IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

In vitro 3D Spheroid Wound Modeling: An Alternative to Experimental Animal Studies

Esila Ece Akkaya✉, Oğuz Kul

[1]*Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, Kırıkkale, Türkiye.*

ARTICLE INFO

Received: 25/03/2024
Accepted: 08/11/2024

DOI: 10.5281/zenodo.14578551

✉**Corresponding Author:** esila.ece.23@gmail.com

Keywords

In vitro
Animal testing alternative
Experimental
Spheroids
Wound modeling
3D cell culture

Cite this article as: *Akkaya EC, Kul O. 2024. In vitro 3D Spheroid Wound Modeling: An Alternative to Experimental Animal Studies. International Journal of Veterinary and Animal Research, 7(3): 59-65. DOI: 10.5281/zenodo.14578551.*

ABSTRACT

Laboratory animals have frequently been used in scientific and preclinical pharmaceutical drug safety and efficacy research. Although the introduction of new in silico assays and computer modeling for drug discovery has shown promise in reducing laboratory animal trials, there is still a need to develop *in vitro* alternatives to in vivo animal models. The *in vitro* spheroid wound model is one of the best options for developing alternative techniques to animal research as it is the most widely used laboratory animal model. The aim of the study is to using 3D *in vitro* wound modeling as an alternative to in vivo wound healing assays. In the study, a three-dimensional cell culture (organoid culture) with cell/cell and cell/matrix junctions was generated using the most common Fibroblast and HaCaT cell lines hanging drop technique to replicate the healing stages in the injured skin area. After spheroid epidermal structures were formed, inhibitors and activators were added to the culture medium and their effects on the wound line and 3D cells produced were determined. It was noted that the number of spheroid structures increased significantly and cell-cell interactions became visible in the additional activator groups compared to the control groups. When the inhibitor-treated group was compared with the control groups, it was observed that the formed structures completely disappeared or decreased in amount and cell-cell interactions could not be established. In conclusion, this study offers an alternative to using laboratory animals to evaluate potential medicines and/or extracts in wound healing experiments.

INTRODUCTION

The wound is a phenomenon that will never lose its importance as long as life exists, and the efforts to understand healing and regeneration mechanisms have been grown out of a long-held fascination and remain current. The wound is the breakdown of living tissue's anatomical and functional integrity (Robson et al., 2001). Wound formation can be superficial or deep, depending on the degree of the force applied to the tissue (Ekizoğlu and Arican, 2009). Continuous therapy research and innovative applications for wound and wound healing have been conducted utilizing various materials, methodologies, and animal models. In vivo and in vitro modeling are the most preferred ways for wound creation and healing modeling. In vivo modeling can be defined as the designation of the experimental mechanism for the processing or development process of some physiological or pathological phenomena using biological similarities between animals and humans (Kaya and Çevik, 2011). The animals used in animal experiments are generally mice, rats, guinea pigs, rabbits, cats, dogs, chickens, sheep, and pigs. The most preferred of these are mice, rats, and guinea pigs. In vitro is a term that means in the laboratory or artificial conditions, and in vitro modeling is created outside of the living organism in a controlled environment, usually using Petri dishes, flasks, or test tubes. In vitro wound modeling involves cell and tissue cultures as with 3D matrices and is often used to assay intercellular interactions and intracellular signal transduction (Jing et al., 2014). Fibroblasts, endothelium and keratinocytes are the most successful cells in wound healing, making them the most widely used cell types in in vitro wound modeling. In these models, the cells are employed separately or in combination (Inan and Duman, 2020). The wound healing phenomena in experimental animals, which is frequently utilized in vivo modeling for wound formation and repair, differs from that in humans. Contrarily, human wound healing is typified by the production of granulation tissue, whereas experimental animals rarely experience this type of healing; instead, they have contraction. Pigs are the most closely related experimental animal model to human physiology, although they are not usually chosen because of the challenges associated with study design, care, and application. Since the healing process after wound formation is a cellular phenomenon, the proliferation of cells active in this process, cell-cell interactions, intercellular signal pathways, cell-matrix interactions, and the activities of cells in the region are evaluated. In vitro wound healing modeling allows the observation of cellular activities and gives a great advantage to see which cells play an active role directly in the region. In vitro experiment designs prevent ethical concerns in vivo studies and animal use is reduced by acting in the light of human experimental technique principles (4R Principles).

In this study, it is aimed to construct a 3D skin matrice and to make it a valid in vitro healing model by testing the effects of well-known healing inhibitor/activator compounds. It is envisaged that this model can be conveniently used in further wound experiments, without the need for in-vivo experiments.

MATERIALS AND METHODS

Fibroblast and keratinocyte cell lines were chosen to create 3D spheroid forms because they represent wound healing and complete skin regeneration stages, respectively. Cell lines L929 (NCTC clone 929) and Hacat (CLS; 300493) were obtained from the cell culture archive of The Center for Application and Research of Scientific and Technological Researches (KÜBTUAM) at Kırıkkale University. All cell culture work was performed in the Class IIB biosafety cabinet in accordance with biosafety regulations (Fig. 1). For the cultivation of the cells; DMEM (Dulbecco's Modified Eagle's Medium, 4.5 g/L Glucose, w/ Sodium Pyruvate, w/out L- Glutamine), 10% polyline-streptomycin, 1% penicillin-streptomycin were used. Briefly, 5ml culture medium was added, and following the necessary warming to 37ºC in an incubator, Thawed cells were washed in PBS and following centrifugation, the cell pellet was resuspended as being their concentrations of 10^6/ml each cell culture suspension. Each fibroblast and skin epithelia cell was cultured indivudually at 37°C 5% CO_2 and they were trypsinized when their confluence reached 80%. Matrigel (Corning Matrigel matrix) and platelet-rich plasma were used to create 3D cytoskeletons and replicate the intercellular matrix of the skin. PRP was prepared as follows; 10 ml total blood was collected by venipuncture and the frozen cells were transferred to the PRP kit tube (Rich Cell Prp Kit 15cc; PRP15/Lot:316/2102-01) and centrifuged at 570g -7 minutes according to the kit instructions. The remaining platelet-rich plasma was collected into a separate falcon tube using a syringe with a 21G needle. Then 2 ml solution of 0.06 mM CaCl2 2H2O and 2% penicillin/streptomycin were added. The gel was kept at room temperature for two hours for gel formation. Trentilin Ampoule (active ingredient: Pentoxyfilin 100mg/5ml) was used by suspension at a rate of 10µg/ml to increase cellular activity during wound healing. As an inhibitor, 6% hydrogen peroxide was used at a rate of 0.4µM/L. In the study, spheroids were prepared using both matrigel and PRP and were allocated into three groups each as follows; control group, activator group, and inhibitor group.

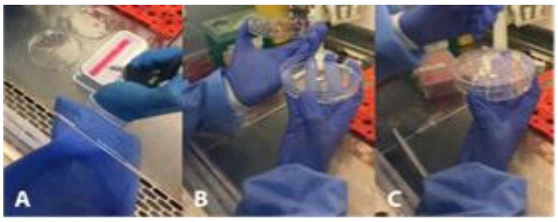

Figure 1. Stages of creating spheroids with the hanging drop technique**.** A) Transfer from cell suspension to petri dish for hanging drop technique. B) Inverting the petri dish in which the cell suspension was transferred. C) View of the petri dish after the formation of hanging drops

PRP–Fibroblast Group

Previously frozen fibroblast(L929) cells were thawed and transferred into a T-75 flask and the study was initiated. The culture medium was changed and the transplanted cells were passaged until they covered 80% of the flask surface. Fibroblast cells reaching 80% growth were trypsinized and separated from the flask surface. 15ml was taken into a flask and centrifuged. The medium formed at the upper end was poured off and the cell pellet at the bottom was diluted with 1 ml medium. To determine the cell viability, cells were stained with trypan blue and live cells were counted. The result was found to be 4.5x106. Fibroblast cells (L929) were suspended 100µl in 24-well plates at 40,000 cells per well, 100µl was spilled into the bottom of the first six wells and the plate was placed in an oven (4% CO2 at 37°C for 2 hours). After removing the plate from the oven, 100µL of PRP in gel form was added to the fibroblasts in the first two wells of the plate and 100µL of PRP with 10µg/ml Trentilin was added to the fibroblasts in the next 2 wells and the surface was covered. The third two wells were closed by adding 100µL of PRP to which 0.4µM/L hydrogen peroxide was added and after this process, the study was terminated by adding another 100µL of fibroblast cell suspension to all wells. After the procedure, fibroblasts were examined under a microscope and photographed and recorded at 0, 24, 48, and 72 hours. The spheroid diameters were measured with the 'Image J' program and recorded and graphed in Microsoft Excel. After the procedure, fibroblasts were examined under a microscope and photographed and recorded at 0, 24, 48, and 72 hours (Fig. 2). The resulting spheroid diameters were measured with the 'Image J' program and recorded and graphed in Microsoft Excel (Fig. 3).

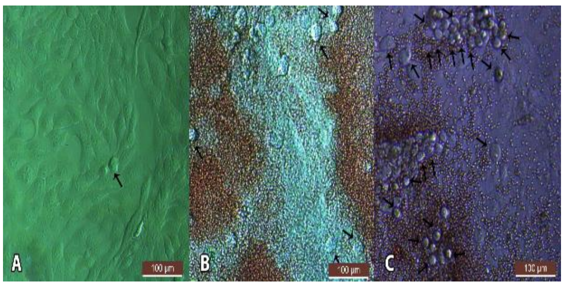

Figure 2. PRP – Fibroblast (L929) Group Spheroid formation Inverted Microscope Image (100 µm) Indicator group B) Control group C) Activator group

Figure 3. Images of measurement in 'Image J'

Matrigel - Fibroblast Group

Previously frozen fibroblast(L929) cells were thawed and transferred to a T-75 flask and the study was started. The culture medium was changed and passaged until the transplanted cells covered 80% of the flask surface. Fibroblast cells reaching 80% growth were trypsinized and separated from the flask surface. After staining with trypan blue, viability determinations, and cell counts were performed. The result was found to be 3.9 x 10^6. After cell counting, the cell suspension was diluted to 1.25 x 105 in 20 µL with the addition of a culture medium. Using the resulting cell suspension, 20 µL droplets were added to the lid of a 10 cm diameter sterile petri dish to form spheroids by the hanging droplet method. 5ml PBS was added to the petri dish bottom to maintain ambient humidity during the propagation of the cell clusters. The lid was quickly inverted and closed without deteriorating the drop structure and left to reproduce for 72 hours in a study with 4% CO_2 at 37°C. For this, the matrigel matrix at -20 ^{o}C was replaced to +4°C the night before. The pipette tips to be used before the procedure were cooled sterile at -20 ^{o}C for 10 minutes and all procedures with the matrigel matrix were performed on an ice battery. The wells to be used on the plate with 24 wells were marked and placed on the base with a 100 µL matrix pipette, and the plate 37 ^{o}C was removed and waited for 30min. Thus, the matrix was gelled, which is found in liquid form in a cold environment. At the end of 30 minutes, the spheroids in the Petri dishes we had prepared before were taken with a 21G needle tip and placed in the middle of the gelled matrigels, taking care not to form bubbles. The second spheroid was added just above the previous spheroid following the same steps as before. Added to the control wells; 500µL of Trentilin Ampoule (Trentilin Ampoule: Pentoxyfilin 100mg/5ml) suspended with 10µg/ml standard culture medium. Added to the migration activation group 500µL of %6 H2O2 suspended at 0.4µM/L was added to the migration inhibition group. The areas of the spheroid structures formed were photographed during the 0, 24, 48, and 72 hours (Fig. 4). The photographs taken were measured and graphed with the 'Image J' program (Fig. 3).

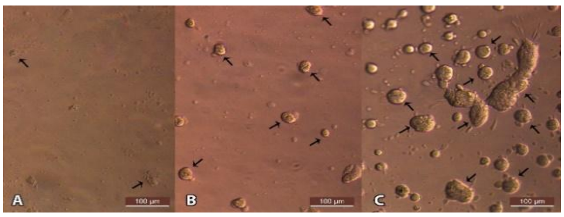

Figure 4. Matrigel–Fibroblast (L929) Control Group Spheroid formation Inverted Microscope Image (100 µm) A) Indicator group B) Control group C) Activator group

PRP–Fibroblast–HaCaT Group

Previously frozen HaCaT and fibroblast (L929) cells were dissolved in a hot water bath and cultured in T-75 flasks. Cells that reached 80% proliferation were trypsinized, and cell counts were 3.6 x 10^6 for HaCaT; and 4.9x10^6 for fibroblast (L929). L929 fibroblast cells and HaCaT cells were previously passaged and were dissolved and transferred into T-75 flasks and the process started. The stock cells at -80^{o}C were removed and dissolved in 37^{o}C water bath. The previous cell transfer procedure was followed, and nd suspended when they reached 80% proliferation; the viability of the cells was measured. Trypan blue was used for vitality measurement and cell count. 10µl of HaCaT cell suspension and 10µl of trypan blue were taken into an Eppendorf tube. 10µl were taken from the new suspension and added to the 'A' compartment of the cell, counting slide and counting with a hemocytometer (3.6 x 10^6). At the same procedure was performed with the L929 fibroblast cell line (4.9 x 10^6). For 24 well plates, new cell suspensions were prepared with 40,000 cells per well, and 100µL of the fibroblast suspension was added to the desired wells in the plate, and the plate was incubated at 37 ^{o}C for 2 hours. The cell surfaces were coated by adding 100 µL each of the previously used PRP Activator- inhibitor and control group. 100µL of HaCaT cell suspension was added. The diameters of the spheroids were evaluated by photographing the hours 0, 24, 48, and 72. Measurements were made with the 'Image J' program, graphed the results. The photographing and examination of the spheroid structures were carried out with an inverted microscope (Marka: Leica Model: DMI 4000 B) (Fig. 5). Spheroid density measurement and graphing in a microscopic field were performed with the " Image J " program (Fig. 3). The areas and environments of the spheroids were measured as quantitative evaluation criteria. The measurements were recorded in Microsoft Excel and compared (Fig. 6 and Fig. 7).

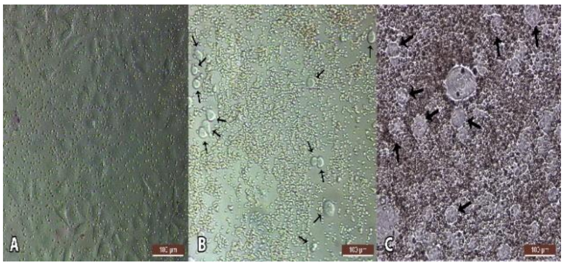

Figure 5. Fibroblast (L929)–PRP-HaCaT Control Group Spheroid formation Inverted Microscope Image. A) Indicator group B) Control group C) Activator group

GROUPS/AREA	24.hour	48.hour	72.hour
CONTROL GROUP PRP-FIBROBLAST	4492	7000	5532
CONTROL GROUP FIBROBLAST-MATRIGEL	12.699	21.118	18.076
CONTROL GROUP FIBROBLAST-PRP-HACAT	25.984	10384	24.864
ACTIVATOR PRP-FIBROBLAST	6956	23925	6643
ACTIVATOR FIBROBLAST- MATRIGEL	17.952	56.016	47.632
ACTIVATOR FIBROBLAST-PRP-HACAT	14.496	9.676	5.268
INDICATOR PRP-FIBROBLAST	4779	3968	0
INDICATOR FIBROBLAST- MATRIGEL	0	0	0
INDICATOR FIBROBLAST-PRP-HACAT	4.780	3.904	0

Figure 6. Area measurements were calculated and tabulated in micrometersquare with the 'Image j' program

GROUPS/ ENVIRONMENTAL MEASURE	24th hour	48th hour	72th hour
CONTROL GROUP PRP-FİBROBLAST	238.761	298.451	263.894
CONTROL GROUP FİBROBLAST-MATRİGEL	436.123	539.886	489.230
CONTROL GROUP FİBROBLAST-PRP-HACAT	571.770	361.283	559.203
ACTIVATOR PRP-FİBROBLAST	296.881	549.779	289.027
ACTIVATOR FİBROBLAST-MATRİGEL	543.035	930.224	858.649
ACTIVATOR FİBROBLAST-PRP-HACAT	427.257	348.717	259.181

Figure 7. Environmental measurements were calculated and tabulated in micrometers with the 'Image j' program

RESULTS

Microscopic examination showed that cell-cell complex structures formed spheroids in all three methods. It was also revealed whether the formed spheroid structures increased or not by using activators and inhibitors. Activator and inhibitor substances were selected from substances that promote and inhibit healing in the wound healing process. Spheroid structures (spherules) formed by the proliferation of cells and their interaction with each other were significantly increased and enlarged in the activator groups using the preparation that clinically activates wound healing. In the inhibitor groups, spheroid formation was seen in some groups, but in small numbers and in small shapes, while in some groups it was seen in very small structures or not seen because it disrupted the structure that would form a skeleton for the cells used and caused death in the cells. Since our aim was not to measure the drug effect, no further evaluation was made on this issue.

As seen in the microscopic examination, it was seen that activator and inhibitor substances that promote wound healing can increase or decrease the formation of cell-cell complex structures in 3D cell culture and increase or decrease migration to the wound site. The area and perimeter measurements of the spheroid structures formed in our study were made with 'Image J'.

The results were compared graphically. In this comparison, the results showed that spheroid formation occurred in the groups in which the gel form of PRP was used as a cell support network, but spheroid structures were not as large as in the groups in which matrigel was used as a cell support network. Although smaller spheroids were formed in the groups using PRP compared to the groups using matrigel, it has an advantage over matrigel, which is that spheroid structures are formed and prominent in the inhibitor groups in the groups using PRP.

In our study, fibroblast and keratinocyte cells, which are most effective in wound healing, were used and the spheroid structures formed by them were examined. The size and multiplicity of the spheroid structures formed give us information about the speed and formation of the migration phenomenon. PRP and Matrigel used in the study form a skeleton for the cells to form a complex structure. Matrigel fills the cavity of the colloagin and PRP contains platelets that accelerate cell migration to the wound area. The activator and inhibitor substances used are clinically used materials.

When the control groups were examined, it was observed that spheroid formations were present but not as large and prominent as in the matrigel control group. Another difference between the control groups was that the spheroid structures formed in the study with HaCat were more prominent and larger than the fibroblasts in the study with fibroblasts only. It was observed that the spheroids formed in the activator groups were small but numerous at the 72nd hour, while at the 24th and 48th hours, the formations had just started and larger structures were also observed. In the inhibitor group studies, no results could not be reached because the inhibitor we used in the matrigel group disrupted the matrigel form, but in studies with fibroblast and keratinocyte cells, spheroid formations were observed at 24 and 48 hours, but the spheroid structures formed were small structures. At 72 hours, no spheroid structures were observed and the structures formed were observed to be disrupted.

DISCUSSION AND CONCLUSION

Wound formation, the healing mechanism, and the quest for healing treatment approaches are among the most frequently investigated and experienced issues worldwide (Daunton et al., 2012). Wound modeling uses a variety of models, including in vivo, in silico, ex vivo, and in vitro (Ud-Din and Bayat, 2017).

In vivo modeling is the method of preference for studying complicated events in wound healing. Although in vivo investigations are favored, the skin structures of experimental animals do not demonstrate physiology in wound formation and healing in people because they are not similar to those in humans, and the cases that arise during the healing process differ from humans (Greenhalgh, 2005).

The group of rodents that are often studied are creatures because they are easy to produce and durable, partly because of the ease of modeling. The fact that wound healing in rodents is by contraction, which does not develop granulation tissue as in humans, as well as the different placement of skin add-on glands and layers, has a negative impact on the study's results (Dorsett-Martin, 2004) Its skin composition is the closest to that of a living pig. The dermis and epidermis are thick in humans and pigs, but thin in rodents. In rodents, wound healing is accomplished by contraction; in humans and pigs, it is accomplished by granulation and epithelialization. Although the architecture of human and pig hair follicles are similar, one feature that sets rodents apart from humans is the abundance of hair follicles. While apocrine sweat glands are confined to the udder in rodents, they are widely distributed throughout the human perineal and axillae (Dorsett-Martin, 2004; Sulivan et al., 2001; Wong et al., 2011). When Google Scholar searches based on the last 5 years, it is seen that there are 18,800 results under the name of 'wound healing mouse model', 19,200 results in the 'wound healing rat model' search, 18,200 results in the 'wound healing rabbit model' search and 17,700 results in the wound healing pig model search. The use of animals in studies brings ethical problems and causes undesirable situations to develop during studies. In the interventions performed during the study, the development of sepsis in animals, shock development during the intervention, or interventions do not have the desired effects on the animal, and the intervention is completely tolerated by the animal (Kaya and Çevik, 2011). Situations such as the care and feeding of animals, the replacement of dead animals, and the repetition of work require extra time and extra-economic burden (Yeğen and Gören, 2005). Sauer and his friends, in their study, argued that animal experiments should be applied after all alternative methods have been tried. In experimental animal modeling, the inability to prevent the animal from being affected by environmental conditions, the fact that the drug applied in drug applications is metabolized first, and the full effect cannot be clearly demonstrated, the animals' reactions to the applications are restrictive factors in studies. In light of all this, the use and development of cell cultures reflect all the characteristics of human tissues, and cells have started to come to the fore in recent years. Considering the ethical concerns in animal studies and the Principles of Human Experimental Technique (4R Principle), the move of the experiments to cell culture eliminates these concerns. Cell culture studies dating back a long time are used in 2D to study the activities, structures, and development of cells in living tissue (Duval et al., 2017). Cell culture studies used in many different fields such as toxicological, pharmacological, microbiological, and oncological examinations and paving the way for important developments in living life are also used in wound modeling and the study of cellular activities developing in wound healing (Huh et al., 2011; Duval et al., 2017). Until recent years, these studies have been modeled in 2D cell culture, first causing mechanical damage to the cells covered in the base to characterize wound formation, and then looking at the migration of cells to this region, the cellular effects of drugs used or developed, whether they are toxic, and cellular responses to mechanical injuries (Liang et al., 2007; Jonkman et al., 2014). Although measurable results were obtained through these studies, it was concluded that 2D cell cultures are alternatives in terms of easy repeatability to animal experiments, control of environmental conditions, and economics, but are incomplete in reflecting the complex event developing in the in vivo environment (Mazzoleni et al., 2009). The fact that cell reproduction is limited to the media in the culture dish and the base in the culture container and the limited cell and cell ECM interaction is one of the limitations of 2D cell cultures in reflecting the organoid structure in life (Bonnier et al., 2015; Choi et al., 2010). As a result of the elimination of these limitations and the increase of studies on in vivo organoid structure and reactions, and as a result of developing research techniques, 3D cell cultures were started to be made (Duval et al., 2017). As a result of cell-cell and cell-ECM interactions in spheroid structures formed in 3D cell cultures, it has been observed that many cellular behaviors such as proliferation, polarization gene, and protein expressions provide traceability as in vivo conditions (Steinwechs et al., 2016; Marby et al., 2016). The basis of 3D cell culture is the secretion of structural proteins and molecules in vivo conditions of spheroids formed in an extracellular matrix-like structure (liquid, solid) (Weaver et al., 1997; Lin et al., 2008). In many studies using 3D cell cultures, 3D cell cultures were closer to in vivo cases than 2D cell cultures, and the results were close to the results of experimental animal studies (Polat, 2020). In light of these studies, this study was carried out to investigate whether 3D wound modeling can be performed and how the cells that play an active role in wound healing will react to the factors used in wound healing that accelerate healing. The culture of the cells most involved in wound healing in 3D in three different cell collections created using two different scaffoldings and the effect of substances that accelerate and inhibit wound healing were examined. The multiplicity and size of spheroid structures formed in the evaluation of the study groups were evaluated. Since the migration and proliferation of fibroblasts in wound healing were related to activation, the evaluation was accurately proportioned with the number and size of spheroids. The fact that while the number of spheroids was evaluated qualitatively, the area and environment measurements of the spheroid structures were evaluated quantitatively with the 'Image J' program (Fig. 8-13). The more spheroid structures formed or larger, the more fibroblast activity they had and were evaluated as migration. The PRP part of the blood used in this study increased the activation of fibroblast cells and acted as a skeleton for cell connections (Fig. 10). PRP has been shown in studies with positive effects on wound healing (Hudgens et al., 2016). Spheroid formation was observed in this 3D cell culture study, and fibroblast migration increased with the activator (Fig.11and Fig. 13). PRP- HaCaT- It was observed that the size and number of spheroid structures formed in the fibroblast group were more intense than in the study in the fibroblast –PRP group. It was thought that the source of this may be the formation of cross-bonds between the HaCat and fibroblast cells that allow scar formation (Ghahary and Ghaffari, 2007). The group where fibroblast migration is best evaluated and seen is the working group with matrigel (Fig. 12 and Fig. 13). Spheroid formation was seen in other groups, but it was not as pronounced in size and number as in the matrix. When scratch modeling was required to be performed for wound work and to see the migration on this line, the cells were collected at the edges due to the gel-shaped structures, and this study could not be done due to the deterioration of the gel form. The environment and areas of the spheroid structures formed in the study were calculated, and it was observed that the matrigel group also stood out compared to these (Fig. 6 and Fig. 7). In the case of migration in the wound, it was considered acceptable as a model used before experimental animal modeling to examine cell connections, cell-matrix connections, and fibroblast activation. Cell-cell connections that play an active role in wound healing and cell ECM can be used in the examination of both 2D and in vivo studies as an alternative study; since activators and inhibitory substances to be used in wound healing can be used as preferable modeling in the study of cases in cell-cell interactions and ligaments, and alternative to 2D cell culture for the study of fibroblast and keratinocyte activation involved in wound healing, there may be an alternative preliminary study to experimental animal studies, both economically and recurrent before applying to the experimental animal study. Considering the study construction and evaluation stages, it is thought that the study can be expanded using different cell skeleton structures and using different cells. Studies can be carried out where complex phenomena can be examined by making more complex 3D cultures and showing cells and cellular interactions. When the materials used in the study were evaluated, it was observed that the matrix better mimics ECM in the cell and retains spheroid structures for longer when preferred as a cell skeleton. The formation continues for a long time. It is thought to be preferable as a working material when the chronic wound healing process is examined.

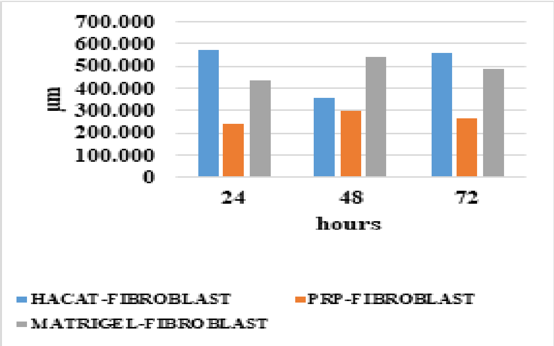

Figure 8. Comparison of Environmental Measurements of Spheroid Formation in Control Groups

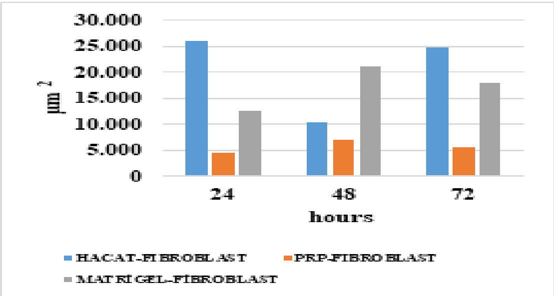

Figure 9. Comparison of The Formation of Spheroid Areas of Control Groups.

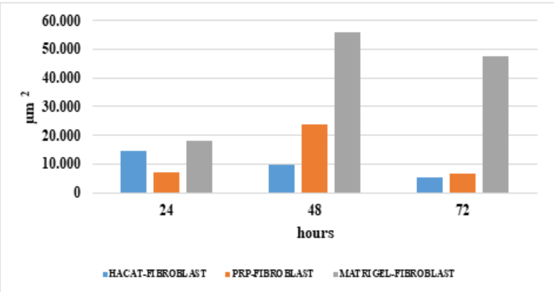

Figure 10. Comparison of The Formation of Spheroid Areas of Activator Groups

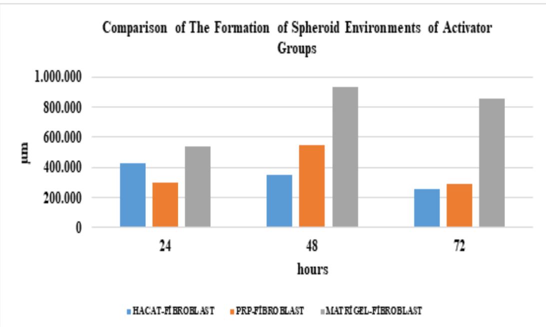

Figure 11. While there was an increase in the spheroid area and circumference measurements until the 48th hour due to the administration of a single dose of the drug to the groups to which the activator substance was added, there was a decrease in these results as the activator effect decreased at the 72nd hour measurements

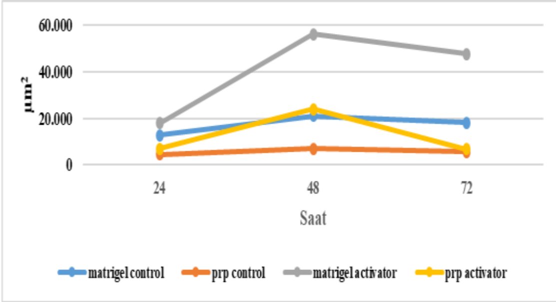

Figure 12. Fibroblast (PRP) Groups Comparison of The Formation of Areas of Matrigel Groups

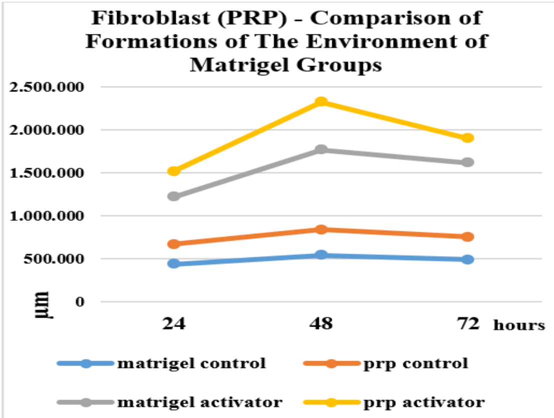

Figure 13. Matrigel fibroblast and prp fibroblast groups were compared because they contain the same cell lines. In the comparison of activator groups between the groups, the matrigel-fibroblast group stands out as the area, while the prp-fibroblast group stands out in the measurement of the circumference

Acknowledgement

This study is based on the master's thesis entitled 'Alternative to Experimental Animal Studies In vitro Wound Modelling', which was completed in 2022 under the supervision of Prof. Dr. Oğuz KUL. (Master's Thesis, Kırıkkale University Faculty of Veterinary Medicine, Kırıkkale, Turkey, 2022).

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: E.E.A., O.K., Design: O.K., E.E.A, Data Collection or Processing: E.E.A., O.K., Analysis or Interpretation: O.K., E.E.A., Literature Search: E.E.A., O.K., Writing: E.E.A., O.K.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Barbul A, Efron DT, Kavalukas SL. 2015. Wound healing. Brunicardi F, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Matthews JB, Pollock R.E. (Eds.), Schwartz's Principles of Surgery, 10e. McGraw Hill. https://accessmedicine.mhmedical.com/content.aspx?bookid=980&sectionid=59610850.

Bonnier F, Keating M, Wrobel TP, Majzner K, Baranska M, Garcia-Munoz A, et al. 2015. Cell viability assessment using the Alamar blue assay: a comparison of 2D and 3D cell culture models. Toxicology in vitro, 29(1):124-131.

Choi SW, Yeh YC, Zhang Y, Sung HW, Xia Y. 2010. Uniform beads with controllable pore sizes for biomedical applications. Small, 6(14):1492-1498.

Daunton C, Kothari S, Smith L, Steele D. 2012. A history materials and practices for wound management. Wound Practice and Research, 20(4):174-85.

Dorsett-Martin WA. 2004. Rat models of skin wound healing: a review. Wound Repair Regen, 12: 591-599.

Duval K, Grover H, Han L-H, Mou Y, Pegoraro AF, Fredberg J, et al. 2017. Modeling physiological events in 2D vs. 3D cell culture. Physiology, 32(4):266-277.

Ekizoğlu O, Arıcan N. http://klinikgelisim.org.tr/eskisayi/klinik_2009_22/05.pdf

Ghahary A., Ghaffari A. 2007. Role of keratinocyte–fibroblast cross-talk in development of hypertrophic scar. Wound Repair and Regeneration, 15, S46-S53.

Greenhalgh DG. 2005. Models of wound healing. J Burn Care Rehabil, 26:293-305.

Hudgens JL, Sugg KB, Grekin JA, Gumucio JP, Bedi A, Mendias CL. 2016 Platelet-Rich Plasma Activates Proinflammatory Signaling Pathways and Induces Oxidative Stress in Tendon Fibroblasts. Am J Sports Med., 44(8):1931-1940. doi: 10.1177/0363546516637176. Epub 2016 Jul 8. PMID: 27400714; PMCID: PMC4970921.

Huh D, Hamilton GA, Ingber DE. 2011. From 3D cell culture to organs-on-chips. Trends in Cell Biology, 21(12):745-754.

İnan NB, Duman N. 2020. Yeni ex vivo ve in vivo sikatris/ yara (dokusu) modelleri. Serarslan G, editör. Sikatris Tedavisine Güncel Yaklaşım. 1. Baskı. Ankara: Türkiye Klinikleri, p.66-70.

Jing X, Shi Q, Bi W, Zeng Z, Liang Y, Wu X, et al. 2014. Rifampicin protects PC12 cells from rotenone-induced cytotoxicity by activating GRP78 via PERK-eIF2alpha-ATF4 pathway. PLoS One, 9: doi: 10.1371/journal.pone.0092110

Jonkman JEN, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM, et al. 2014. An introduction to the wound healing assay using live-cell microscopy. Cell Adh Migr., 8:44-51.

Kaya M, Çevik A. 2011. Hayvan Deneylerinde Planlanma ve Model Seçimi. Deneysel Tıp Araştırma Enstitüsü Dergisi, 1(2):36-39.

Liang C-C, Park AY, Guan J-L. 2007. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc., 2:32-33.

Lin RZ, Chang HY. 2008. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. Biotechnology Journal: Healthcare Nutrition Technology, 3(9-10):1172-1184.

Mabry KM, Payne SZ, Anseth KS. 2016. Microarray analyses to quantify advantages of 2D and 3D hydrogel culture systems in maintaining the native valvular interstitial cell phenotype. Biomaterials, 2016;74:31-41.

Mazzoleni G, Di Lorenzo D, & Steimberg N. 2009. Modelling tissues in 3D: the next future of pharmaco-toxicology and food research?. Genes & Nutrition, 4(1):13.

Polat E. 2020. Üç boyutlu hücre kültürü sistemlerine güncel yaklaşımlar. Namık Kemal Tıp Dergisi, 8(1):84-92.

Robson MC, Steed DL, Franz MG. 2001. Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg., 38(2):72-140.

Steinwachs J, Metzner C, Skodzek K, Lang N, Thievessen I, Mark C, et al. 2016. Three-dimensional force microscopy of cells in biopolymer networks. Nature Methods, 13(2):171.

Sullivan TP, Eaglstein WH, Davis SC, Mertz P. 2001. The pig as a model for human wound healing. Wound Repair Regen, 9(2):66-76.

Ud-Din S, and Bayat A. 2017. Non-animal models of wound healing in cutaneous repair: In silico, in vitro, ex vivo, and in vivo models of wounds and scars in human skin. Wound Rep and Reg, 25:164-176.

Weaver VM, Petersen, OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ. 1997. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. The Journal of Cell Biology, 137(1):231-245.

Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC. 2011. Surgical approaches to create murine models of human wound healing. J Biomed Biotechnol. 2011:969618. Doi: 10.1155/2011/969618.

Yeğen B, Gören Z. 2005. Biyomedikal Araştırmalarda Deney Hayvanı: Temel Bilgiler ve Etik İlkeler; Yüce Yayımları.