cell level to a more sophisticated organ-level. It allows for a direct, side-by-side comparison of theoretical potential in a controlled but complex environment, stepping further to the real situation, thereby offering more predictive insights for improving safety as well as efficiency of future therapies related with stem cells [38, 39].

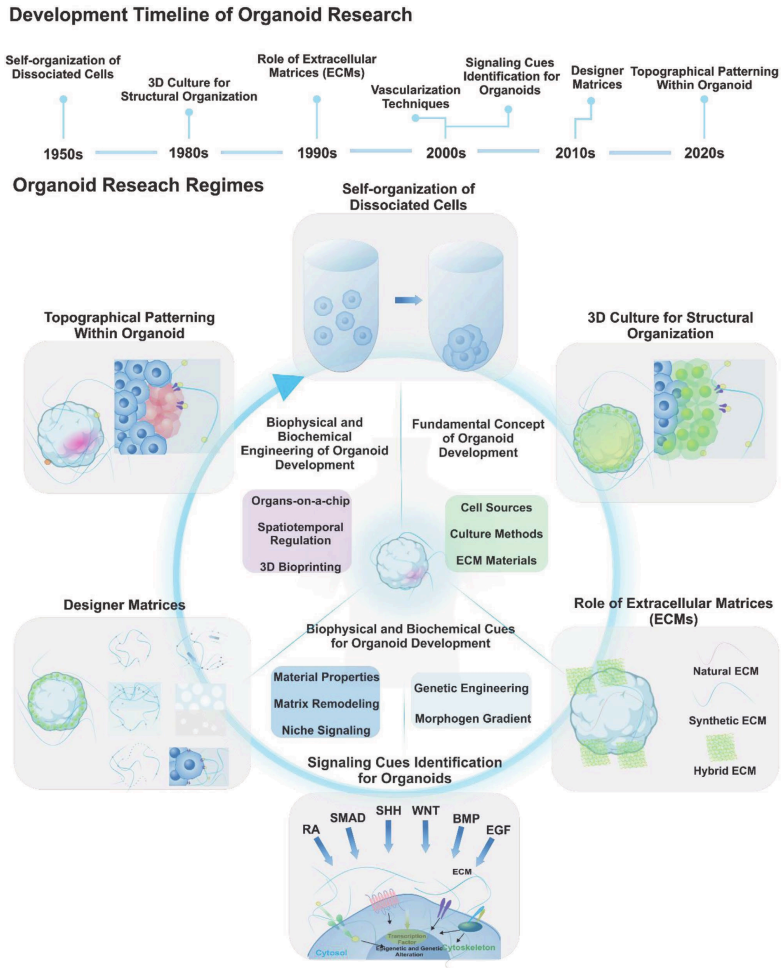


Figure 3. Developmental progress in organoid technologies (the upper part). From basic principles to engineering, showing a complete framework of organoid procedures (the lower part) [40]

DOI: 10.54254/2753-8818/2025.AU38993

5. Conclusion

This study outlines a systematically-designed proposal which aims to investigate the radiation response of naïve versus primed human pluripotent stem cells. By controlling for genetic background and focusing on intrinsic differences in pluripotency states, the experiment has the potential to fill a gap in our understanding of how different states in pluripotent stem cells can act as individuals to face radiation stress. The expected results, whether indicating superior resistance in the naïve or primed state, will provide valuable insights for optimizing stem cell-based therapies. An observed difference in radio-resistance would encourage the selective use of one cell type over the other for transplantation into radiation-damaged tissues. Furthermore, the findings would establish a foundation for future work, such as utilizing organoids and therefore enabling the future design of more effective regenerative medicine approaches aiming to help cancer patients' post-radiotherapy.

References

[1] Lander, A. D. (2009). The 'stem cell' concept: Is it holding us back? *Journal of Biological Chemistry, 8*(8), 70. doi: 10.1186/jbiol177.
[2] Jin, J. (2017). Stem cell treatments. *JAMA, 317*(3), 330. doi: 10.1001/jama.2016.17822.
[3] Mikkelsen, T. S., Hanna, J., Zhang, X., et al. (2008). Dissecting direct reprogramming through integrative genomic analysis. *Nature, 454*(7200), 49–55. doi: 10.1038/nature07056.
[4] Chin, M. H., Mason, M. J., Xie, W., et al. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell, 5*(1), 111–123. doi: 10.1016/j.stem.2009.06.008.
[5] Zeyu, T., Tao, Y., and et al. (2023). Introduction to stem cells. *Progress in Molecular Biology and Translational Science, 199*, 1877-1173. doi: 10.1016/bs.pmbts.2023.02.012
[6] Jennifer, N., and Austin, S. (2009). Naïve and primed pluripotent states. *Cell Stem Cell, 4*. doi: 10.1016/j.stem.2009.05.015.
[7] Tsogatbaatar, E., et al. (2020). Energy metabolism regulates stem cell pluripotency. *Front. Cell Dev. Biol., 8*: 87. doi: 10.3389/fcell.2020.00087.
[8] Atilgan, Y., & Nissim, B. (2019). Defining human pluripotency. *Cell Stem Cell, 25*. doi: 10.1016/j.stem.2019.06.010.
[9] He, S., Nakada, D., & Morrison, S. J. (2009). Mechanisms of stem cell self-renewal. *Annu Rev Cell Dev Biol, 25*: 377–406. doi: 10.1146/annurev.cellbio.042308.113248.
[10] Mahshid, B., Hossein, M., & Farshid, Y. (2023). Stem cell therapy, the market, the opportunities and the threat. *International Journal of Molecular and Cellular Medicine, 12*(3): 310-319. doi: 10.22088/IJMCM.BUMS.12.3.310.
[11] Saul, J., et al. (2012). Pluripotent stem cell-based cancer therapy: Promise and challenges. *Sci Transl Med, 4*(127): 127ps9. doi: 10.1126/scitranslmed.3003920.
[12] Zhang, P., et al. (2019). Oxidative stress in radiation-induced cardiotoxicity. *Oxidative Medicine and Cellular Longevity*. 15. doi. 10.1155/2020/3579143.
[13] Varintra, E. K., et al. (2020). Tumor microenvironment as a regulator of radiation therapy: New insights into stromal-mediated radioresistance. *Cancers, 12*: 2916. doi: 10.3390/cancers12102916.
[14] Yuyang, T., et al. (2017). Effect of a hypoxic microenvironment after radiofrequency ablation on residual hepatocellular cell migration and invasion. *Cancer Sci, 108*: 753-762. doi: 10.1111/cas.13191.
[15] Magdalena, J.-B., et al. (2019). Tumor microenvironment as a 'game changer' in cancer radiotherapy. *Molecular Science, 20*: 3212. doi: 10.3390/ijms20133212.
[16] Rossi, D. J., et al. (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature, 447*(7145): 725-9. doi: 10.1038/nature05862.
[17] Nishimura, H., & Li, Y. (2024). Human pluripotent stem cell-derived models of the hippocampus. *Int J Biochem Cell Biol, 177*: 106695. doi: 10.1016/j.biocel.2024.106695.
[18] Nakajima, T., & Ikeya, M. Development of pluripotent stem cell-based human tenocytes. *Dev Growth Differ, 63*(1): 38-46. doi: 10.1111/dgd.12702.
[19] Poling, H. M., et al. (2024). Human pluripotent stem cell-derived organoids repair damaged bowel in vivo. *Cell Stem Cell, 31*(10), 1513–1523.e7. doi: 10.1016/j.stem.2024.08.009.

DOI: 10.54254/2753-8818/2025.AU38993

[20] Sacchetti, B., et al. (2016). No identical "mesenchymal stem cells" at different times and sites: Human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels. *Stem Cell Reports, 14*(6), 897–913. doi: 10.1016/j.stemcr.2016.05.011.
[21] Yu, Z., et al. (2018). Hoxc-dependent mesenchymal niche heterogeneity drives regional hair follicle regeneration. *Cell Stem Cell, 23*(4), 487–500.e6. doi: 10.1016/j.stem.2018.07.016.
[22] Nguyen, D. H., et al. (2020). Apoptosis in the fetal testis eliminates developmentally defective germ cell clones. *Nat Cell Biol, 22*(12), 1423–1435. doi: 10.1038/s41556-020-00603-8.
[23] Wojciech, Z., et al. (2019). Stem cells: Past, present, and future. *Stem Cell Research & Therapy, 10*, 68. doi: 10.1186/s13287-019-1165-5.
[24] Gafni, O., et al. (2013). Derivation of novel human ground state naive pluripotent stem cells. *Nature, 504*(7479), 282–286. doi: 10.1038/nature12745.
[25] Fang, R., et al. (2014). Generation of naive induced pluripotent stem cells from rhesus monkey fibroblasts. *Cell Stem Cell, 15*(4), 488–497. doi: 10.1016/j.stem.2014.09.004.
[26] Samanta, M., & Kalantry, S. (2020). Generating primed pluripotent epiblast stem cells: A methodology chapter. *Current Topics in Developmental Biology, 138*, 139–174. doi: 10.1016/bs.ctdb.2020.01.005.
[27] Sullivan, S., et al. (2018). Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. *Regenerative Medicine, 13*(7), 859–866. doi: 10.2217/rme-2018-0095.
[28] Gayen, S., et al. (2015). A primary role for the Tsix lncRNA in maintaining random X-chromosome inactivation. *Cell Reports, 11*(8), 1251–1265. doi: 10.1016/j.celrep.2015.04.039.
[29] Lan, M. L., et al. (2012). Characterizing the radioresponse of pluripotent and multipotent human stem cells. *PLoS One, 7*(12), e50048. doi: 10.1371/journal.pone.0050048.
[30] James, N., et al. (2022). Comparative evaluation of corneal storage medias used as tooth avulsion medias in maintaining the viability of periodontal ligament cells using the cell counting kit-8 assay. *Clinical, Cosmetic and Investigational Dentistry, 14*, 87–94. doi: 10.2147/CCIDE.S314478.
[31] Zhu, Y., et al. (2023). lncRNA ENST00000422059 promotes cell proliferation and inhibits cell apoptosis in breast cancer by regulating the miR-145-5p/KLF5 axis. *Acta Biochim Biophys Sin, 55*(12), 1892–1901. doi: 10.3724/abbs.2023226.
[32] Martinez-Pastor, B., et al. (2021). Assessing kinetics and recruitment of DNA repair factors using high content screens. *Cell Rep., 37*(13), 110176. doi: 10.1016/j.celrep.2021.110176.
[33] Nozhat, Z., et al. (2022). Different methods for cell viability and proliferation assay: Essential tools in pharmaceutical studies. *Anticancer Agents Med Chem, 22*(4), 703–712. doi: 10.2174/1871520621999201230202614.
[34] Furda, A. M., et al. (2012). Analysis of DNA damage and repair in nuclear and mitochondrial DNA of animal cells using quantitative PCR. *Methods Mol Biol., 920*, 111–32. doi: 10.1007/978-1-61779-998-3_9.
[35] Yakes, F. M., & Van Houten, B. (1997). Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A., 94*(2), 514–9. doi: 10.1073/pnas.94.2.514.
[36] Zaffaroni, N., et al. (1994). DNA double-strand break repair and radiation response in human tumour primary cultures. *Int J Radiat Biol, 66*(3), 279–85. doi: 10.1080/09553009414551211.
[37] Zhao, Z., et al. (2022). Organoids. *Nature Reviews Methods Primers, 2*: 94. doi: 10.1038/s43586-022-00174-y.
[38] Das, D., et al. (2020). Human forebrain organoids from induced pluripotent stem cells: A novel approach to model repair of ionizing radiation-induced DNA damage in human neurons. *Radiation Research, 194*(2): 191-198. doi: 10.1667/RR15567.1.
[39] Pasch, C. A., et al. (2019). Patient-derived cancer organoid cultures to predict sensitivity to chemotherapy and radiation. *Clinical Cancer Research, 25*(17): 5376-5387. doi: 10.1158/1078-0432.CCR-18-3590.
[40] Yi, S. A., et al. (2021). Bioengineering approaches for the advanced organoid research. *Advanced Materials, 33*(45): e2007949. doi: 10.1002/adma.202007949.