Sains Malaysiana 54(8)(2025): 1985-1994
*http://doi.org/10.17576/jsm-2025-5408-09*

# Evaluating the Efficacy of Cryopreservation Media for the Preservation and Short-Term Storage of Human Dermal Fibroblast

(Menilai Keberkesanan Media Pengkrioawetan untuk Pemeliharaan dan Penyimpanan Jangka Pendek Fibroblas Dermal Manusia)

TITHTEEYA RATTANACHOT[1], NUR RASYIDAH HAZIMAH MOHD ROSDI[1], NUSAIBAH SALLEHUDDIN[1], FAUZI MH BUSRA[1,2] & MANIRA MAAROF[1,2,3,*]

*[1]Department of Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia*
*[2]Advance Bioactive Materials-Cells UKM Research Group, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
*[3]Ageing and Degenerative Disease UKM Research Group, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*

*Received: 20 November 2024/Accepted: 17 June 2025*

ABSTRACT

Cryopreservation is a vital process for long-term preservation of cells without compromising their viability and functionality. Continuous cell culturing can lead to reduction in cell viability, higher risk of contamination, and increased reagent consumption. Optimizing short-term storage is essential to minimize cell damage and enhance cell adaptability for applications requiring brief storage duration. This study evaluates the effects of short-term storage on human dermal fibroblasts cryopreserved in different cryopreservation media. Redundant skin samples were obtained from surgeries with patient consent, processed, and sub-cultured to passage three (P3). Confluent cells were trypsinised and cryopreserved in three cryopreservation media: Foetal bovine serum with 10% dimethyl sulfoxide (FBS+10%DMSO), CryoStor10 (CS10), and cryo freezing serum-free media (CF-SFM). Cells were stored at -80 °C for 7 days, 14 days, and 1 month. Fibroblasts maintained their spindle-shaped, elongated morphology across all groups post-storage. The total number of live cells slightly decreased after 1 month, but no significant differences were found between the groups. Cell viability in CS10 after 1 month was significantly lower compared to the other storage durations, while no significant differences were observed in the other two media groups. Immunocytochemistry showed positive collagen type I (Col-1) and Ki67 expression at all storage durations. These findings suggest that fibroblasts retain their characteristics after short-term storage at -80 °C in different cryopreservation media. However, further studies are needed to examine the impact of long-term storage on other cell types.

Keywords: Cell characteristics; cryopreservation; fibroblasts; short-term storage

ABSTRAK

Pengkrioawetan merupakan proses penting untuk pemeliharaan jangka panjang sel tanpa menjejaskan daya hidup dan fungsinya. Kultur sel berterusan boleh menyebabkan pengurangan daya hidup sel, peningkatan risiko pencemaran dan penggunaan reagen yang tinggi. Pengoptimuman penyimpanan jangka pendek adalah penting untuk mengurangkan kerosakan dan meningkatkan kebolehsuaian sel bagi aplikasi yang memerlukan tempoh penyimpanan yang singkat. Penyelidikan ini menilai kesan penyimpanan jangka pendek terhadap fibroblas dermal manusia yang dikrioawet dalam media krioawetan berbeza. Sampel kulit berlebihan diperoleh daripada pembedahan dengan kebenaran pesakit, diproses dan dikultur sehingga subkultur 3 (P3). Sel yang mencapai konfluensi akan ditripsinkan dan dibekukan dalam tiga jenis media berbeza: serum janin lembu dengan 10% dimetil sulfoksida (FBS+10%DMSO), CryoStor10 (CS10) dan media bebas serum pembekuan kriogenik (CF-SFM). Sel ini disimpan pada suhu -80 °C selama 7 hari, 14 hari dan 1 bulan. Fibroblas mengekalkan morfologi berbentuk gelendong yang memanjang selepas penyimpanan. Jumlah keseluruhan sel hidup menurun sedikit selepas 1 bulan, namun tiada perbezaan yang ketara ditemui antara kumpulan. Keviabelan sel dalam CS10 selepas 1 bulan adalah lebih rendah berbanding tempoh penyimpanan yang lain, manakala tiada perbezaan diperhatikan dalam dua kumpulan media yang lain. Analisis imunositokimia menunjukkan ekspresi positif kolagen jenis I (Col-1) dan Ki67 pada semua tempoh penyimpanan. Penemuan ini menunjukkan bahawa fibroblas dapat mengekalkan cirinya selepas penyimpanan jangka pendek pada suhu -80 °C dalam media krioawetan berbeza. Namun, kajian lanjut diperlukan untuk menilai kesan penyimpanan jangka panjang pada jenis sel lain.

Kata kunci: Ciri sel; fibroblas; pengkrioawetan; penyimpanan jangka pendek

## INTRODUCTION

Cell cryopreservation has emerged as a crucial technology in recent years for a range of cell-based applications such as tissue engineering, stem cell treatment, assisted human reproduction, and transfusion medicine. This technique allows living cells to be preserved at cryogenic temperature between -80 °C and -196 °C (liquid nitrogen), without affecting their biological viability and functionality. The chemical, biological, and physical processes that typically take place at the cellular level can be halted for an extended period of time at such extremely low temperatures (Yang et al. 2020). At temperature below -130 °C, the kinetic energy and molecular motion within a biological material are greatly reduced. The rates of chemical and biological reactions decrease, leading to the slowing down of metabolism, active transport, enzymatic reactions, and diffusion. This process facilitates the maintenance of the cells in a state of suspended animation until a subsequent temperature elevation is introduced (Murray & Gibson 2022).

Multiple factors can influence the fibroblasts throughout the cryopreservation process. Research indicates that the use of cryoprotectant agents (CPAs), increased frequency of freeze-thaw cycles, and rapid freezing and thawing procedures may have detrimental effects on the cells (Shorokhova et al. 2024). The most effective CPAs should be able to prevent cellular damage, oxidative stress, physical and chemical damages to the cell membranes. To function as a successful CPA, a molecule should possess specific qualities, such as high-water solubility especially at low temperatures, ability to freely penetrate cell membranes and has minimal toxicity (Erol et al. 2021). Dimethyl sulfoxide (DMSO), a chemical compound synthesized from dimethyl sulfide, is an example of CPA. Other examples of CPAs include sucrose and trehalose, which are synthesized by fungi, plants, and invertebrate animals (Marcantonini et al. 2022). These CPAs decrease water crystallization and help maintain the integrity of the cell membranes (Singh et al. 2023). When nucleating chemicals are present, they initiate and catalyse the freezing process. Other factors that affect the ideal cooling rate include volume of the samples, the type of cells, and water permeability (Erol et al. 2021).

Dimethyl sulfoxide ($Me_2SO$), also known as DMSO, is a commonly used cryoprotectant that can minimise cell damages caused by extreme low temperature. DMSO, a dipolar aprotic solvent, has a variety of characteristics that allow it to participate in a wide range of chemical processes. DMSO exhibits excellent molecular stability, a significant dielectric constant and fundamental characteristics such as the capability to solvate salts, especially anions. It preferentially serves as a hydrogen bond acceptor, making it well known as an aprotic solvent (Awan et al. 2020). Thus, DMSO is often mixed with high molecular weight macromolecules that act as protectants by not penetrating the cells and providing protection via extracellular mechanisms. High molecular weight polymers can decrease the freezing point based on the principles of cooling kinetics, thus, preventing excessive freezing and lowering the rate of cooling which is necessary for cell survival. This will prevent water molecules from crystallizing during freezing and inhibit recrystallization after thawing (Awan et al. 2020). A mixture of 90% foetal bovine serum (FBS) and 10% DMSO is a commonly used cryoprotectant for fibroblasts and many other cell types. The function of FBS is to provide essential nutrients and proteins that aid in maintaining cell viability. Some FBS have differentiation growth factors that could cause cell differentiation (Uhrig, Ezquer & Ezquer 2022).

CryoStor® CS10 is defined a serum-free, protein-free and animal component-free cryopreservation medium based on the HypoThermosol® formulation. It contains 10% DMSO and is specifically formulated to maintain the ionic balance of cells at low and freezing temperatures, thus promoting faster recovery by minimizing stress and damage caused by the cryopreservation process. The formulation of CS10 includes pH buffering, free radical scavenging, osmotic support and ionic concentrations optimisation to enhance cell stability at ultralow temperatures (Valyi-Nagy et al. 2021). It is easy to use because there is no need for dilution or addition of ad hoc components. They are also standardised with consistent composition with minimal batch-to-batch variation.

Cryo Freezing Serum-Free Medium (CF-SFM) is a specialized culture medium designed to support the growth and maintenance of cells without the use of FBS. The SFM is formulated based on a mixture of Dulbecco's Modified Eagle Medium (DMEM) and Ham's F12, supplemented with adenine and L-Glutamax. The components in SFM provide essential nutrients and growth factors that can aid in maintaining cell viability during cryopreservation to ensure that the cells remain healthy and functional after thawing (Caneparo et al. 2022).

To determine which medium best preserves cell viability during short-term storage at -80 °C, it is important to study the effectiveness of different cryopreservation media. Understanding the performance of various cryopreservatives at low temperatures can help optimise protocols for short-term storage, minimising potential damage to cells and improving the recovery rates. Therefore, the aim of this study was to evaluate and compare the characteristics of human dermal fibroblasts (HDFs) after short-term cryopreservation at -80 °C using three different cryopreservation media, namely FBS+10%DMSO, CryoStor10, and CF-SFM, for the storage duration of 7 days, 14 days, and 1 month.

## MATERIALS AND METHODS

This study is approved by UKM research ethics committee with the reference number: UKM PPI/111/8/JEP-2023-007. The cryopreserved HDFs for this study were obtained from the Department of Tissue Engineering and Regenerative Medicine (DTERM) primary cell bank with ethics approval number: [UKM1.5.3.5/224/FF-2015-376].

### REVIVAL OF HDFs

The cryopreserved cells were thawed and resuspended in F12: Dulbecco's Modified Eagle Medium (F12: DMEM) (Sigma, USA) supplemented with 10% fetal bovine serum (FBS) (Capricorn Scientific, Germany). The cells were cultured in a T25 flask and maintained at 37 °C with 5% carbon dioxide ($CO_2$). The medium was changed every two to three days. The cells were subsequently subcultured into T75 flasks with medium changes every two to three days until it reaches 70 to 80% confluency.

### CRYOPRESERVATION OF HDFs

The HDFs cell pellet was resuspended with three different cryo media (FBS+10%DMSO, CryoStor10 and CF-SFM) at a final concentration of $3 \times 10^5$ cells/mL. One mL of each cell suspension was transferred into sterile cryovials and labelled as FBS-DMSO, CS10 and CF-SFM, respectively. The cryovials were then transferred into Mr. Frosty™ cell freezing container which had been equilibrated to room temperature and transferred to a -80 °C freezer for 24 h. After 24 h, the frozen cryovials were transferred to a cryobox and continuously stored at -80 °C for 7 days, 14 days, and 1 month.

At each timepoint, the cells were thawed using the same protocol. The cells were examined to determine the percentage of cell attachment, an indicator for cell viability and growth. Every image from the cell observation was captured. Immunocytochemistry analysis were performed for all cell cultures.

### CELL GROWTH AND VIABILITY

The fibroblasts viability was assessed by using the Trypan Blue solution and is expressed as the percentage of live cells relative to the total cell count.

$$\text{Cell Count} = \text{Average number of cells per square} \times \text{Dilution factor} \times 10^4 \times \text{Volume}$$

$$\text{Viability percentage} = \frac{\text{Live cells count}}{\text{Total cell count}} \times 100\%$$

### IMMUNOCYTOCHEMISTRY STAINING AND ANALYSIS

10,000 cells were seeded into three wells of 24-well plate and incubated at 37 °C with 5% $CO_2$ incubator for 24 h. After 24 h, the cells were fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature before being permeabilised with 0.5% Triton X-100 solution for 20 min. Prior to staining, cells were rinsed three times with phosphate buffered saline (PBS). Cell blocking was performed by incubating the cells in 10% goat serum for one hour at 37 °C. The cells were then incubated overnight at 4 °C with primary antibodies: mouse anti-Collagen Type 1 (Col-1) and rabbit anti-human Ki67. On the following day, the cells were incubated by a secondary antibody: goat anti-mouse Alexa Fluor 594 (red) for 2 h at 37 °C. Nuclei staining was performed using DAPI (4',6-diaminidino-2-phenylindole) at 1:15 000 dilution for 20 min at room temperature. Fluorescent photomicrographs were captured using Nikon confocal microscope (Nikon, A1R, Japan).

### STATISTICAL ANALYSIS

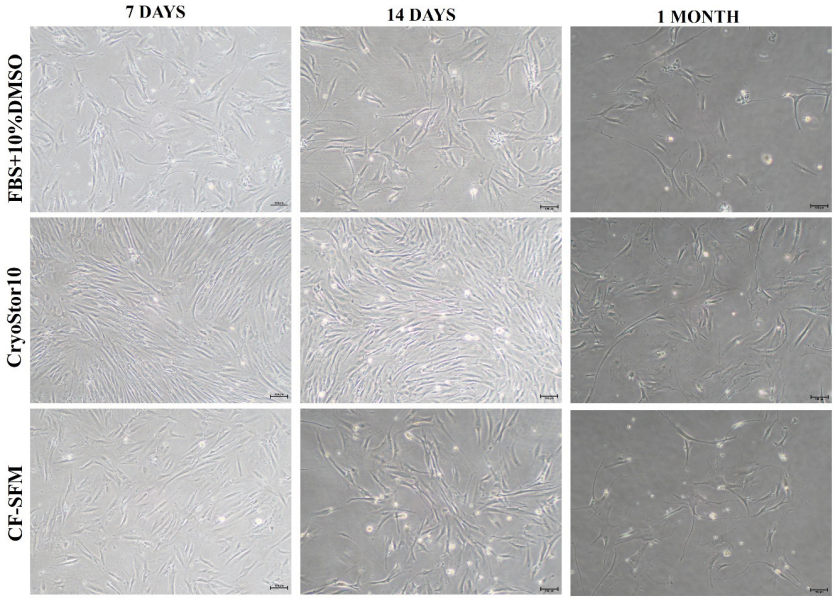
The statistical analysis was performed using GraphPad Prism (Version 10.0; GraphPad Software Inc., San Diego, USA). Statistical significance was interpreted using the two-way analysis of variance (ANOVA) and shown as the mean ± standard deviation (SD). The differences were considered significant if the p-value is less than 0.05.

### RESULTS AND DISCUSSION

After cryopreservation in different cryopreservation media (FBS+10%DMSO, CryoStor10 and CF-SFM), fibroblasts retained their elongated and spindle-shaped morphology (Figure 1). This observation is consistent with storage duration of 7 days, 14 days and 1 month at -80 °C. This indicates that the fibroblasts were able to maintain their morphologies even after 1 month of cryopreservation in ultralow temperature. Cell count was performed for all 3 groups at all time points and the results indicated a slight decrease in cell numbers for 1 month storage period in all groups. However, no significant differences were observed among the groups regardless of the storage duration (Figure 2(A)). The percentage of cell viability was also measured after 7 days, 14 days, and 1 month of cryopreservation (Figure 2(B)). The results showed that both FBS+10%DMSO and CF-SFM maintained consistent cell viability percentages, whereas CryoStor10 showed significantly lower cell viability after 1 month compared to the other storage durations.

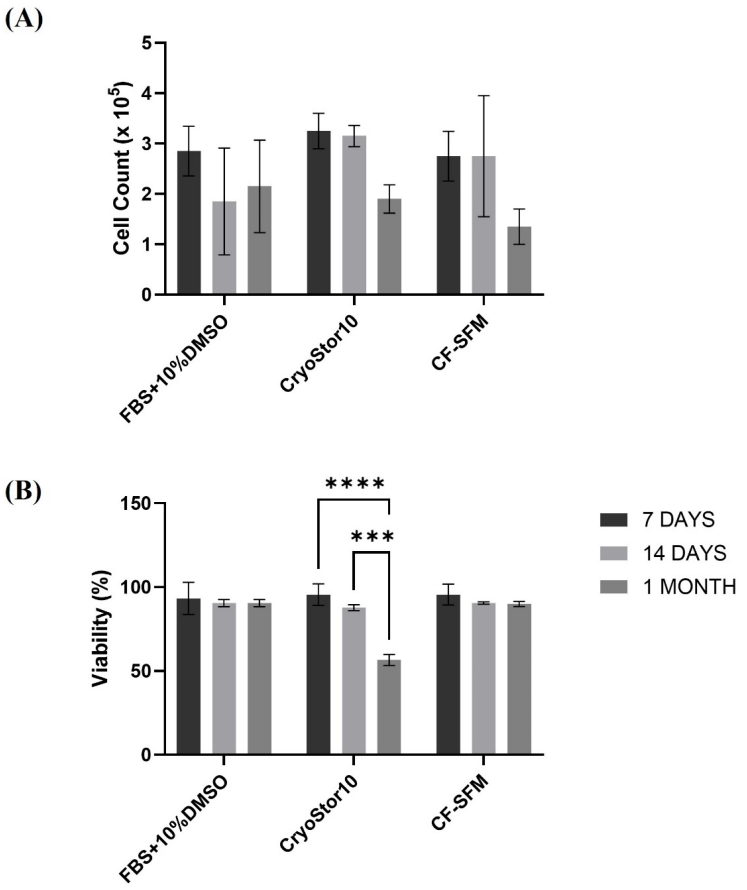
Immunocytochemistry (ICC) analysis was performed to determine the expression of specific surface protein markers in fibroblasts after 7 days, 14 days, and 1 month of cryopreservation. The results showed positive expression of collagen type I (Col-1) (Figure 3) and Ki67 (Figure 4) for all groups. Quantitative evaluation of expression levels for the antibodies are shown in Figure 5. No significant differences were observed in Col-1 and Ki67 expression levels among the three different cryopreservation media after different storage durations. However, the percentage of Col-1 and Ki67 expression levels were slightly higher in cells cryopreserved in FBS+10%DMSO compared to the other two cryopreservation media for 7 days' and 14 days' time points.

Preserving cells at ultralow temperatures while they remain unfrozen offers several advantages over traditional freezing methods. These include simpler processes for collecting, storing and retrieving cells, lower costs, and the ability to use non-harmful preservation agents. This technique is widely used in fields such as stem cell research, regenerative medicine and biobanking, where maintenance of cell viability is essential. Cells can be preserved at -80 °C for an extended period (Ma et al. 2021). However,



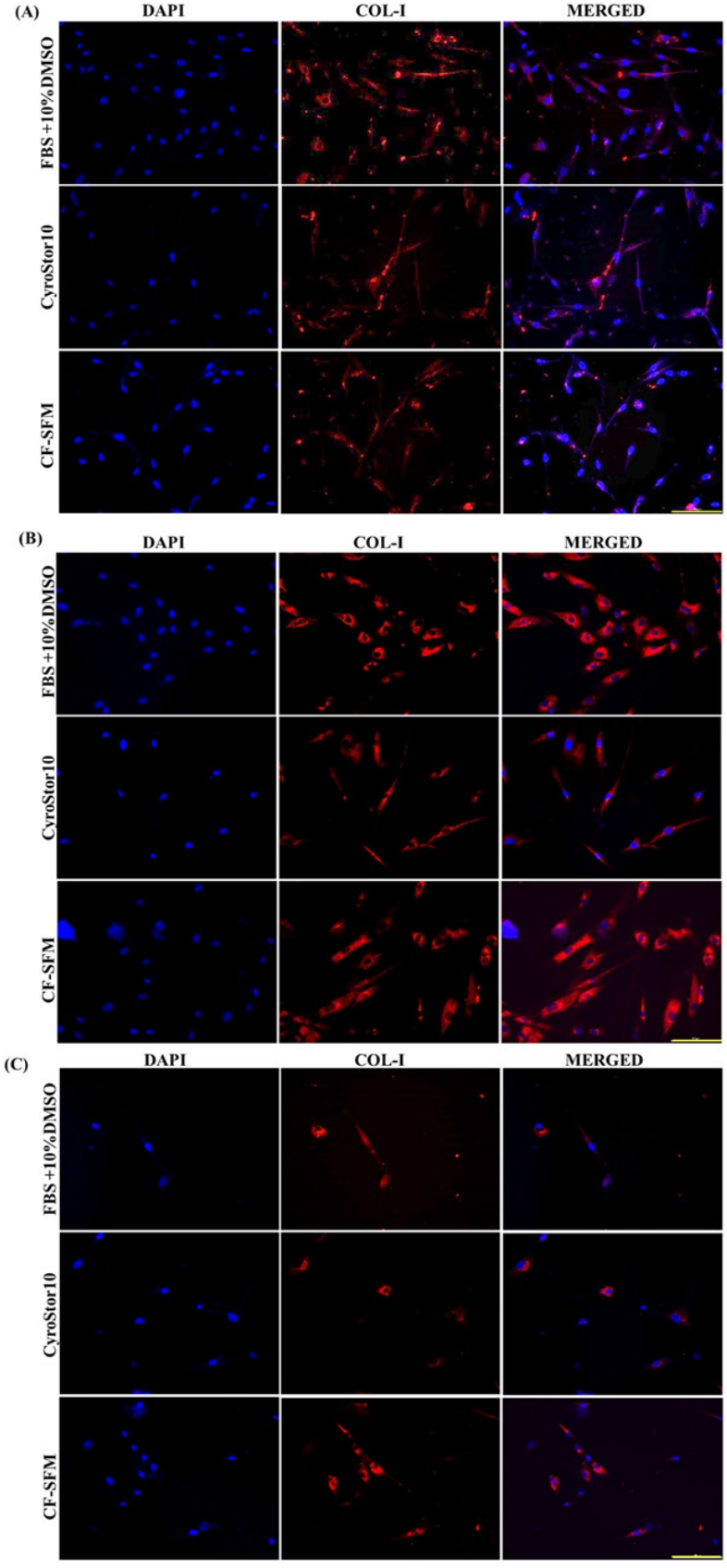

Scale bar is 100 µm

FIGURE 1. Morphology of revived fibroblast after 7 days, 14 days, and 1 month cryopreserved in different cryomedium: FBS+10%DMSO, CryoStor10 and CF-SFM



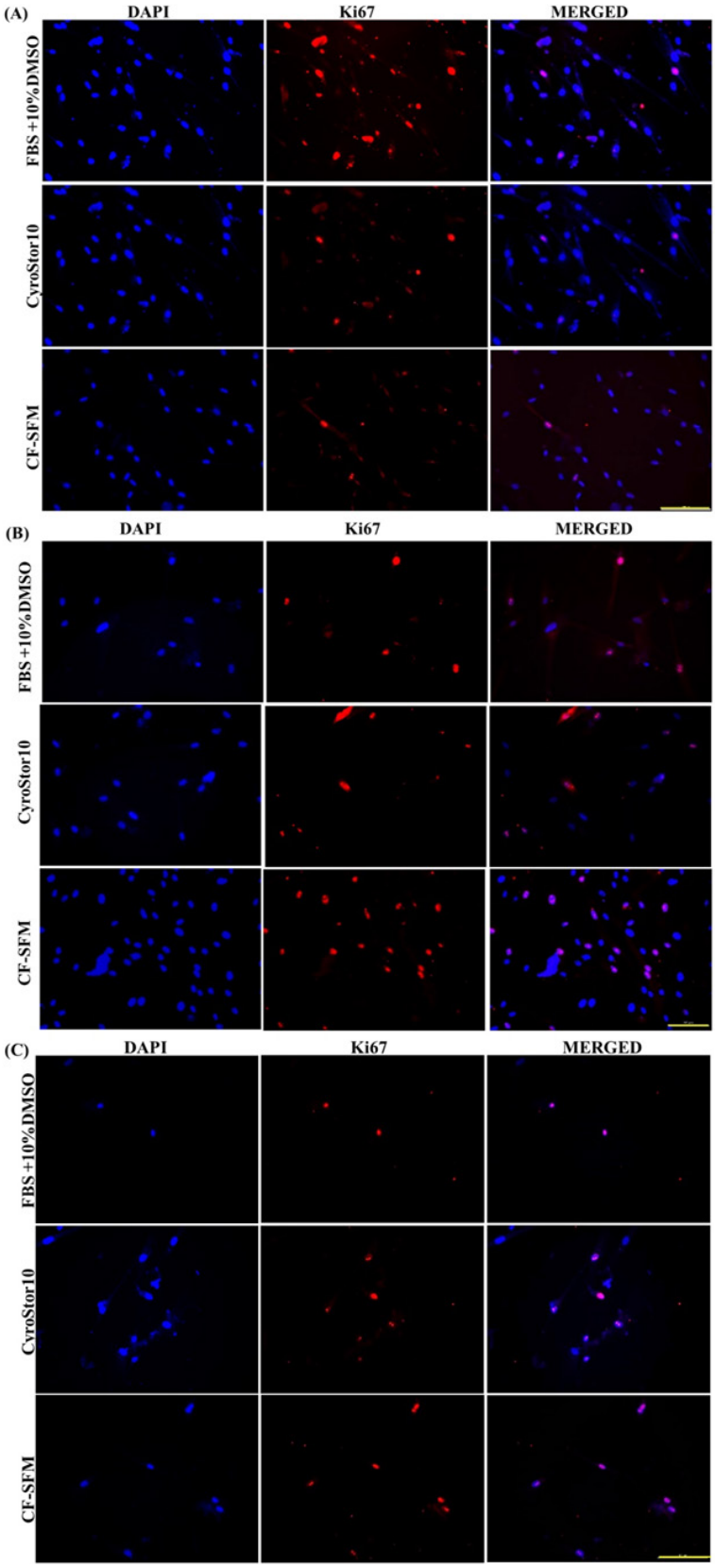

*** and **** indicate significantly higher compared to the 1-month CryoStor10 group

FIGURE 2. (A) Live cell count of fibroblasts and (B) viability percentage of fibroblasts after storage for three different durations in different cryopreservation media




Scale bar is 100 µm

FIGURE 3. Representative images of immunocytochemistry staining of fibroblasts, with nuclear staining (blue) and anti-collagen type 1 (red) after (A) 7 days, (B) 14 days, and (C) 1 month storage




Scale bar is 100 µm

FIGURE 4. Representative images of immunocytochemistry staining of fibroblasts, with anti-Ki-67 (red) and nuclear staining (blue) after (A) 7 days, (B) 14 days, and (C) 1 month storage

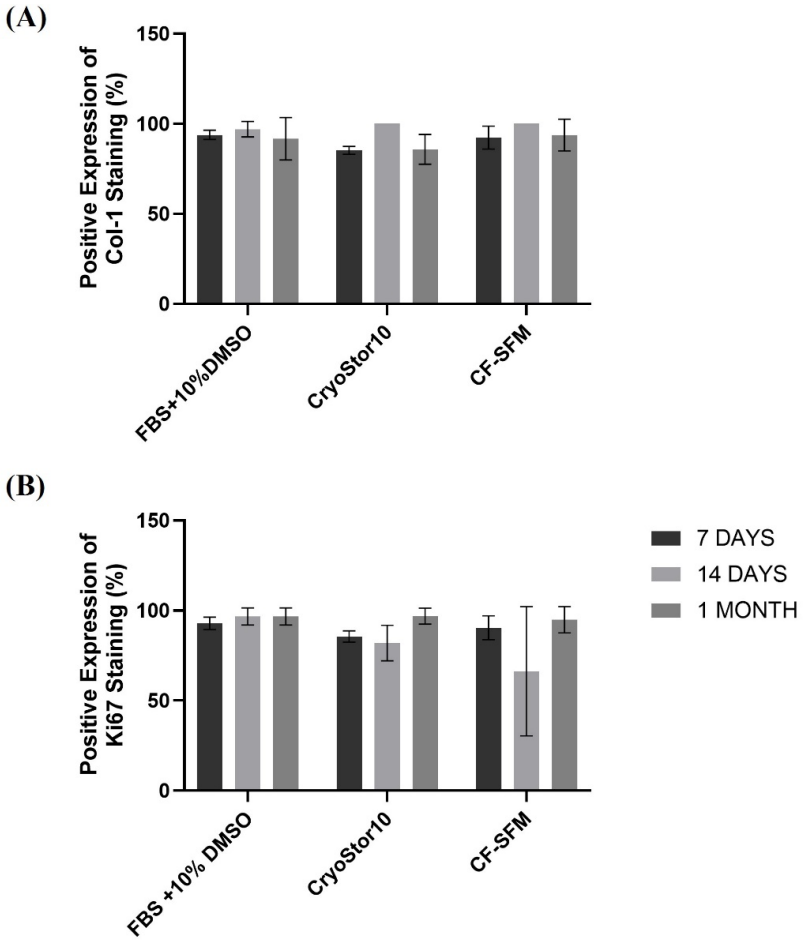



FIGURE 5. The percentage of positive expression for (A) collagen type 1 (Col-1) staining and (B) Ki67 staining in fibroblasts stored in different cryopreservation media

cryopreservation in liquid nitrogen is a method employed to preserve biological samples by swiftly freezing them at -196 °C. This technique entails immersing the samples in liquid nitrogen, facilitating rapid cooling and reducing the formation of ice crystals that could harm cellular structures (Lisan et al. 2024).

In this cryopreservation study, fibroblasts were evaluated to assess their viability and functionality following extended storage at -80 °C. The capability of these cells to maintain their characteristics after cryopreservation is crucial for potential clinical applications in regenerative medicine (Ishak et al. 2019). Fibroblasts play an important role in the synthesis and maintenance of the extracellular matrix (ECM), which provides structural support to tissues. They are used in research to investigate a range of skin conditions, the aging process, and fibrosis (Law et al. 2016; Manira et al. 2014). Due to their resilience, rapid doubling time, and endurance in *in vitro* culture, fibroblasts are renowned for their adaptability and functionality (Mohamed et al. 2024).

Fibroblasts maintained their elongated and spindle-shaped morphology after cryopreservation for 7 days, 14 days, and 1 month (Figure 1). This shows that the fibroblasts can maintain their characteristics and morphologies after 1 month of cryopreservation at -80 °C. Whaley et al. (2021) reported that numerous cellular abnormalities can result from cryopreservation, such as alterations to cell morphology, features, capability to proliferate, function and increased cell death. A reduction in temperature has the potential to initiate stress response pathways, and upon thawing, it may initiate necrotic and apoptotic cascades due to the formation of ice crystals. During thawing process, the ice fraction will increase causing an increase in extracellular osmolarity which will result in cells injuries (Baust et al. 2022). Hence, cryoprotectants such as FBS+10%DMSO, CryoStor10 or CF-SFM are added as these substances have comparatively low cell toxicity at low temperatures and do not ionise in aqueous solutions. By limiting the exposure of cryoprotectant to low temperatures as well as by minimizing the pre-freeze exposure duration, the toxicity can be reduced. Hence, CryoStor10 containing 10% DMSO is commonly used for stem cells cryopreservation (Meneghel, Kilbride & Morris 2020).

In clinical practice, storing samples in -80 °C is more convenient as ultralow temperature freezers are widely available. It has been proven that peripheral blood specimens can be kept up to 180 days at this temperature. On the other hand, mesenchymal stem cells and tissue samples can be cryopreserved for up to 10 years in -80 °C (Linkova, Rubtsova & Egorikhina 2022). Several studies have shown that viability of the thawed cells when stored at -80 °C for a short time, such as several months is comparable to cells stored in liquid nitrogen (Hunt 2019). If cryopreservation at -80 °C is intended for an extended period (more than a year), ultralow temperature freezers should be used as they are capable of maintaining a consistent temperature (Linkova, Rubtsova & Egorikhina 2022).

The ICC results depicted the presence of nucleus, as well as type I collagen (Col-1) and Ki67 expression. The expression of Col-1 is an important marker for fibroblasts, confirming their activity and functionality. Its presence indicates that fibroblasts are actively producing extracellular matrix components, which are essential for tissue repair and regeneration (Ishak et al. 2019; McAndrews et al. 2022). In early stages of wound healing, fibroblasts are stimulated by growth factors produced by inflammatory cells to migrate towards the injury site, multiply, and release an extracellular matrix rich in collagen (Nur Izzah, Mh Busra & Manira 2024). In addition, Ki67 marker can be used to examine proliferation of cells during wound healing (Nur Izzah, Mh Busra & Manira 2024). Ki67, a non-histone nuclear protein associated with the cell cycle, is only produced by proliferating cells and is absent from latent cells. Ki67 antibodies interact with the intranuclear antigens of actively proliferating cells in the active proliferative phase (Prabhu et al. 2022).

## LIMITATION OF STUDY

Cryopreservation can cause cellular damage, or cryodamage, even with optimal protocols. This happens due to ice crystal formation, osmotic stress, or the toxicity of cryoprotectants. It is challenging to find techniques to reduce these damages in fibroblast cell lines. Variation in fibroblasts susceptibility to freezing and thawing can cause conflicting results in research. Furthermore, fibroblasts can have different freezing tolerances and recovery rates because they are obtained from a variety of tissues, including but not limited to skin and lungs tissues. Standardization may be hampered by cryopreservation techniques optimised for fibroblast from one tissue source but not optimal for fibroblasts derived from other sources.

## FUTURE STUDY

Cryopreservation of fibroblasts has great potential, especially in medical, research, and biotechnological field. Cryopreserved fibroblasts play a key role in regenerative medicine, particularly in tissue engineering. They can be seeded onto scaffolds and used in wound healing due to their ability to produce extracellular matrix and collagen. Furthermore, fibroblast biobanks can be established for research and clinical purposes. For example, cryopreserved fibroblasts may be used to study rare diseases and for future cellular therapies application.

## CONCLUSION

In conclusion, the optimal cryopreservation medium for short-term storage of human dermal fibroblasts at -80 °C is FBS+10%DMSO. It allows storage for up to 1-month while still preserving optimal cell viability and cellular characteristic. Based on the ICC staining results, the cells retained the ability to express Col-1 and Ki67 surface protein markers specific for human dermal fibroblasts. Cryopreservation holds considerable promise in basic science and several applications including tissue engineering. There is an ongoing need to create innovative cryopreservation procedures to support the continuous progress of cell therapies in regenerative medicine. It is widely acknowledged that successful cryopreservation techniques provide enabling technologies that may eventually lead to curative cell therapies.

## ACKNOWLEDGEMENTS

We would like to express immense gratitude to the Faculty of Medicine, UKM for the guidance and resources to complete this manuscript. This study has been performed under good quality management of ISO 9001:2015 for research facilities in DTERM. This study was supported by Geran Fundamental Fakulti Perubatan (GFFP), Universiti Kebangsaan Malaysia (Grant Code: FF-2023-038). Our contributions are as follows: Conceptualization, T.R., N.S., and M.M.; validation, T.R., N.R.H.M.R., N.S., and M.M.; writing - original draft preparation, T.R., N.R.H.M.R., N.S., and M.M.; drawings of figures, T.R., N.R.H.M.R. and N.S.; writing - review and editing, T.R., F.M.B., M.M., and; visualization and supervision F.M.B., M.M.; project administration, M.M.; and funding acquisition, M.M. We declared that all data and material has been embedded in the manuscript. We also declare that we have no conflict of interest with any step of the article preparation.

## REFERENCES

Awan, M., Buriak, I., Fleck, R., Fuller, B., Goltsev, A., Kerby, J., Lowdell, M., Mericka, P., Petrenko, A., Petrenko, Y., Rogulska, O., Stolzing, A. & Stacey, G.N. 2020. Dimethyl sulfoxide: A central player since the dawn of cryobiology, is efficacy balanced by toxicity? *Regenerative Medicine* 15(3): 1463-1491. https://doi.org/10.2217/rme-2019-0145

Baust, J.M., Snyder, K.K., Van Buskirk, R.G. & Baust, J.G. 2022. Assessment of the impact of post-thaw stress pathway modulation on cell recovery following cryopreservation in a hematopoietic progenitor cell model. *Cells* 11(2): 278. https://doi.org/10.3390/cells11020278.

Caneparo, C., Chabaud, S., Fradette, J. & Bolduc, S. 2022. Evaluation of a serum-free medium for human epithelial and stromal cell culture. *International Journal of Molecular Sciences* 23(17): 10035. https://doi.org/10.3390/ijms231710035

Erol, O.D., Pervin, B., Seker, M.E. & Aerts-Kaya, F. 2021. Effects of storage media, supplements and cryopreservation methods on quality of stem cells. *World Journal of Stem Cells* 13(9): 1197-1214. https://doi.org/10.4252/wjsc.v13.i9.1197

Hunt, C.J. 2019. Technical considerations in the freezing, low-temperature storage and thawing of stem cells for cellular therapies. *Transfusion Medicine and Hemotherapy* 46(3): 134-150. https://doi.org/10.1159/000497289

Ishak, M.F., Manira, M., Ng, M.H., Khairul, B., Gargy, L., Aminuddin, B.S. & Ruszymah, B.H.I. 2019. Long term effect of cryopreservation on primary human skin cells. *Sains Malaysiana* 48(1): 137-144. https://doi.org/10.17576/jsm-2019-4801-16

Law, J.X., Musa, F., Ruszymah, B.H., El Haj, A.J. & Yang, Y. 2016. A comparative study of skin cell activities in collagen and fibrin constructs. *Medical Engineering and Physics* 38(9): 854-861. https://doi.org/10.1016/j.medengphy.2016.05.017

Linkova, D.D., Rubtsova, Y.P. & Egorikhina, M.N. 2022. Cryostorage of mesenchymal stem cells and biomedical cell-based products. *Cells* 11(17): 2691. https://doi.org/10.3390/CELLS11172691

Lisan, R.A., Mahyudin, F., Edward, M. & Buwana, D.S. 2024. Role of preservation methods using deep-freezing and liquid nitrogen in bone allograft characteristics: An *in vitro* study. *Narra J*. 4(1): e757. https://doi.org/10.52225/narra.v4i1.757

Ma, Y., Gao, L., Tian, Y., Chen, P., Yang, J. & Zhang, L. 2021. Advanced biomaterials in cell preservation: Hypothermic preservation and cryopreservation. *Acta Biomaterialia* 131: 97-116. https://doi.org/10.1016/j.actbio.2021.07.001

Manira, M., Khairul Anuar, K., Seet, W.T., Ahmad Irfan, A.W., Ng, M.H., Chua, K.H., Mohd Heikal, M.Y., Aminuddin, B.S. & Ruszymah, B.H. 2014. Comparison of the effects between animal-derived trypsin and recombinant trypsin on human skin cells proliferation, gene and protein expression. *Cell and Tissue Banking* 15(1): 41-49. https://doi.org/10.1007/s10561-013-9368-y

Marcantonini, G., Bartolini, D., Zatini, L., Costa, S., Passerini, M., Rende, M., Luca, G., Basta, G., Murdolo, G., Calafiore, R. & Galli, F. 2022. Natural cryoprotective and cytoprotective agents in cryopreservation: A focus on melatonin. *Molecules* 27(10): 3254. https://doi.org/10.3390/molecules27103254

McAndrews, K.M., Miyake, T., Ehsanipour, E.A., Kelly, P.J., Becker, L.M., McGrail, D.J., Sugimoto, H., LeBleu, V.S., Ge, Y. & Kalluri, R. 2022. Dermal αSMA$^+$ myofibroblasts orchestrate skin wound repair via β1 integrin and independent of type I collagen production. *EMBO Journal* 41(7): e109470. https://doi.org/10.15252/EMBJ.2021109470/SUPPL_FILE/EMBJ2021109470-SUP-0007-SDATAEV.ZIP

Meneghel, J., Kilbride, P. & Morris, G.J. 2020. Cryopreservation as a key element in the successful delivery of cell-based therapies - A review. *Frontiers in Medicine* 7: 592242. https://doi.org/10.3389/FMED.2020.592242/BIBTEX

Mohamed, H.M., Sundar, P., Ridwan, N.A.A., Cheong, A.J., Mohamad Salleh, N.A., Sulaiman, N., Mh Busra, F. & Maarof, M. 2024. Optimisation of cryopreservation conditions, including storage duration and revival methods, for the viability of human primary cells. *BMC Molecular and Cell Biology* 25(1): 20. https://doi.org/10.1186/s12860-024-00516-6

Murray, K.A. & Gibson, M.I. 2022. Chemical approaches to cryopreservation. *Nature Reviews Chemistry* 6: 579-593. https://doi.org/10.1038/s41570-022-00407-4

Nur Izzah Md Fadilah, Mh Busra Fauzi & Manira Maarof. 2024. Effect of multiple-cycle collections of conditioned media from different cell sources towards fibroblasts in *in vitro* wound healing model. *Pharmaceutics* 16(6): 767. https://doi.org/10.3390/pharmaceutics16060767

Prabhu, V., Rao, B.S.S., Rao, A.C.K., Prasad, K. & Mahato, K.K. 2022. Photobiomodulation invigorating collagen deposition, proliferating cell nuclear antigen and Ki67 expression during dermal wound repair in mice. *Lasers in Medical Science* 37(1): 171-180. https://doi.org/10.1007/S10103-020-03202-Z/FIGURES/5

Shorokhova, M., Pugovkina, N., Zemelko, V., Lyublinskaya, O. & Grinchuk, T. 2024. Long-term cryopreservation may cause genomic instability and the premature senescence of cells. *International Journal of Molecular Sciences* 25(3): 1467. https://doi.org/10.3390/ijms25031467

Singh, M., Henry, C., Ma, X., Abolude, A.T., Moawad, A.R., Stephens, T. & Chandra, R. 2023. Effect of different cryopreservation temperatures on recovery of goat skin derived fibroblast cells. *Journal of Biotech Research* 15: 355-359.

Uhrig, M., Ezquer, F. & Ezquer, M. 2022. Improving cell recovery: Freezing and thawing optimization of induced pluripotent stem cells. *Cells* 11(5): 799. https://doi.org/10.3390/CELLS11050799/S1

Valyi-Nagy, K., Betsou, F., Susma, A. & Valyi-Nagy, T. 2021. Optimization of viable glioblastoma cryopreservation for establishment of primary tumor cell cultures. *Biopreservation and Biobanking* 19(1): 66. https://doi.org/10.1089/BIO.2020.0050

Whaley, D., Damyar, K., Witek, R.P., Mendoza, A., Alexander, M. & Lakey, J.R.T. 2021. Cryopreservation: An overview of principles and cell-specific considerations. *Cell Transplantation* 30: 963689721999617. https://doi.org/10.1177/0963689721999617

Yang, J., Gao, L., Liu, M., Sui, X., Zhu, Y., Wen, C. & Zhang, L. 2020. Advanced biotechnology for cell cryopreservation. *Transactions of Tianjin University* 26(6): 409-423. https://doi.org/10.1007/s12209-019-00227-6

*Corresponding author; email: manira@ukm.edu.my