

ORIGINAL ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

Comparative analysis of KnockOut serum replacement and fetal bovine serum for in vitro culture of human dermal fibroblasts

Nur Ilham Risma Hidayati¹ , Indra Kusuma^{2,3,4*} , and Eko Poerwanto² 

ABSTRACT

BACKGROUND

Human dermal fibroblast (HDF) cultures can be used as a regenerative agent for wound healing. Fetal bovine serum (FBS) as a culture supplement is derived from animals, therefore not being constant in composition, causes variations in research results, thus requiring a substitute such as KnockOut serum replacement (KOSR). This study evaluated the defined KOSR as FBS substitute for HDF culture by measuring the relative expression of basic fibroblast growth factor (bFGF) and keratinocyte growth factor (KGF) messenger RNA (mRNA), HDF cell proliferation, and HDF migration.

METHODS

Human dermal fibroblast culture was divided into 2 intervention groups receiving KOSR 5% and KOSR 10%, respectively, and a control group receiving FBS 10%. Reverse transcription polymerase chain reaction (RT-PCR) was used for bFGF and KGF mRNA relative expression at the fifth passage (P5). Cell counting kit-8 (CCK-8) reagent was used for the HDF cell proliferation assay at P5 and the scratch assay was used for HDF cell migration at P6. Data were analyzed using dependent t-test, One-way ANOVA, or Kruskal-Wallis test.

RESULTS

There were no significant differences in bFGF and KGF mRNA relative expression and HDF migration velocity between the intervention and control groups ($p > 0.05$ and $p > 0.05$, respectively). The doubling time of the KOSR 5% group showed no significant difference ($p > 0.05$), but KOSR 10% and FBS 10% showed significant differences between treatment days 2-6 and treatment days 6-10 ($p < 0.05$).

CONCLUSIONS

The KOSR 10% was comparable to FBS 10% in supporting bFGF and KGF mRNA relative expression, HDF cell proliferation, and HDF cell migration in HDF culture.

Keywords: Humandermal fibroblast, FBS, KOSR, bFGF, KGF, mRNA relative expression.

¹Biomedical Science Masters Study Program, Postgraduate School, YARSI University, Jakarta, Indonesia

²Physiology Department, Faculty of Medicine, YARSI University, Jakarta, Indonesia

³Stem Cell Research Center YARSI University, Jakarta, Indonesia

⁴Research and Education Department, YARSI Hospital, Jakarta, Indonesia

*Correspondence:

Dr. dr. Indra Kusuma, M.Biomed Physiology Department, Faculty of Medicine, YARSI University Cempaka Putih, Jakarta, 10510
Phone: 6221-4206674 ext. 3091
Email: indra.kusuma@yarsi.ac.id
ORCID ID : 0000-0002-7689-451X

Date of first submission, June 9, 2023

Date of final revised submission, September 18, 2023

Date of acceptance, September 27, 2023

This open access article is distributed under a Creative Commons Attribution-Non Commercial-Share Alike 4.0 International License

Cite this article as: Hidayati NIR, Kusuma I, Poerwanto E. Comparative analysis of KnockOut serum replacement and fetal bovine serum for in vitro culture of human dermal fibroblasts. Univ Med 2023;42:283-93. doi: 10.18051/UnivMed.2023.v42:283-293



INTRODUCTION

Fibroblasts play a role in collagen formation, normal cellular growth, inflammatory processes, wound healing, angiogenesis, and the physiological activity of every tissue and organ in the body.⁽¹⁾ Fibroblasts in wound healing involve various biological and molecular processes, such as cell migration, proliferation, extracellular matrix deposition, and remodeling. Fibroblasts can be a regenerative agent for wound healing.⁽²⁾ Fibroblasts synthesize extracellular matrix components, such as collagen, reticular, and elastin. Fibroblasts also synthesize anionic macromolecules, such as glycosaminoglycans, proteoglycans, multi-adhesive glycoproteins, laminin, and fibronectin, which can promote cell attachment to substrates. On the other hand, fibroblasts require growth supplements for cell growth in vitro.⁽³⁾

A growth supplement is one of the components that influence the success of cell culture, including fibroblast culture. The use of growth supplements in cell culture includes growth factor sources derived from fetal bovine serum (FBS) and other growth factors of animal origin. Fetal bovine serum is the most commonly used growth supplement for cell culture, but may potentially carry pathogenic organisms and pose a risk of immune rejection in humans. The compounds contained in FBS can cause variations in research results, therefore serum replacement supplements are needed that do not cause variations in research results, such as KnockOut serum replacement (KOSR).⁽⁴⁾

KnockOut serum replacement is a serum replacement supplement supporting the growth of embryonic and pluripotent stem cells. The compounds contained in KOSR include amino acids, vitamins, antioxidants, trace elements, and three types of proteins: insulin, transferrin, and albumin.⁽⁵⁾ Results from previous studies using KOSR showed that the addition of KOSR to porcine blastocyst medium (PBM) enhanced the survival of porcine blastocysts, and improved development to the hatching stage and blastocyst

quality.⁽⁶⁾ KnockOut serum replacement also supported spermatogonia stem cell growth in vitro and spermatogonia stem cell activity in vivo in combination with mouse embryonic fibroblasts as feeder cells.⁽⁷⁾ KnockOut serum replacement supplementation at the minimum concentration of 10% affected germ cell maturation and testosterone production.⁽⁸⁾

These findings provide a useful reference that KOSR can be used to replace FBS as a serum supplement for human dermal fibroblast (HDF) cultures that could potentially be used as a regenerative agent for wound healing. KnockOut serum replacement will enable HDFs to become a regenerative agent in clinical settings. This is related to the ability of fibroblasts to produce collagen fibers and repair damaged epidermal tissue.⁽⁹⁾ Fibroblasts are also accessible to culture because they can attach and regenerate.⁽¹⁾ Fibroblasts also play a role in wound healing by secreting cytokines and several growth factors, such as basic fibroblast growth factor (bFGF) and keratinocyte growth factor (KGF), which can stimulate cell proliferation and inhibit cell differentiation.⁽³⁾

This study evaluated the ability of KOSR to replace FBS in bFGF and KGF mRNA relative expression, HDF cell proliferation, and HDF cell migration in vitro.

METHODS

Research design

This in-vitro study of experimental laboratory design was conducted from November 2022 to March 2023 at the Stem Cell Laboratory, Laboratorium Penelitian Terpadu Universitas YARSI (LPT-UY).

Cell culture

In this study, HDFs were obtained from the biorepository of the Stem Cell Laboratory, Laboratorium Penelitian Terpadu Universitas YARSI (LPT-UY). Cell culture was conducted using Dulbecco's Modified Eagle medium (DMEM) in the control group, with FBS 10%



Figure 1. Measurements of open area with the wound healing assay plugin

(Gibco) and 1% antibiotics-antimycotics (Gibco). In the intervention groups 1 and 2, DMEM was supplemented with KOSR 10% and KOSR 5% (Gibco), respectively as a substitute for FBS. The HDFs were kept in an incubator at 37°C and 5% CO₂.

Sample preparation

Human dermal fibroblast cells were thawed in a T75 flask and cultured to 80% confluence. The experiment was conducted in a T75 flask for the bFGF and KGF mRNA relative expression at the fifth passage (P5), on 24-well plates for the cell proliferation assay at P5, and on six-well plates for the cell migration assay at P6. The T75 flask was seeded with 1 million viable cells, the 24-well plates were seeded with 30.000 cells/well, and the six-well plates were seeded with 300.000 cells/well. Each plate was divided into three treatment groups in duplicate (bFGF and KGF mRNA relative expression, cell migration assay) and in triplicate (cell proliferation assay). The study used reverse transcription polymerase chain reaction (RT-PCR) for bFGF and KGF mRNA relative expression, cell counting kit-8 (CCK-8) reagent for the cell proliferation assay, and scratches for the cell migration assay.

bFGF/FGF-2 and KGF/FGF-7 mRNA relative expression

Cells were harvested after ten days or when 80-100% confluent. One million cells from each treatment group were isolated using an RNA Extraction kit (Zymo Research). Total RNA was quantified using a Qubit Fluorometer (Invitrogen). Then, the RNA was converted into cDNA using the cDNA Synthesis kit (Toyobo). Reverse

transcription polymerase chain reaction (RT-PCR) was performed using the Realtime qPCR SensiFAST SYBR No-ROX kit (Bioline). Forward and reverse primers for bFGF were 5'-AGAGCGACCCTCACATCAAG-3' and 5'-ACTGCCAGTTCGTTTCAGT-3', respectively, with 234 base pairs and a temperature of 64°C.⁽¹⁰⁾ Forward and reverse KGF primers were 5'-TGGCAATCAAAGGGG TGGAA-3' and 5'-GCCATAGGAAGAAAGT GGGCT-3' with 253 base pairs and a temperature of 60°C.⁽¹¹⁾ Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene with the forward primer sequence 5'-CCACCCATGGCAAATTCAT GGCA-3' and reverse primer 5'-TCTAGACGGC AGGTCAGGTCCACC-3' with 598 base pairs and a temperature of 60°C.⁽¹²⁾ The primary sequence was confirmed to be specific only in humans by examining it using the Nucleotide Blast facility from National Center for Biotechnology Information (NCBI). The mRNA transcriptional expression profile is shown as cycle threshold (Ct) relative to GAPDH.

Cell proliferation assay

The treatment group was incubated for 24 hours, followed by measurement of the cell viability using cell counting kit-8 (CCK-8) reagent (Sigma), according to the cell proliferation assay protocol. The result was measured using a Safas MP96 UV microplate reader at a wavelength of 450 nm. Each treatment group was subjected to the proliferation assay at five time points (day 2, day 6, day 8, day 10, and day 13). The measured absorbance was converted to cell numbers using the normal curve equation. The resulting cell

numbers were used to calculate the doubling time of segments A (days of treatment 2-6) and B (days of treatment 6-10). Doubling time was calculated using the formula:

$$n = 3.32 (\log UCY - \log I) + X$$

where n = population doubling, UCY = cell yield, I = inoculum numbers, X = population doubling rate of inoculum.⁽¹³⁾

Cell migration assay

The cell migration assay was performed to assess the ability of the HDF cells to close a scratch in a monolayer culture. After 100% confluence was achieved, the migration assay was performed using a 10 µl pipette tip, which was positioned at the left end of the well and moved horizontally to the right, thereby scratching a linear open area to mimic a wound. The procedure was performed in all groups (treatment groups and control group). The cells were then rinsed using phosphate-buffered saline (PBS, Gibco) to remove debris or dead cells resulting from the scratching and given medium according to the treatment group. Cell migration ability was observed using the Evos XL Core microscope (Invitrogen) at 4x magnification. Photomicrographs of the cells were obtained every two hours and taken at the same hour every day or until the open area in the control group (FBS 10%) was closed (0-50 hours). Migration analysis was conducted using the ImageJ Fiji version Windows 64-bit application with the wound healing assay plugin from <https://github.com/AlejandraArnedo/Wound-healing-size-tool/wiki>.⁽¹⁴⁾ This plugin will detect the open area in the image and its percentage (as shown in Figure 1). Migration velocity was to be analyzed using three time segments, namely segment A (0-6 hours), segment B (6-26 hours), and segment C (26-50 hours), then compared between the segments.

Statistical analysis

All data obtained were presented as mean ± standard deviation using Microsoft Office Excel 2019. Analysis was performed using the Statistical Program for Social Sciences (SPSS) version 22

by performing the dependent t-test, One-way ANOVA, or Kruskal-Wallis test. One-way ANOVA was followed by the Post Hoc LSD test, whereas the Kruskal-Wallis test was followed by the Mann-Whitney test at the significance level of $p < 0.05$.

RESULTS

bFGF and KGF mRNA relative expression

Figures 2A and 2B show that all treatment groups had a bFGF and KGF mRNA relative expression, and there was no difference in bFGF and KGF mRNA relative expression between the FBS 10%, KOSR 10%, and KOSR 5% treatment groups ($p > 0.05$).

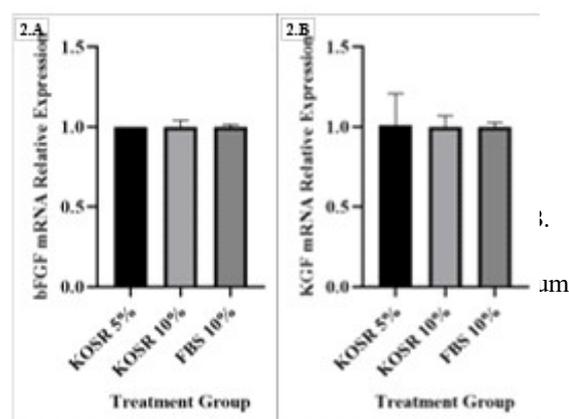


Figure 2.A. bFGF mRNA Relative Expression. **2.B** KGF mRNA Relative Expression. messenger RNA (mRNA), KOSR (Knockout Serum Replacement), FBS (Fetal Bovine Serum)

Cell proliferation assay

Figure 3A shows that all treatment groups experienced increased viability until treatment day 10. On treatment day 13, the number of fibroblast cells decreased in all treatment groups compared to day 10. The FBS 10% treatment group experienced the highest increase in viability compared to other treatment groups. This study revealed a significant difference in cell viability of the FBS 10% group as compared to the KOSR 10% and KOSR 5% treatment groups ($p < 0.05$), respectively.

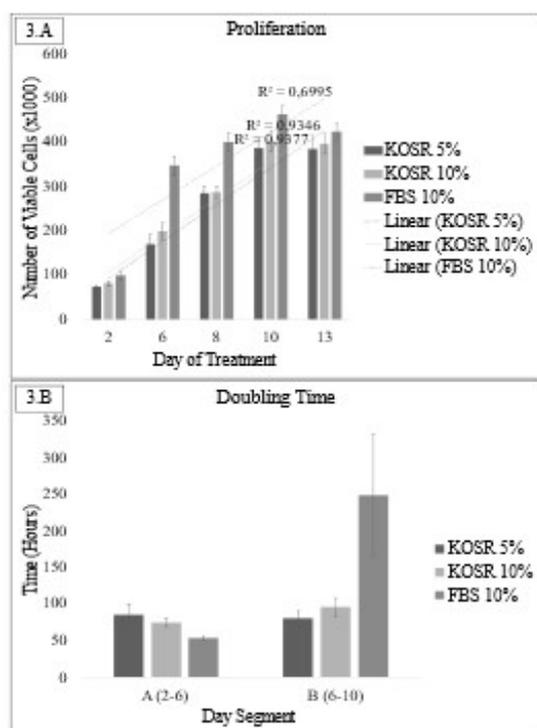


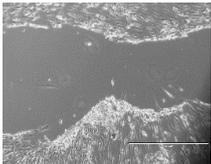
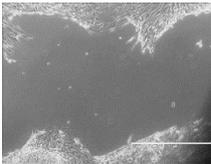
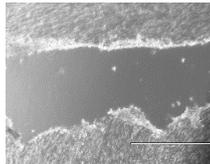
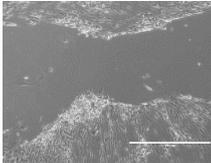
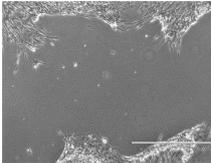
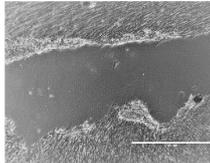
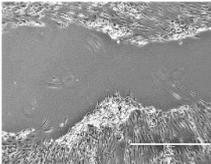
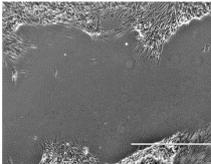
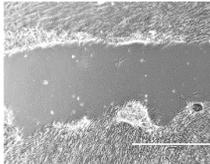
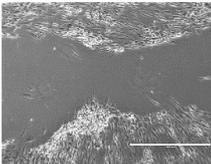
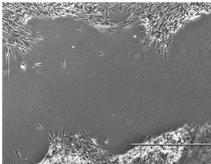
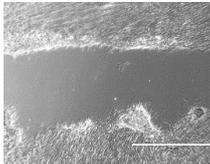
Figure 3A. Cell proliferation of human dermal fibroblasts (HDFs). **3B.** Doubling time of human dermal fibroblasts (HDFs). KOSR = KnockOut serum replacement. FBS = fetal bovine serum

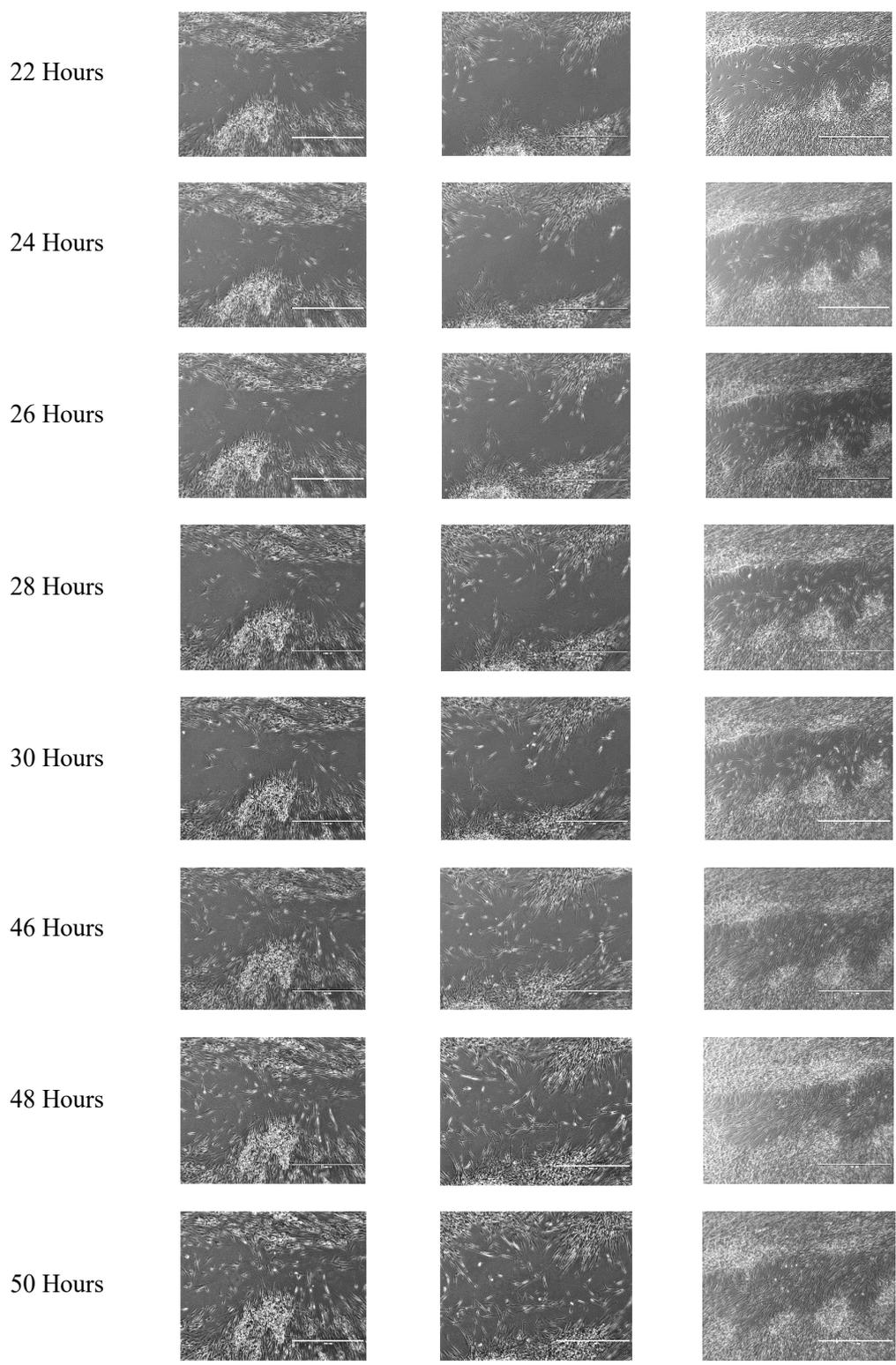
Figure 3B on doubling time between treatment groups in the same segment shows that in segment A (day of treatment 2-6), the doubling time of the FBS 10% group was significantly lower than that of the KOSR 10% and KOSR 5% % treatment groups ($p < 0.05$). In segment B (day of treatment 6-10), the doubling time of FBS 10% was significantly higher than that of KOSR 10% and KOSR 5% ($p < 0.05$). The doubling time between segments A and B in the same treatment group showed that the KOSR 10% and FBS 10% treatment groups in the segment experienced an increase ($p < 0.05$) in doubling time while the KOSR 5% treatment group was stable ($p > 0.05$).

Cell migration assay

The results of the migration assay showed that the cells migrated over the open area produced by the scratch within 50 hours (Table 1).

Table 1. Cell migration assay

Time	KOSR 5%	KOSR 10%	FBS 10%
0 Hours			
2 Hours			
4 Hours			
6 Hours			



Note: Table 1 shows the results of the scratch assay in the KOSR 5%, KOSR 10%, and FBS 10% treatment groups which were observed every 2 hours and performed at the same hour every day or until the control group (FBS 10%) was closed (0-50 hours). KOSR (Knockout Serum Replacement), FBS (Fetal Bovine Serum)

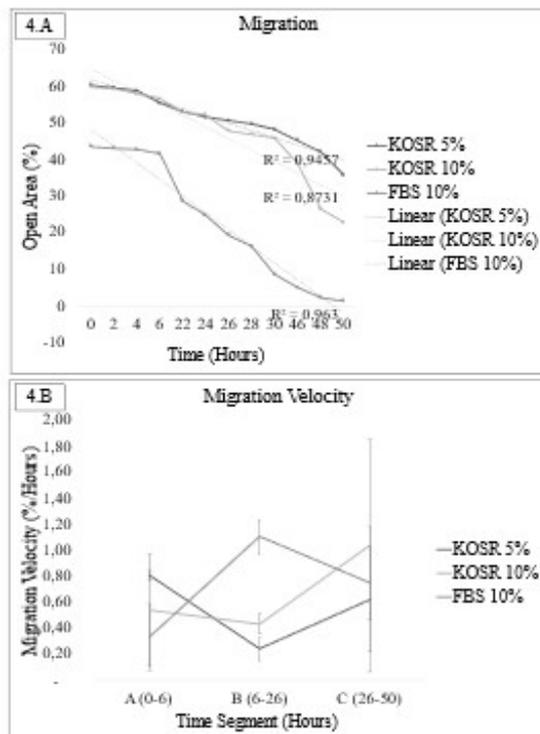


Figure 4A. Cell migration of human dermal fibroblasts (HDFs). 4B. Cell migration velocity of human dermal fibroblasts (HDFs). KOSR = KnockOut serum replacement. FBS = fetal bovine serum

Figure 4A shows that in the FBS 10% treatment group the open area was closed within 50 hours. The KOSR 10% and KOSR 5% treatment groups also show similar migration patterns. However, due to differences in the open area starting point, the FBS 10% group can close the open area within 50 hours.

Figure 4B shows the peak migration velocity in the three segments A – C of the migration pattern. The KOSR 5% treatment group has its peak migration velocity in segment A (0-6 hours), followed by the FBS 10% treatment group with its peak in segment B (6-26 hours), and the KOSR 10% treatment group with its peak in segment C (26-50 hours). However, statistical calculation shows no significant difference in the migration velocity between treatment groups in the same segment and between the segments in the same treatment group ($p > 0.05$). Therefore, the migration velocity in this study was comparable in each treatment group and segment.

DISCUSSION

Based on the results, KOSR supplementation shows similar support of bFGF and KGF mRNA relative expression and cell migration velocity compared to FBS supplementation. KnockOut serum replacement also supports the proliferation of HDFs. However, analysis of doubling time shows that KOSR 5% was stable during 10-day proliferation period while KOSR 10% and FBS 10% show a significant increase in doubling time during the 10-day proliferation period. This suggests that KOSR 10% and FBS 10% experienced a decrease in proliferation capacity. Results from previous studies found that using KOSR 5% in blastocyte cultures significantly increased the expression of blastocyst-hatching-related mRNA expression, increased survival on day 5, and the blastocyte count on day 7 compared to those cultured in porcine blastocyst medium (PBM) alone or with the addition of FBS 10%.⁽⁶⁾ These results show that the constituents of the KOSR 10% supplement can influence mRNA relative expression, cell proliferation, and cell migration.

Fetal bovine serum and KOSR have vitamins/antioxidants and proteins that can increase FGF mRNA relative expression, leading to increased proliferation, differentiation, and migration of fibroblasts that contribute to a faster wound-healing process.⁽¹⁵⁾ The presence of serum in cell culture constitutes an extracellular stimulatory process that uses intracellular signals via the Rho-actin/myocardin-related transcription factor (MRTF) and Ras ERK/ternary complex factor (TCF) pathways. These factors act as a cofactor for serum response factor (SRF) in the nucleus to regulate cell differentiation, migration, motility, proliferation, cytoskeleton, signaling, adhesion, development, myogenesis, apoptosis, etc. SRF binds to the serum response element (SRE) in the promoter region of target genes, including immediate early genes (IEGs). SRE is a DNA cis-element known as the CA_nG box; through its binding to CA_nG sequences, SRF activates genes that respond to mitogens.⁽¹⁶⁾

Based on the results of this study, it was found that KOSR can act as an external stimulus to obtain similar functions such as gene expression, cell proliferation, and cell migration.

Serum response factors are transcription factors that play a role in regulating gene expression in all cell types. bFGF acts through the MRTF pathway, MRTF being a cofactor of SRF for cell proliferation. The bFGF is a growth factor secreted by fibroblasts for tissue repair, epithelial regeneration, and remodeling.⁽¹⁷⁾ Previous studies have shown that human mesenchymal stromal cells (hMSCs) reduce FGFR1 expression, leading to unresponsiveness to bFGF, whereas fibroblasts maintain high FGFR1 expression. In hMSCs and fibroblasts, inhibition of actin-myosin interaction and MRTF/SRF activity decreases FGFR1 expression. Fibroblasts maintain high SRF and FGFR1 expression and remain responsive to bFGF.⁽¹⁸⁾ Fibroblasts produce endogenous bFGF which acts via autocrine mechanisms on FGFR1. Cultures using FBS contain exogenous and endogenous bFGF, as well as other growth factors, such as endothelial cell growth factor (ECGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF).⁽¹⁹⁾ Our results showed that bFGF synthesis was equivalent for both FBS and KOSR supplements. This means that bFGF synthesis is independent of MRTF, which is a cofactor of SRF.

Keratinocyte growth factor is also a growth factor secreted by fibroblasts which plays a role in wound healing through the re-epithelialization process in supporting the migration and proliferation of keratinocytes. Several KGF therapeutic strategies can enhance wound healing by targeting re-epithelialization.⁽²⁰⁾ Keratinocyte growth factor is a downstream target of SRF that regulates KGF expression through its binding to the SRE in the promoter region of the gene. The specific pathway for SRF to regulate KGF expression is not yet known, so further research

is needed to fully understand the mechanism of SRF regulation of KGF expression. The role of SRF in keratinocyte biology can be analyzed through SRF expression in skin conditions associated with increased keratinocyte migration and/or proliferation and SRF plays a role in the normal function and maintenance of skin.⁽²¹⁾ Reduced SRF expression in keratinocytes affects epidermal homeostasis. The underlying mechanisms involve cytoskeleton disruption and impaired cell adhesion. Expression of dermally-derived KGF stimulates wound re-epithelialization by paracrine signaling.⁽²⁰⁾

Fetal bovine serum and KOSR contain ingredients that affect cell proliferation and migration. FBS provides the necessary proteins to regulate the cell culture system,⁽²²⁾ one of the FBS proteins being vimentin which interacts with Extracellular Signal-Regulated Kinase (ERK) to increase cell proliferation or division.⁽²³⁾ Vimentin also promotes cell migration into normal tissue during wound repair.⁽²⁴⁾ KnockOut serum replacement contains amino acids, vitamins, antioxidants, trace elements, and three types of proteins: insulin, transferrin, and albumin.⁽⁵⁾ Collagen formed from the amino acids of these proteins by fibroblasts will modulate fibroblast proliferation, migration, and survival. Antioxidants in KOSR can activate the expression of collagenase which helps in the degradation of the extracellular matrix and in cell migration.⁽²⁵⁾ KnockOut serum replacement does not contain undefined growth or differentiation-promoting factors, therefore it can be used for various applications using cells, tissues, or embryos, such as in the selection of drugs for gene-targeted cells and as a handling medium for embryo transplantation.⁽²⁶⁾

Fibroblast proliferation is influenced by SRF interacting with its cofactors from the Ras ERK/TCF pathway,⁽²⁷⁾ which plays a role in controlling proliferation, invasion, and metastasis.⁽²⁸⁾ Serum response factor activates immediate-early genes (IEG), such as c-fos and c-jun. C-fos acts as a transcriptional activator of IEGs and binds to c-jun to form the AP-1 complex. Inhibition of c-fos

can cause a decrease in AP-1 expression and suppress the proliferation process.⁽²⁹⁾ Cyr61 is the product of an IEG that can mediate cell survival, proliferation, differentiation, migration, adhesion, and extracellular matrix synthesis.⁽³⁰⁾ SRF also affects migration through the actin cytoskeleton.⁽³¹⁾ Actin stabilization is influenced by troponin t, tropomyosin, and calponin, which are IEGs regulated by SRF.^(32,33,34) The Rho-actin pathway plays a role in cell migration by regulating shape, polarity, and cell movement through actin-myosin contraction mechanisms and cell adhesion. The Rho-actin pathway also relies on actin dynamics that provide traction for cells to move forward during the cell migration process.⁽³⁵⁾ The limitation of this study is that the open area is dependent on the scratch results. This study shows that KOSR supplementation in serum-free culture systems for fibroblast production is an Advanced Therapeutic Medicinal Product (ATMP). Furthermore, the KGF mRNA expression by fibroblasts in this study shows the potential of the fibroblast secretome as an active substance for wound healing.

CONCLUSIONS

This study showed that KOSR 10% was comparable to FBS 10% in supporting the functions of bFGF and KGF mRNA relative expression, cell proliferation, and cell migration. Suggestions for future research are the examination of SRF expression in serum-free cultures using KOSR 10% in fibroblast, keratinocyte, and stem cell cultures.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

ACKNOWLEDGEMENTS

We thank Anggi Puspa Nur Hidayati and Intan Razari for laboratory assistance and RT-PCR procedures.

FUNDING

Funding was provided by PDP Kemendikbudristek 2023 grant No. 179/E5/PG.02.00/PL/2023.

AUTHOR CONTRIBUTIONS

NIRH, IK, and EP: conceived and planned the experiment. IK and EP: supervised the experiment. NIRH: carried out the experiment, processed the experimental data, performed the analysis, and drafted the manuscript with input from all authors. IK: conceived the original idea. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study is available from the corresponding author upon request.



REFERENCES

1. Kurniawati Y, Adi S, Achadiyani, Erlangga D, Putri T. Kultur primer fibroblas: penelitian pendahuluan. *MKA* 2015; 38: 34–40. DOI: <https://doi.org/10.22338/mka.v38.i1.p33-40.2015>.
2. Santi. Peranan sel punca dalam penyembuhan luka. *Cermin Dunia Kedokteran* 2018;45: 374–379. DOI: <https://dx.doi.org/10.55175/cdk.v45i4.681>.
3. Churiyah, Kusuma I, Kusumastuti SA, Hadi RS., Wibowo AE, Fabiola FK. Isolasi sel punca pluripoten dengan penanda CD105+ dan SSEA3+ dari sel fibroblas kulit asal jaringan preputium. *Jurnal Ilmu Kefarmasian Indonesia* 2015;14: 233–239. DOI: <http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/36>.
4. Zhang S, Liu Z, Su G, Wu H. Comparative analysis of KnockOut™ serum with fetal bovine serum for the in vitro long-term culture of human limbal epithelial cells. *J Ophthalmol* 2016;2016:7304812. doi: 10.1155/2016/7304812.
5. Arslan HO, Keles E, Rostami B, et al. An overview of adding ROCK and KSR with trehalose to a low glycerol tris-based semen extender. *Black Sea J Agric* 2023;6:211–4. DOI: <https://doi.org/10.47115/bsagriculture.1155604>.

6. Sakurai M, Suzuki C, Yoshioka K. Effect of KnockOut serum replacement supplementation to culture medium on porcine blastocyst development and piglet production. *Theriogenology* 2014;83:1–8. DOI: <http://dx.doi.org/10.1016/j.theriogenology.2014.11.003>.
7. Aoshima K, Baba A, Makino Y, Okada Y. Establishment of alternative culture method for spermatogonial stem cells using knockout serum replacement. *PLoS One* 2013;8:e77715. doi: 10.1371/journal.pone.0077715.
8. Reda A, Albalushi H, Montalvo SC, et al. KnockOut serum replacement and melatonin effects on germ cell differentiation in murine testicular explant cultures. *Ann Biomed Eng* 2017; 45:1783-94. doi: 10.1007/s10439-017-1847-z.
9. Damayanti F, Wathon S. Peningkatan performa pertumbuhan kultur sel fibroblas dan aplikasinya untuk perbaikan jaringan yang rusak. *J BioTrends* 2017;8:32–9.
10. Veltmann M, Hollborn M, Reichenbach A, Wiedemann P, Kohen L, Bringmann A. Osmotic induction of angiogenic growth factor expression in human retinal pigment epithelial cells. *PLoS One* 2016;11:e0147312. DOI: <https://doi.org/10.1371/journal.pone.0147312>.
11. Lämmermann I, Terlecki-Zaniewicz L, Weinmüller R, et al. Blocking negative effects of senescence in human skin fibroblasts with a plant extract. *NPJ Aging Mech Dis.* 2018;4:4. doi: 10.1038/s41514-018-0023-5.
12. Bhushan B, Gopinath P. Antioxidant nanozyme: a facile synthesis and evaluation of the reactive oxygen species scavenging potential of nanoceria encapsulated albumin nanoparticles. *J Mater Chem B* 2015; 3: 4843–52. DOI: <https://doi.org/10.1039/C5TB00572H>.
13. Kusuma I, Hadi RS. Geraniin supplementation increases human keratinocyte proliferation in serum-free culture. *Univ Med* 2013; 32: 3–10. DOI: <https://doi.org/10.18051/UnivMed.2013.v32.3%20-%2010>.
14. Suarez-Arnedo A, Figueroa FT, Clavijo C, Arbeláez P, Cruz JC, Muñoz-Camargo C. An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. *PLoS One* 2020;15:e0232565. doi: 10.1371/journal.pone.0232565.
15. Rosada A, Mujayanto R, Poetri AR. Ekstrak daun salam dalam meningkatkan ekspresi fibroblast growth factor pada ulkus traumatik rongga mulut. *Odonto Dental J* 2020;7: 90. DOI: <http://dx.doi.org/10.30659/odj.7.2.90-96>.
16. Onuh JO, Qiu H. Serum response factor-cofactor interactions and their implications in disease. *FEBS J* 2021;288:3120-34. doi: 10.1111/febs.15544.
17. Febrianti N, Tahir T, Yusuf S. Study literature peran epidermal growth factor dalam proses penyembuhan luka. *J Keperawatan Muhammadiyah* 2019;4:7–13. DOI: <http://dx.doi.org/10.30651/jkm.v4i1.1852>.
18. Zonderland J, Rezzola S, Moroni L. Actomyosin and the MRTF-SRF pathway downregulate FGFR1 in mesenchymal stromal cells. *Commun Biol* 2020;3:576. doi: 10.1038/s42003-020-01309-1.
19. Lee DY, Lee SY, Yun SH, et al. Review of the current research on fetal bovine serum and the development of cultured meat. *Food Sci Anim Resour* 2022;42:775-99. doi: 10.5851/kosfa.2022.e46.
20. Bártolo I, Reis RL, Marques AP, Cerqueira MT. Keratinocyte growth factor-based strategies for wound re-epithelialization. *Tissue Eng Part B Rev* 2022;28:665-76. doi: 10.1089/ten.TEB.2021.0030.
21. Sumitomo A, Siritwach R, Thumkeo D, et al. LPA induces keratinocyte differentiation and promotes skin barrier function through the LPAR1/LPAR5-RHO-ROCK-SRF axis. *J Invest Dermatol* 2019;139:1010-22. doi: 10.1016/j.jid.2018.10.034.
22. Waryastuty H, Irianingsih SH, Wasito R. Deteksi kontaminasi bovine viral diarrhea virus pada fetal bovine serum yang tersedia secara komersial. *J Veteran* 2021;22:229–36. DOI: <https://doi.org/10.19087/jveteriner.2021.22.2.229>.
23. Rohmah MK. Pengaruh jenis substrat dan serum terhadap aktivitas penempelan, proliferasi dan diferensiasi kultur sel myoblast C2C12. *LenteraBio Berkala Ilmiah Biologi* 2021;10:134–9. DOI: <https://doi.org/10.26740/lenterabio.v10n2.p134-139>.
24. Cheng F, Eriksson JE. Intermediate filaments and the regulation of cell motility during regeneration and wound healing. *Cold Spring Harb Perspect Biol* 2017;9:a022046. DOI: <https://doi.org/10.1101%2Fcsbperspect.a022046>.
25. Arief H, Widodo MA. Peranan stres oksidatif pada proses penyembuhan luka. *J Ilmiah Kedokteran Wijaya Kusuma* 2018;5: 22–29. DOI: <http://dx.doi.org/10.30742/jikw.v5i2.338>.
26. Jiang Y, Zhu WQ, Zhu XC, et al. Cryopreservation of calf testicular tissues with knockout serum replacement. *Cryobiology* 2020;92:255-7. doi: 10.1016/j.cryobiol.2020.01.010.
27. Dinsmore CJ, Soriano P. Differential regulation of cranial and cardiac neural crest by serum response factor and its cofactors. *Elife* 2022;11:e75106. doi: 10.7554/eLife.75106.
28. Gualdrini F, Esnault C, Horswell S, Stewart A, Matthews N, Treisman R. SRF co-factors control the balance between cell proliferation and contractility. *Mol Cell* 2016;64:1048–61. DOI: <http://dx.doi.org/10.1016/j.molcel.2016.10.016>.

29. Elliyanti A. Peran C-Fos sebagai agen proliferasi dan pro-apoptosis sebagai strategi pengembangan pengobatan kanker. *Majalah Kedokteran Andalas* 2016;39:73–8. DOI: <http://dx.doi.org/10.22338/mka.v39.i2.p73-78.2016>.
30. Chen N, Chen CC, Lau LF. Adhesion of human skin fibroblasts to Cyr61 is mediated through integrin $\alpha 6 \beta 1$ and cell surface heparan sulfate proteoglycans. *J Biol Chem* 2000;275:24953–61. DOI: 10.1074/jbc.M003040200.
31. Costa A, Gozzellino L, Urbini M, et al. SRF rearrangements in soft tissue tumors with muscle differentiation. *Biomolecules* 2022;12:1678. DOI: <https://doi.org/10.3390/biom12111678>.
32. Schulte SRC, Scott B, Barrick SK, Stump WT, Blackwell T, Greenberg MJ. Single molecule mechanics and kinetics of cardiac myosin interacting with regulated thin filaments. *bioRxiv [Preprint]* 2023:2023.01.09.522880. doi: 10.1101/2023.01.09.522880.
33. Khaitlina SY. Tropomyosin as a regulator of actin dynamics. *Int Rev Cell Mol Biol* 2015;318:255-91. doi: 10.1016/bs.ircmb.2015.06.002.
34. Jensen MH, Morris EJ, Gallant CM, Morgan KG, Weitz DA, Moore JR. Mechanism of calponin stabilization of cross-linked actin networks. *Biophys J* 2014;106:793-800. doi: 10.1016/j.bpj.2013.12.042.
35. Esnault C, Stewart A, Gualdrini F, et al. Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. *Genes Dev* 2014;28:943-58. doi: 10.1101/gad.239327.114.