ORIGINAL ARTICLE

High-Resolution Ultrasound Imaging for Non-Invasive Characterization of Acute Wound Healing in Radiation Injury on Guinea Pig Skin Tissue

Zeinab Hormozi-Moghaddam [1,2*], Manijhe Mokhtari-Dizaji [3*], Mohammad Ali Nilforoshzade [4], Mohsen Bakhshande [5], Sona Zare [4]

[1] *Radiation Biology Research Center, Iran University of Medical Sciences (IUMS), Tehran, Iran*

[2] *Department of Radiation Sciences, Allied Medicine Faculty, Iran University of Medical Sciences (IUMS), Tehran, Iran*

[3] *Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

[4] *Skin and Stem Cells Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[5] *Department of Radiology Technology, Allied Medical Faculty, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

*Corresponding Authors: Zeinab Hormozi-Moghaddam, Manijhe Mokhtari-Dizaji
Email: hormozimoghadam.z@iums.ac.ir, Mokhtarm@modares.ac.ir

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Abstract

Purpose: High-resolution ultrasound imaging is a non-invasive and objective appraisal. Ultrasound imaging accomplishes the target assessment and follow-up of radiation-induced skin injury.

The study aimed to investigate the complete anatomical and structural alternations of acute wound healing in skin tissue radiation injury after cell therapy with high-frequency ultrasound imaging techniques.

Materials and Methods: Female guinea pigs (250 g) were divided into 3 groups: (a) controls, consisting of non-treated guinea pigs; (b) radiation-treated; (c) radiation-treated receiving adipose-derived mesenchymal stem cells. Acute radiation-induced skin injury was induced by a single fraction of X-ray irradiation of 60Gy to a 3.0×3.0-cm area with a 1.3-cm bolus on 100-cm SSD in the abdominal skin tissue. Ultrasonic imaging of the depth and quality of healing in the skin tissue was performed by processing ultrasound images at 40-MHz and 75-MHz frequencies.

Results: Skin thickness indicated a significant difference between the treatment and control groups on Day 10 after 60 Gy irradiation ($P<0.05$). The highest skin thickness was observed in the irradiated group, and the lowest skin thickness was found in the stem cell treatment group.

Conclusion: Evaluation of skin thickness, wound depth, and scar formation is important for the proper assessment and management of wound healing in stem cell therapy of radiation-induced skin damage. High-resolution ultrasound at 40- and 75-MHz frequencies is a major non-invasive method providing unprecedented insight into determining the characterization of the skin, particularly in the context of wound healing.

Keywords: High-Resolution Ultrasound Imaging; Radiation; Skin; Stem Cell Therapy; Wound Healing.

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1. Introduction

High-resolution ultrasound imaging is a non-ionizing (mechanical wave), non-invasive, and real-time diagnostic procedure. Ultrasound imaging of the skin tissue is becoming more and more popular and is performed to evaluate healthy and pathologically altered skin, as well as to monitor the alterations in the skin layers [1]. Many diagnostic methods can help skin tissue disorders, including photography, dermoscopy, reflective confocal microscopy, optical coherence tomography, and high-frequency ultrasound [2, 3]. High-frequency apparatuses are equipped with transducers of the frequency of 20–100 MHz [1-4].

Acoustic waves constitute mechanical longitudinal waves, which can be described in terms of particle displacement or pressure variations. High-frequency ultrasound imaging uses the propagation of mechanical waves in the propagation environment and the interactions of reflection, refraction, scattering, and absorption in the environment affecting the formation of the image [5]. The percentage of reflected waves (echo) from the interface in the tissue may be anechoic (black), hyper-echoic (bright), hypo-echoic (dark), and iso-echoic images compared with adjacent subcutaneous tissues on the screen. Differences in tissue characteristics such as density, attenuation coefficient, and speed of ultrasound waves determine the amount of echo [6].

Some of the more important quantities affected by ultrasound imaging quality include frequency/wavelength, propagation speed, pulsed mode, the interaction of ultrasound with the tissue, the angle of incidence, and attenuation. Frequency has a direct relationship with resolution in imaging and an inverse relationship with depth [5]. An increase in frequency leads to a decrease in wavelength, an increase in the depth of the near-field zone, and a higher axial resolution in the evaluation of surface tissue textures. Note that frequency has a direct relationship with absorption; with an increase in frequency, the absorption of ultrasound waves increases and the depth of penetration decreases, but resolution, especially axial resolution, rises [5-7].

The skin tissue, as the most superficial soft tissue of the body, consists of different layers. If high-frequency ultrasound imaging is used, many details of the skin tissue can be recorded [5]. The main structure of the skin tissue is the epidermis layer the outermost layer of keratinocyte cells. Although the epidermis layer forms a thin layer of about 0.02-0.15 mm, it can act as an effective protective barrier against many pathogens [6]. The epidermis layer contains proliferative cells that are dividing and are sensitive to radiation. These cells migrate to the upper layers and replace the upper layers [7]. The dermis layer is the thickest layer of the skin, located under the epidermis layer. The thickness of this skin layer in humans ranges from about 0.6 to 3.0 mm. Its main function is to regulate body temperature and provide blood to the epidermis layer [8].

The cellular structure of skin tissue layers, especially basal cells, is affected by radiation in radiation therapy methods. This type of skin damage should be controlled in the early stages. Lack of healing in the early stages will lead to loss of function of skin tissue and permanent degeneration of cells. In this type of damage, it is necessary to assess the effect of radiation on the skin tissue to obtain the best treatment method in the early stages [9]. Because the tissue is sensitive, invasive evaluations are not possible. If the radiation therapy method is accompanied by surgery and invasive methods, this side effect will be more severe and lead to the loss and complete damage of the skin tissue.

Recently, stereotactic body radiotherapy methods that deliver a high dose to the target and skin tissue have been introduced. A small part of the skin is constantly irradiated and is damaged in total treatment fractions [9]. Therefore, radiation damage to the skin tissue is known as a common side effect and the main limitation of radiation therapy methods. Acute radiation injury of the skin is caused by damage to keratinocyte cells in the epidermis layer, fibroblast cells in the dermis layer, skin vessels, hair follicles, and reduction of cell proliferation capacity [7-9].

The presence of mesenchymal stem cells can increase the function and number of cells in the area under radiation damage. In addition, mesenchymal stem cells derived from the adipose tissue (AdMSCs) help heal the damaged tissue by creating suitable environmental conditions in the tissue, by secreting the fibroblast growth factor [9, 10]. For a complete skin evaluation, the use of high-resolution ultrasound is essential because the high frequency allows detailed real-time visualization of the epidermis, dermis, and subcutaneous tissue, allowing an in-depth characterization of various skin parameters [11].

In this context, the purpose of this study was to describe the main data collected in the evaluation of anatomical and structural changes in irradiated skin after stem cell therapy. Ultrasound imaging at 40- and 75-MHz frequencies provides a high-resolution assessment and follow-up of radiation-induced skin injury.

2. Materials and Methods

2.1. Radiation Injury Model on Skin Tissue

To investigate skin tissue alterations after radiotherapy and treatment with AdMSCs, the skin tissue of female guinea pigs (weighing 250 g) was selected. The animals were anesthetized under the conditions of irradiation and treatment by injection of ketamine and xylazine (Alfasan, Woerden, and the Netherlands). Radiation doses of 20, 30, 40, 50, 60, and 80 Gy were exposed to the skin tissue of the guinea pigs in a single fraction through the linear accelerator radiation of 6 MeV (Elekta Compact, Au055, Stockholm, Sweden). The irradiation conditions included a 3×3 cm radiation field, 1.3 cm blus, and SSD=100 cm. Then, the radiation dose was selected to evaluate the structural changes of the tissue, and the animals were treated with stem cells.

2.2. Stem Cell Culture

The adipose tissue was extracted from the neck of the guinea pigs and was then placed in a falcon tube and 0.1% collagenase enzyme was added to it. The tissue was incubated in a 37-°C incubator for 30 to 45 minutes. The collagenase enzyme was neutralized by DMEM 10% FBS medium and centrifuged at 1200 rpm for 10 minutes. The obtained cell was pipetted with 2 ml of DMEM 10% FBS medium, and the cell suspension was transferred to 25 ml flasks and cultured in an incubator at 37 °C, 95% humidity, and 5% Carbon dioxide. The cell culture medium was replaced every two days. When the cell population reached 80 or 90%, cell passage was performed.

2.3. Treatment Procedure

To prevent progress in radiation injury of the skin tissue in the early stages, AdMSCs were injected 24 to 48 hours after the irradiation of the skin tissue by the accelerator. AdMSCs (2× 106) were injected intradermally under sterile conditions. The animals were divided into three groups: (a) normal (n=5), consisting of non-treated guinea pigs (n=5); (b) radiation injury of the skin tissue (60 Gy) (n=5); (c) radiation injury of the skin tissue treated with adipose-derived mesenchymal stem cells (AdMSCs) (n=5).

2.4. High-Resolution Ultrasound Imaging

Ultrasound imaging of the depth and quality of healing in skin layers was assisted by a high frequency of 40 MHz (Ultrasonix company, Richmond, Canada, resolution 0.01 mm in longitudinal and transverse directions) and 75 MHz (skin scanner). B-Mode images of a 40-MHz ultrasound system (depth: 1 cm, gain: 60%) were taken from the left, right, and center of the irradiated area. To reduce error, the thickness of the layers in each image was measured five times. Then, the size of the skin tissue layers, including the dermis and epidermis layers, was extracted via the ImageJ software (Java-based image processing program, 1.52 v for Windows). In imaging with a frequency of 75 MHz, a scan was performed from the beginning of the irradiation area to the end, and then, the impression of the skin texture was automatically evaluated in terms of thickness (depth and width), density, and the number of pixels (Figure 1). Finally, the results of two ultrasound imaging methods were measured via the linear regression function and Pearson's correlation coefficient.

2.5. Statistical Analysis

The data of skin tissue anatomical structure before and after radiation with or without treatment were estimated as mean ± standard deviation. A t-test and one-way ANOVA were performed to analyze differences between groups at a significance level of 0.05 ($P< 0.05$) (SPSS v.20, Chicago, LL).

3. Results

The difference in the thickness of different skin layers in normal, radiation injury, and groups treated with AdMSCs was significant. The thickness of skin tissue layers, including the epidermis and dermis, was scanned by high-frequency ultrasound imaging. Ultrasound imaging with a frequency of 40 MHz showed the changes in the dermis and epidermis layers of the guinea pig skin tissue. To achieve an animal model of acute radiation injury, doses of 20, 30, 50, 60, and 80 Gy were used. The increase in the skin tissue thickness after radiation was investigated 10 days after radiation and before the appearance of skin injuries.

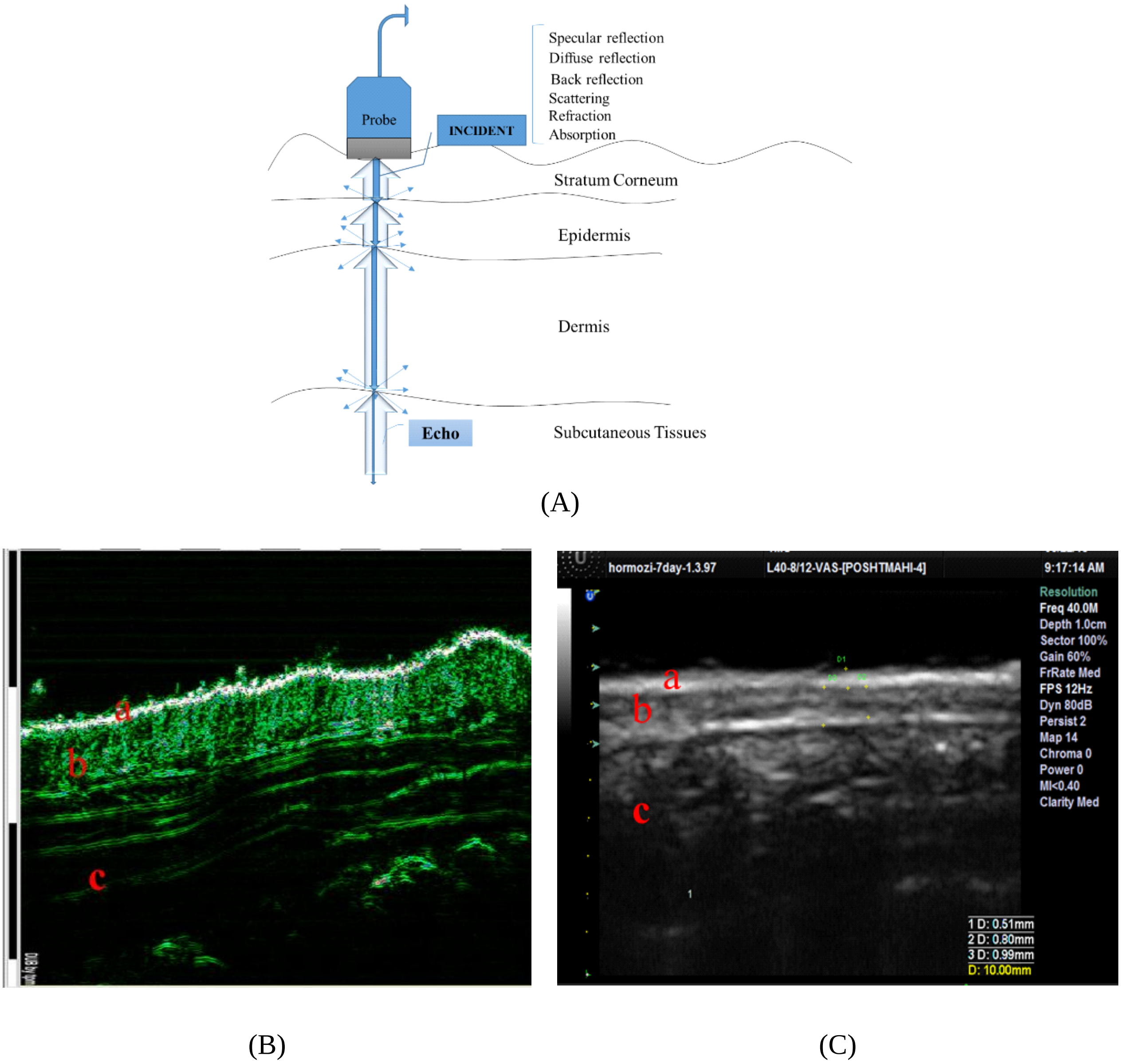

Figure 1. High-frequency ultrasound image principle. A) Interactions of ultrasound with skin layers, B) 75 MHz frequency probe; C) 40 MHz frequency probe (B-Mode); a: Stratum corneum and epidermis, b: dermis, c: subcutaneous tissue

The results of the 40-MHz ultrasound images (B Mode) clearly showed the thickening of the epidermis and dermis tissue at radiation doses of 20, 30, and 50 Gy. Furthermore, the thickness of epidermal and dermal tissues was significantly higher at radiation doses of 60 and 80 Gy. Higher thickness was achieved in the dermis layer of high doses after 10 days. The results of the thickness of the epidermis, dermis, and their total layers are presented in Table 1.

According to the results obtained in the investigated groups before radiation (Day zero) in the abdominal area, the thickness of the epidermis layer was 0.14 mm, the thickness of the dermis layer was 0.75 mm, and the total thickness was approximately 0.90 mm. Ten days after the radiation therapy, an increase in the average thickness of the epidermis, dermis, and total thickness was observed following acute damage caused by radiation at different doses.

Finally, based on the photographic images and changes in the thickness of the dermis and epidermis layers of ultrasound images at radiation doses of 60 Gy and above in the abdominal area causing acute radiation injury, this radiation dose was selected as the target radiation dose to investigate the combined treatment protocol of radiation waves. In previous studies, Hemotoxylin and Eosin (H&E) staining of skin tissue samples under 60-Gy radiation confirmed

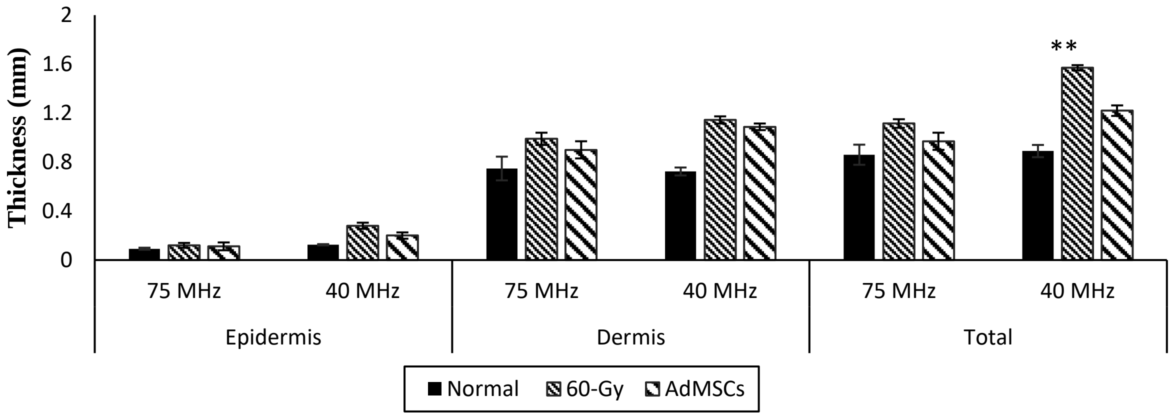

Figure 2. The average and standard deviation of the thickness of the skin tissue layers in millimeters of the 40 and 75 MHz ultrasound imaging

Table 1. Average and standard deviation of the thickness of skin tissue layers after irradiation of different doses from B-mode ultrasound images with 40 MHz frequency

Tissue layer thickness (mm)	Normal	20 Gy	30 Gy	50 Gy	60 Gy	80 Gy
Epidermis with stratum corneum	0.14±0.05	0.20±0.03	0.30± 0.04	0.30± 0.03	0.33± 0.04	2.1± 0.28
Dermis	0.75± 0.03	0.87± 0.02	1.13± 0.10	1.20±0.09	1.60± 0.16	1.85± 0.26
Total	0.90± 0.04	1.13±0.05	1.35± 0.10	1.52± 0.07	1.90± 0.13	2.1± 0.28

the change in tissue structure, which led to the recording of changes in ultrasound imaging [12].

The combined therapeutic protocol of AdMSCs on acute radiation injury of the skin tissue was applied. The results of measuring the thickness of the epidermis layer, dermis, and total skin tissue with a 40-MHz ultrasound imaging method and 75 MHz after 14 days were obtained. The ultrasound images of the thickness of the epidermal layers, the dermis of the skin tissue in the control, 60-Gy radiation, and treatment groups are presented in Figure 2. An ultrasound image was recorded, and then, the depth of the layers was measured in each ultrasound image. The thickness of the layers was presented as mean and standard deviation.

Figure 2 depicts a significant difference between the control, 60-Gy radiation, and AdMSCs treatment groups in 40 MHz ultrasound images. The normal skin tissue of the animal models had a thickness of 0.89±0.04 mm with a dermis layer of 0.72±0.03 mm and an epidermis layer with a thickness of 0.13±0.01 mm. The maximum skin tissue thickness in the 60 Gy radiation group was 1.57±0.02 mm with the dermis layer of 1.14±0.03 mm and the epidermis of 0.28±0.03 mm ($P<0.05$). The thickness of the skin tissue in AdMSCs was 1.22±0.04 mm, the dermis layer was 1.08±0.03 mm, and the epidermal layer was 0.20±0.02 mm. The AdMSCs group after 10 days post-radiation showed a less altered thickness than the control group (Figure 3).

Ultrasound imaging well depicted the effect of real-time diagnosis on the prevention of acute radiation complications in stem cell therapy. The results of the 40-MHz and 75-MHz ultrasonic devices also confirmed the findings (Figure 4).

The correlation between skin thickness of 40-MHz and 75-MHz ultrasound imaging was checked by Pearson analysis (Figure 4). There was a significant correlation between 40-MHz and 75-MHz ultrasonography ($P<0.05$).

The result of the 40-MHz ultrasound system was obtained through image analysis with ImageJ software, but the results of the 75-MHz ultrasound system were extracted automatically. Finally, the results of both systems were obtained with a correlation coefficient of $R^2 = 0.9493$.

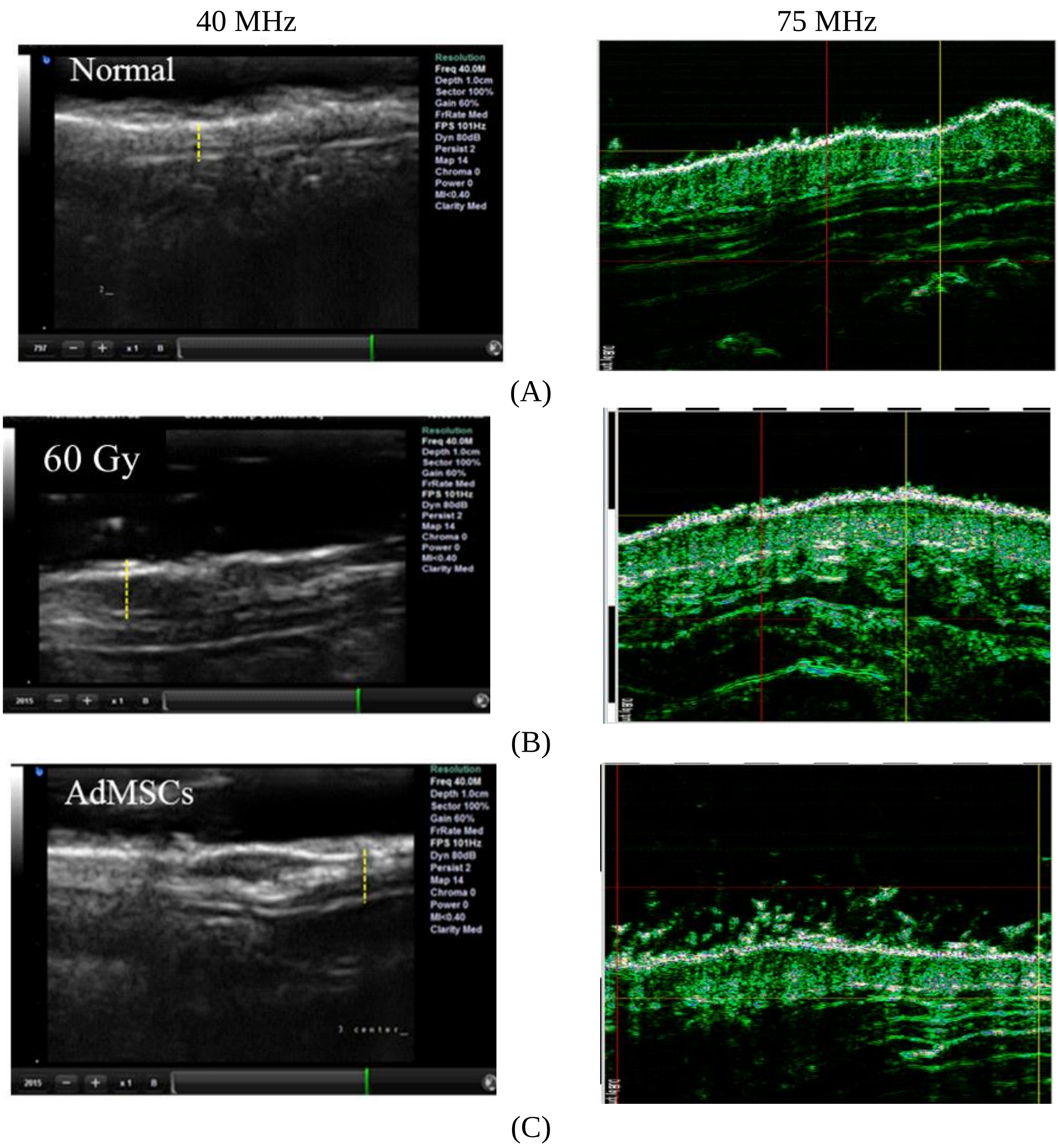

Figure 3. High-frequency ultrasound image of 40 (B-Mode) and 75 MHz of skin tissue group: A) control, B) 60 Gy, and c) AdMSCs

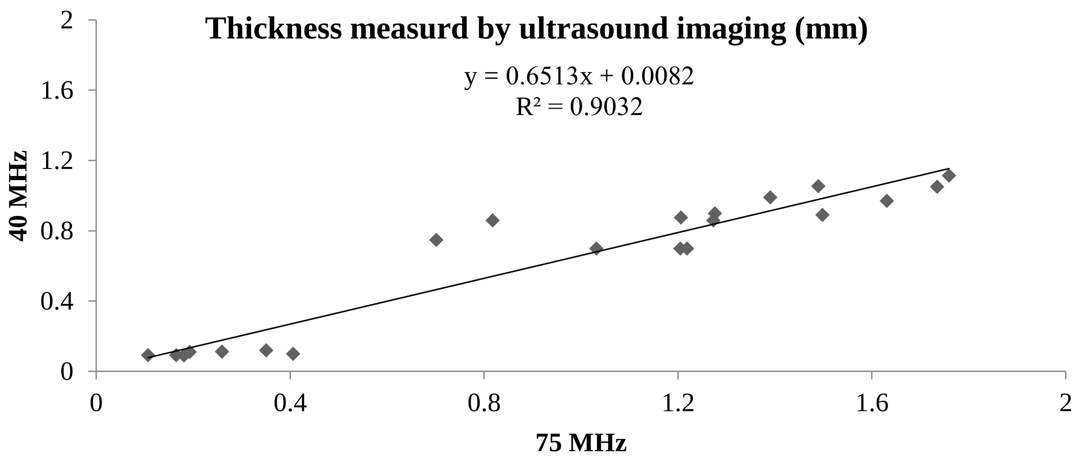

Figure 4. The regression curve between thicknesses evaluated by 40 MHz and 75 MHz frequencies ultrasound imaging systems

4. Discussion

This study investigated and developed a high-frequency ultrasound diagnostic method as a powerful approach to evaluate anatomical wound healing of an acute radiation damage model in response to stem cell therapy. Ultrasound imaging with 75- and 40-MHz can monitor and assess wound healing in all three dimensions allowing scaling depth. High-resolution ultrasound measures the changes in anatomical structures as discriminated by their acoustic properties as a non-invasive and non-ionizing imaging evaluation. The evaluation and treatment of acute radiation damage are prioritized to prevent damage to deeper organs, as well as the systemic damage and sudden death of the model due to radiation. In studies such as that by Rodgers *et al.* (2016), the radiation damage model of guinea pig skin tissue was investigated due to the similarity of its structure and physiology to human skin tissue. In this study, single doses of 25 to 79 Gy were irradiated on the skin tissue in a field of 4 x 4 cm using an XRAD320ix orthovoltage X-ray machine [13]. The results showed that the alterations in acute radiation damage of the skin tissue in the dermis, epidermis, and hair follicles of guinea pigs are similar to the human tissue. In this model, radiation damage healing was observed at radiation doses up to 32 Gy after 17 days of radiation. However, at high doses, radiation damage was progressive, and no repair was observed. Our results were consistent with the findings of this study at 20- and 30-Gy doses (Table 1). An orthovoltage device was used in the cited study, which nowadays has no therapeutic priority.

To achieve high degrees of acute radiation damage to the skin tissue, in addition to increasing the radiation dose, the location of radiation should be changed [14]. According to the evidence presented in this study, the areas near the eyes, abdomen, and legs can properly show radiation effects. Therefore, in this study, radiation doses were irradiated on the abdominal tissue. Systemic damage, side effects, and death were observed in none of the guinea pigs [15, 16].

Non-invasive evaluation of the anatomical structure (damage depth) of skin tissue changes after irradiation at doses of 20, 30, 50, 60, and 80 Gy using 40-MHz ultrasound imaging is presented in Table 1. The measurement of skin tissue layers in the acute radiation damage model shows a significant difference in the thickness of the layers between different radiation doses on the 10th day after radiation. Based on the results, there is a significant difference between the irradiated and control groups with 60- and 80-Gy doses. The average thickness of the epidermis layer increased to 0.33 mm, the dermis to 1.60 mm, and the total thickness of the skin tissue to 1.90 mm after 60-Gy ionizing radiation in 40-MHz ultrasound imaging (Table 1). Khalin *et al.* (2013) also proposed the guinea pig model as an available, effective, and more efficient model than other in vivo animal models [17]. This study observed all degrees of radiation damage to the skin tissue with a dose of 60 Gy. Therefore, this radiation dose was chosen as the dose used in the radiation damage model to provide the treatment protocol. In this study, the selected dose of 60 Gy was further evaluated based on ultrasound imaging and stem cell therapy.

Meirelles *et al.* (2013) conducted a study to investigate the model of radiation damage in a rabbit model as a medium-sized animal model [18]. The skin of the back area was stretched to separate it from the internal organs and fixed between two wooden clamps. Two rabbits died due to visceral necrosis and skin tissue necrosis. The Cobalt ALCYONII teletherapy device was used with doses of 20, 30, and 45 Gy. An increase in the thickness of the layers, loss of elasticity, increased collagen deposition, and a decrease in glands and blood vessels were observed in the skin tissue of the rabbits [18]. It seems that ionizing radiation increased the thickness due to inflammation in the dermis layer and collagen deposition.

Based on recent studies, to prevent the occurrence of acute radiation damage, the mesenchymal stem cell treatment protocol was applied. The results showed a significant difference between the treatment groups and the radiation group compared to the control group ($P<0.05$). Ling *et al.* (2017) reported that the degree of inflammation and apoptotic death was lower in the bladder tissue treated with stem cells, and the bladder function improved after radiotherapy [19]. The results of this study are similar to the results of stem cell transplantation on the healing of skin tissue radiation damage. The injection of a mesenchymal stem cell culture medium derived from the adipose tissue in Sun *et al.'s* (2019) study showed that the mesenchymal stem cell culture medium contains factors causing cell proliferation on the radiation damage of the rat skin tissue. It has been reported that the size of the wound in the treatment group is completely closed after 8 weeks [20]. Francois *et al.* (2007) examined the effect of mesenchymal stem cells derived from bone marrow on skin radiation damage. A dose of 30 Gy from a cobalt source was applied to the legs of mice. In the treated mice, the development of radiation side effects was delayed, and the development of the complication to more than scaling was observed in none of them [21]. The results of the present study on the guinea pig model of radiation damage also showed that the degree of acute radiation damage decreased in the treatment groups with mesenchymal stem cells derived from the adipose tissue.

Based on 75-MHz ultrasound imaging, the nearest size of skin tissue thickness to healthy skin tissue belonging to the AdMSCs group is 0.97±0.07 mm, the dermis layer is 0.90±0.07 mm, and the epidermis layer is 0.11±0.03 mm. The skin tissue of a healthy guinea pig has a thickness of 0.86±0.08 mm with a dermis layer of 0.75±0.09 mm and an epidermis layer of 0.09±0.01 mm. The maximum thickness of the skin was 1.12±0.03 mm, with the dermis layer of 0.99±0.09 mm and the epidermis of 0.12±0.02 mm at 60-Gy radiation. ($P<0.05$) (Figures 30-3). Similar to Gnyawali's (2015) study that introduced ultrasound for the management of burn injury as non-invasive imaging on the pig model, B-Mode results indicated that wound depth increased until the 21st day. Doppler and ultrasound elastography measures blood flow and skin tissue stiffness during injury. Evaluation of skin thickness, wound depth, scar formation, blood flow pulse, and elasticity is critical for the proper assessment and management of wound healing. The ultrasound imaging system has been evaluated as non-invasively monitoring the process of wound healing, including measurements of tissue elasticity and microcirculation against invasive histological and biomechanical data [22].

The results of imaging with 75-MHz ultrasound waves (specific application of skin tissue) have a good correlation (R^2 = 0.9493) with the results of the 40-MHz image (general application). The results of the 40-MHz system have been determined through the operator and the ImageJ software to determine the area of the epidermis and dermis and the entire skin tissue. However, in the 75-MHz system, the measurements are obtained automatically. In a similar study, skin cancer was evaluated by high-frequency ultrasound. Results indicated that high-frequency ultrasound could evaluate benign and malignant skin lesions (AUC = 0.959). Moreover, sensitivity and specificity were 93.8 and 97.3%, respectively. Therefore, this method can detect skin malignancy with an accurate, non-invasive, and real-time method [23]. Bezugly investigated normal skin and various skin pathologies. High frequency ultrasound is useful in distinguishing between inflammation, hypertrophy, scars, keloids, and skin atrophy. Ultrasound imaging has been used to diagnose and manage skin tumors. It can also provide essential information about the size and margin of the tumor and the depth of invasion for skin treatment and post-treatment skin monitoring [24]. In this study, after the treatment period, the results of ultrasound imaging showed a significant reduction in the severity of acute radiation damage to the skin tissue in stem cell transplantation. The therapeutic effect of these cells on acute radiation damage of skin tissue in humans was discussed by Portas *et al.* (2016) and Lataillade *et al.* (2007) [9, 25]. The results demonstrate that local grafting can play a key role in the early stages of radiation injury healing. A non-invasive evaluation of ultrasound imaging has an opportunity to assist with the damage, record the details, and follow up on the treatment conditions.

Specific imaging of the skin tissue can provide more information with a high resolution. It is hoped that through proper training and increasing the use of skin tissue ultrasound imaging through artificial intelligence and deep learning, much information about skin tissue can be provided to therapists.

5. Conclusion

Currently, these measurements require repeated biopsies that necessitate the removal of a portion of the wound to assess biomechanics, morphology, and biochemical properties. High-resolution ultrasound with 40- and 75-MHz frequencies could be widely applied in research involving the skin, particularly in the context of wound healing.

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References

1- X. Wortsman *et al.*, "Guidelines for Performing Dermatologic Ultrasound Examinations by the DERMUS Group." (in eng), *J Ultrasound Med*, Vol. 35 (No. 3), pp. 577-80, Mar (2016).

2- Fulvia Elia, Giovanni Paolino, M Donati, and FM %J Journal of ultrasound Solivetti, "Ultrasound pattern of a rare skin disease: multiple miliaryosteoma cutis." Vol. 19 (No. 2), pp. 145-47, (2016).

3- Ximena Wortsman and Gregor Jemec, Dermatologic ultrasound with clinical and histologic correlations. *Springer Science & Business Media*, (2013).

4- Orlando Catalano and Ximena Wortsman, "Dermatology Ultrasound. Imaging Technique, Tips and Tricks, High-Resolution Anatomy." Vol. 36 (No. 4), pp. 321-27, (2020).

5- R. K. Mlosek and S. Malinowska, "Ultrasound image of the skin, apparatus and imaging basics." (in eng), *J Ultrason,* Vol. 13 (No. 53), pp. 212-21, Jun (2013).

6- Sarah T. Arron, "Anatomy of the Skin and Pathophysiology of Radiation Dermatitis." (2016).

7- E. F. Bernstein *et al.*, "Collagen gene expression and wound strength in normal and radiation-impaired wounds. A model of radiation-impaired wound healing." (in eng), *J Dermatol Surg Oncol,* Vol. 19 (No. 6), pp. 564-70, Jun (1993).

8- Frank Haubner, Elisabeth Ohmann, Fabian Pohl, Jürgen Strutz, and Holger G. Gassner, "Wound healing after radiation therapy: Review of the literature." *Radiation Oncology,* Vol. 7 (No. 1), p. 162, 2012/09/24 (2012).

9- M. Portas *et al.*, "Use of Human Cadaveric Mesenchymal Stem Cells for Cell Therapy of a Chronic Radiation-Induced Skin Lesion: A Case Report." (in eng), *Radiat Prot Dosimetry,* Vol. 171 (No. 1), pp. 99-106, Sep (2016).

10- S. Akita, K. Akino, A. Hirano, A. Ohtsuru, and S. Yamashita, "Mesenchymal stem cell therapy for cutaneous radiation syndrome." (in eng), *Health Phys,* Vol. 98 (No. 6), pp. 858-62, Jun (2010).

11- Esperanza Naredo *et al.*, "Performance of ultra-high-frequency ultrasound in the evaluation of skin involvement in systemic sclerosis: a preliminary report." *Rheumatology,* Vol. 59 (No. 7), pp. 1671-78, (2019).

12- Zeinab Hormozi Moghaddam, Manijhe Mokhtari-Dizaji, Mohammad Ali Nilforoshzadeh, Mohsen Bakhshandeh, and Sahar Ghaffari Khaligh, "Low-intensity ultrasound combined with allogenic adipose-derived mesenchymal stem cells (AdMSCs) in radiation-induced skin injury treatment." *Scientific Reports,* Vol. 10 (No. 1), p. 20006, 2020/11/17 (2020).

13- K. E. Rodgers *et al.*, "Development of a guinea pig cutaneous radiation injury model using low penetrating X-rays." (in eng), *Int J Radiat Biol,* Vol. 92 (No. 8), pp. 434-43, Aug (2016).

14- E. B. Collen and M. N. Mayer, "Acute effects of radiation treatment: skin reactions." (in eng), *Can Vet J,* Vol. 47 (No. 9), pp. 931-2, 34-5, Sep (2006).

15- S. Kumar, A. Kolozsvary, R. Kohl, M. Lu, S. Brown, and J. H. Kim, "Radiation-induced skin injury in the animal model of scleroderma: implications for post-radiotherapy fibrosis." (in eng), *Radiat Oncol,* Vol. 3p. 40, Nov 24 (2008).

16- L. H. Rifkin *et al.*, "An athymic rat model of cutaneous radiation injury designed to study human tissue-based wound therapy." (in eng), *Radiat Oncol,* Vol. 7p. 68, May 8 (2012).

17- I. Khalin and G. Kocherga, "Arginine glutamate improves healing of radiation-induced skin ulcers in guinea pigs." (in eng), *Int J Radiat Biol,* Vol. 89 (No. 12), pp. 1108-15, Dec (2013).

18- R. P. Meirelles, B. Hochman, A. Helene Junior, R. Lellis, M. F. Fraga, and L. M. Ferreira, "Experimental model of cutaneous radiation injury in rabbits." (in eng), *Acta Cir Bras,* Vol. 28 (No. 11), pp. 751-5, Nov (2013).

19- L. Ling *et al.*, "Effects of low-intensity pulsed ultrasound (LIPUS)-pretreated human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation on primary ovarian insufficiency in rats." (in eng), *Stem Cell Res Ther,* Vol. 8 (No. 1), p. 283, Dec 19 (2017).

20- J. Sun, Y. Zhang, X. Song, J. Zhu, and Q. Zhu, "The Healing Effects of Conditioned Medium Derived from Mesenchymal Stem Cells on Radiation-Induced Skin Wounds in Rats." (in eng), *Cell Transplant,* Vol. 28 (No. 1), pp. 105-15, Jan (2019).

21- S. François *et al.*, "Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in a xenogenic transplant model." (in eng), *Ann Hematol,* Vol. 86 (No. 1), pp. 1-8, Jan (2007).

22- S. C. Gnyawali *et al.*, "High-resolution harmonics ultrasound imaging for non-invasive characterization of wound healing in a pre-clinical swine model." (in eng), *PLoS One,* Vol. 10 (No. 3), p. e0122327, (2015).

23- Shabnam Kia, Saeed Setayeshi, Majid Pouladian, and Seyed Hossein Ardehali, "Early diagnosis of skin cancer by ultrasound frequency analysis." Vol. 20 (No. 11), pp. 153-68, (2019).

24- A. Bezugly and A. Rembielak, "The use of high frequency skin ultrasound in non-melanoma skin cancer." (in eng), *J Contemp Brachytherapy,* Vol. 13 (No. 4), pp. 483-91, Aug (2021).

25- J. J. Lataillade *et al.*, "New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy." (in eng), *Regen Med,* Vol. 2 (No. 5), pp. 785-94, Sep (2007).