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# Far infrared ray (FIR) therapy: An effective and oncological safe treatment modality for breast cancer related lymphedema

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ABSTRACT

*Background:* The incidence of breast cancer related lymphedema is approximately 5%. Far infrared ray (FIR) treatment can potentially reduce fluid volume and extremity circumference as well as the frequency of dermatolymphangitis (DLA). However, there is no published data on the oncological safety of FIR and the potential for activation of any residual breast cancer cells. The aim of this study is to investigate the safety of far infrared ray (FIR) treatment of postmastectomy lymphedema, clinically and *in vitro*.

*Methods:* Patients who underwent mastectomy more than 5 years ago complicated by upper extremity lymphedema for more than 1 year were included. The enrolled patients were divided into an FIR treatment group and a control group (conservative treatment using bandage compression). Outcome measures included tumor markers (CA153, CA125), ultrasonography of relevant structures and monitoring for adverse reactions 1 year after treatment. For the *in vitro* part of the study, the effects of FIR on human breast adenocarcinoma cell lines (MCF7, MDA-MB231) compared to the effects of FIR on human dermal fibroblasts as a control were considered. The viability, proliferation, cell cycle and apoptotic statistics of the adenocarcinoma and human dermal fibroblast cell lines were analyzed and compared.

*Results:* Results demonstrated that after treatment with FIR, tumor marker (CA153, CA125) concentrations in both the FIR and control groups were not elevated. There was no statistically significant difference between FIR and control group marker expression ($p > 0.05$). Furthermore, no patients were diagnosed with lymphadenectasis or newly enlarged lymph nodes in these two groups. Importantly, there were no adverse events in either group. The *in vitro* experiment indicated that FIR radiation does not affect viability, proliferation, cell cycle and apoptosis of fibroblasts, MCF-7 and MDA-MB-231 cells.

*Conclusions:* FIR should be considered as feasible and safe for the treatment of breast cancer related lymphedema patients 5 years after mastectomy. FIR does not promote recurrence or metastasis of breast cancer and is a well-tolerated therapy with no adverse reactions.

## 1. Introduction

Breast cancer-related lymphedema (BCRL) of the upper limb is a well-recognized complication associated with breast cancer surgery, particularly when axillary lymph node clearance and irradiation are included as part of the treatment [1]. The frequency of postmastectomy lymphedema is described to be as high as 9–41% following axillary dissection and 4–10% following sentinel lymph node biopsy for breast

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cancer [2,3]. The affected patients have increased interstitial fluid (lymph) and develop chronic inflammation resulting in fibro-adipose tissue proliferation and deposition as well as nonpitting edema [4,5].

The clinical symptoms of postmastectomy lymphedema include: oedema of the upper extremity, poor skin elasticity and limited joint movements as well as dermato-lymphangitis (DLA) [6,7]. Excess tissue and fluid in the upper limb causes pain and anxiety, affects the patient's activities of daily living and social comfort, decreases physical function due to limb bulkiness and weight, leads to body image disturbances, and has a significant, measurable effect on quality of life [8].

Currently, conventional, non-surgical treatment for BCRL includes exercise and elevation, manual lymphatic drainage, hyperthermia, intermittent air pressure wave therapy, elastic bandage, elastic pants, elastic sleeves, low-level laser therapy, self-nursing, pharmacotherapy and static compression garments.

These methods aim to alleviate symptoms and may have beneficial effects in all clinical stages but it is nearly impossible to achieve significantly improved clinical outcomes utilizing these conservative measures alone. Conservative treatment protocols seem to be most effective in early stage lymphedema (International Society of Lymphology stages I and II). These treatments represent an important supplementary tool for patients who are not able or do not want to undergo surgical procedures [9–13], and may improve lymphedema by promoting collateral lymph flow and removing excess fat tissue thereby slowing down or even preventing the aggravation of pre-existing lymphedema.

Far Infrared Radiation (FIR) treatment affects tissues in a similar way as hyperthermia, acting *via* three main biological effects: radiation, vibration (or resonance), and a thermal effect. The infrared region of the spectrum of radiation lies beyond the red end of the visible range, with wavelengths between 0.01 and $7.5 \times 10^{-5}$ cm. Infrared rays are further divided in: near infrared, medium infrared, and far infrared. Among the different frequencies composing the infrared spectrum, far infrared rays are the most beneficial for living organisms [14,15]. As the majority of human body mass is composed by 55%–60% of water, FIR is able to interact with water molecules and cause a thermal reaction which increases tissue temperature and dilates blood vessels. Far infrared rays are able to penetrate tissue layers up to 4 cm in depth and resonate with water and other organic molecules [16]. In this way, blood circulation is improved and a greater amount of oxygenized blood can reach the soft tissues, reacting with nutrients and removing the accumulated toxins.

These effects lead to clinical improvement of particular pathological modalities such as lymphedema, promoting microcirculation and collateral lymph flow [17]. FIR therapy has been applied to various clinical fields, including vessel-related disorders, [18] and more recently to thousands of patients affected by lymphedema [12,13,17]. It has been shown that FIR effectively reduces extremity fluid volume and circumference as well as the frequency of DLA [18]. However, the oncological safety of FIR has not been investigated yet, as there is a theoretical potential for activation of any residual breast cancer cells in the lymphedematous tissues. Breast cancer recurrence rates during FIR treatment of upper extremity lymphedema has also not been specifically looked at. The purpose of this study is to demonstrate the effectiveness and oncological safety of FIR to treat BCRL clinically and *in vitro*.

## 2. Patients and Methods

### 2.1. Clinical Study

63 female patients suffering from BCRL following modified radical mastectomy, axillary lymphadenectomy, and chemo-radiotherapy were randomised into two groups and treated between April 2014 and April 2016 using FIR ($n = 32$) and compression bandages ($n = 31$).

Only patients 5 years post-mastectomy and presenting with upper extremity lymphedema of more than 1 year were included in the study.

Patients with clinical evidence of potential cancer recurrence or those who suffered from serious comorbidities that could interfere with routine treatment and follow-up were excluded from the study. Furthermore, patients with episodes of DLA or vascular embolization and patients whose affected limbs were too large to be placed in the curing cabin were also excluded from the study.

#### 2.1.1. FIR Therapy Device

The FIR therapy device was developed by the Ninth People's Hospital, affiliated with Shanghai Jiaotong University, China [19]. The infrared radiation producing elements are enclosed in a stainless steel chamber that is resistant to high temperatures. Inside the chamber, installed on an internal ring there are eight quartz lamp lights that emit infrared rays with a wavelength between 6.0 and 14.0 μm [14]. The device is also equipped with a temperature control apparatus that can adjust the temperature inside the chamber.

#### 2.1.2. FIR Group Treatment Measures

Following written consent, FIR therapy was initiated. Treatment duration was 1 h every day for 4 weeks (20 days in total) at a temperature of 42 °C. Patients were advised to implement self-nursing skills such as extremity hygiene, prevention of skin injuries and the avoidance of dermatophytosis [19].

#### 2.1.3. Control Group: Bandage Treatment Measures

Following written consent, compression bandage treatment was commenced. The patients wore bandages for 12 h each day, while awake. They were treated for 4 weeks (20 days in total). Patients were advised to implement self-nursing skills such as extremity hygiene, prevention of skin injuries and the avoidance of dermatophytosis.

#### 2.1.4. Ethics Statement

Informed consent was obtained in writing before treatment. The ethics committee, Ninth People's Hospital, Shanghai, prospectively approved this research study. All relevant regulations, as well as the guidelines of the Declaration of Helsinki were followed accordingly.

#### 2.1.5. Data Collection

Patients were followed up for 1 year after treatment. Follow-up outcome measures included: A) Tumor marker detection: Venous blood was extracted and tumor markers relevant to breast cancer were assayed in the laboratory. Results within normal range were recorded as (−). Results that were higher than normal range were scored as (+). Tested tumor markers were CA153 and CA125. Normal range for CA125 is 0-35 U/ml. Normal range for CA153 is 0-25 U/ml. B) Ultrasonography: The following organs and lymphatic basins were imaged: liver, spleen, kidney, breast, axillary lymph nodes, supra-clavicular and infra-clavicular lymph nodes. If lymphadenectasis was detected or newly enlarged lymph nodes were found in the liver, spleen, kidney, or breast, the case was recorded as (+). If not, it was registered as (−). C) Adverse reactions: If any adverse reaction, such as burns, local infection, pyrexia, discomfort, and pain, induced by FIR or compression bandage occurred, the case was recorded as (+). If no adverse reaction occurred, the case was recorded as (−).

### 2.2. The In Vitro Study

#### 2.2.1. Cell Cultures

Dermal diploid fibroblasts were derived from 2-mm punch biopsies taken from the upper arms of healthy male donors. Human breast adenocarcinoma cell lines MCF-7 and MDA-MB231 were purchased from American Type Culture Collection (ATCC, USA). Cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM glutamine and 10% fetal bovine serum (FBS). Cells were

maintained at 37 °C in an atmosphere containing 5% CO2.

#### 2.2.2. FIR Treatment

FIR treatment was performed using a device releasing FIR with variable wavelengths from 6.0 μm to 14.0 μm. The irradiated groups of cells (fibroblasts, MCF-7 and MDA-MB231) were incubated in 9 cm dishes placed in a square box. The distance between the cells and the source of FIR was 30 cm. Real time temperature detectors monitored the temperature in the incubation chamber, which was maintained at (37 ± 0.5 °C). The irradiation time was 1 h, identical to the radiation protocol in the clinical portion of this study. The unirradiated groups of cells (fibroblasts, MCF-7 and MDA-MB231) were maintained in the same conditions but not exposed to FIR.

#### 2.2.3. Cell Viability and Proliferation

Cells were incubated in 96-well plates with a density of 4000 cells per well. The irradiated groups were treated by FIR for 1 h daily for 7 days (37 ± 0.5 °C), while the unirradiated groups were placed in the same areas but not subjected to irradiation. Cell viability and proliferation capability were evaluated using CCK8 kits immediately after each daily FIR treatment. During the tests, cells were incubated in 10% CCK-8 (Dojindo, Japan) that was diluted in normal culture medium at 37 °C for 3 h until visual color conversion occurred.

#### 2.2.4. FACS Analysis of Cell Cycle

The effect of FIR treatment on cell cycle was analyzed on fibroblasts, MCF-7 and MDA-MB-231 cells. The irradiated groups were treated with FIR for 1 h at 37 ± 0.5 °C. After FIR treatment, the irradiated and non-irradiated groups of cells were collected and fixed in 70% ethanol, washed two times with ice-cold PBS and re-suspended in 500 μl of PBS. Cell suspensions were incubated with RNase A (50 μg/ml) for 30 min at 37 °C, sequentially stained with PI (50 μg/ml) for 1 h and analyzed *via* flow cytometry. Three independent experiments were performed.

#### 2.2.5. Evaluation of Apoptosis

One hour after FIR treatments, apoptotic cells in irradiated groups and non-irradiated groups were scored by flow cytometry. The Annexin V-FITC/PI Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA) was used following the manufacturer's instructions. Cells demonstrating Annexin V + /PI − staining were considered early apoptotic cells, and those demonstrating Annexin V + /PI + staining were considered late apoptotic cells. After the staining, cells were immediately analyzed using a BD FACS Calibur flow cytometer and the CELL Quest software. The experiment was performed in triplicate.

### 2.3. Statistical Analysis

All outcome measures (tumor markers, ultrasonography test and adverse reactions) were statistically analyzed using the chi-square test. For the *in vitro* study, data were presented as means ± S.D. Three independent experiments were performed in triplicate. Unpaired Student's *t*-tests were performed to evaluate the differences between FIR irradiated groups and non-irradiated groups for all cell types. All data were analyzed using version 19.0 of the Statistical Package for Social Sciences (SPSS Inc., Chicago, USA), with a $p$ value $< 0.05$ being considered as statistically significant.

## 3. Results

### 3.1. Clinical Study

A total of 63 female patients with BCRL were treated at our institution. Demographics for each group are summarized in Table 1. The inclusion and exclusion process is depicted in Fig. 1. 3 patients refused to take part in the trial and dropped out before random allocation.

**Table 1**
Population demographics.

| Age | FIR Group | Control Group |
|---|---|---|
| Mean ± SD | 61.3 ± 9.2 | 59.7 ± 9.9 |
| Range | 34–82 | 36–78 |
| Years after mastectomy | | |
| Mean ± SD | 9.6 ± 2.7 | 9.2 ± 2.2 |
| Range | 5–14 | 5–12 |
| Years with lymphedema | | |
| Mean ± SD | 8.2 ± 3.3 | 7.9 ± 3.0 |
| Range | 1–13 | 1–12 |
| Side | | |
| Left | 22(68.8%) | 17(54.8%) |
| Right | 10(31.2%) | 14(45.2%) |
| Classification of lymphedema | | |
| I | 9% | 10% |
| II | 63% | 58% |
| III | 28% | 32% |

FIR, far infrared ray.

### 3.2. Tumor Marker Detection

All 63 patients were randomly allocated to two groups and were treated by FIR treatment or compression bandage treatment. After one year, there was no patient detected with abnormal values of CA125 or CA153 in both FIR and control group. The changes in CA125 and CA153 values in the FIR group were similar to those in the control group ($p > 0.05$) (Table 2).

### 3.3. Ultrasound Test

One year after FIR or compression bandage treatment, patients underwent ultrasonography of their liver, spleen, kidneys, breast, axillary lymph node, supraclavicular lymph node, and infraclavicular lymph node basins. No patient in either the FIR or control group was diagnosed with lymphadenectasis or newly enlarged nodes. The changes in these ultrasound outcomes in the FIR group were similar to those in the control group ($p > 0.05$) (Table 2).

### 3.4. Adverse Reactions

Patients were followed up for possible adverse reactions caused by FIR and compression bandage treatments such as burns, local infections, pyrexia, discomfort, and pain. There were no adverse events reported. There was no significant difference between adverse reaction outcomes in the FIR and control groups ($p > 0.05$) (Table 2).

### 3.5. In Vitro Study

#### 3.5.1. Cell Viability and Proliferation

We evaluated the effect of FIR treatment on cell viability and proliferation rates of human primary fibroblasts and two different human breast adenocarcinoma cell lines, MDA-MB231 and MCF-7. FIR treatment was performed for 1 h, once daily for 7 days. Cell viability and proliferation capability were evaluated using the CCK8 kit immediately after FIR treatments. The CCK8 result was analyzed by cell growth curves and did not demonstrate a significant difference in cell viability and proliferation between irradiated and non-irradiated groups in all cell types (Fig. 2).

#### 3.5.2. Cell Cycle Analysis

To investigate the influence of FIR treatment on the cell cycle, flow cytometry analysis of cellular DNA content was performed. The proportion of cell population in different phases of the cell cycle was

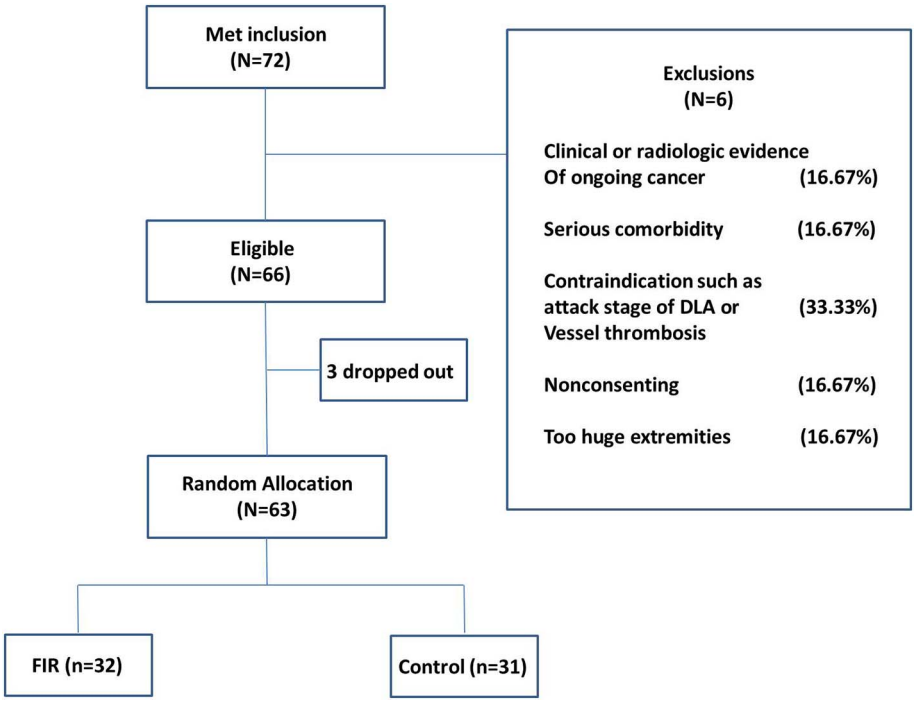


**Fig. 1.** CONSORT diagram. 72 patients met the inclusion criteria. Of those, 6 were excluded and 3 dropped out. The remaining 63 patients were divided into far infrared ray (FIR) and bandage treatment groups.

**Table 2**
Clinical study results.

| Items | FIR (-) (*n* cases) | Control (-) (*n* cases) | FIR *vs* Control (*p*) |
|---|---|---|---|
| CA153 | 32 | 31 | > 0.05 |
| CA125 | 32 | 31 | > 0.05 |
| Liver ultrasound | 32 | 31 | > 0.05 |
| Splenic ultrasound | 32 | 31 | > 0.05 |
| Renal ultrasound | 32 | 31 | > 0.05 |
| Breast ultrasound | 32 | 31 | > 0.05 |
| Axillary lymphatic basin ultrasound | 32 | 31 | > 0.05 |
| Supraclavicular lymphatic basin ultrasound | 32 | 31 | > 0.05 |
| Subclavian lymphatic basin ultrasound | 32 | 31 | > 0.05 |
| Scald | 32 | 31 | > 0.05 |
| Local infection | 32 | 31 | > 0.05 |
| Pyrexia | 32 | 31 | > 0.05 |
| Discomfort | 32 | 31 | > 0.05 |
| Pain | 32 | 31 | > 0.05 |

(-), normal. FIR, far infrared ray.

determined immediately after the FIR treatment. Results (Table 3) did not show significant differences between irradiated and non-irradiated groups of all cell types analyzed, indicating that exposure to FIR treatment did not affect cell cycle progression.

### *3.5.3. Evaluation of Apoptosis*

The influence of FIR treatment on the apoptotic threshold was evaluated by flow cytometry using the Annexin V-FITC/PI apoptosis detection kit. As shown in Fig. 3, FIR treatment did not influence the apoptotic status of normal fibroblasts and breast cancer MCF-7 and MDA-MB231 cell lines.

## 4. Discussion

Infrared radiation is an invisible portion of the electromagnetic spectrum with wavelengths ranging from 750 nm to 100 μm, frequencies ranging from 400THz to 3THz, and a photon energy range of 12.4 meV– 1.7 eV. The infrared spectrum is located between the long wavelength red edge of visible spectrum and the short edge of terahertz (starting at 3 THz) spectral bands. FIR has three main biological effects: radiation, vibration (or resonance), and thermal effect [18]. In cells, radiation and vibration promote the oscillation of free ions, resulting in the denaturation of macromolecules such as proteins leading to an increase in the absorption of proteins in tissue frameworks [14]. Thermal effects may promote microcirculation by expanding blood or lymphatic vessels [11,12]. Liu et al. found that local hyperthermia activates Langerhans cells, macrophages and endothelial cells, enhances immune function, and promotes microcirculation reflow. Activated macrophages may hydrolyze excessive proteins, reduce colloid osmotic pressure, and promote interstitial liquid backflow to the circulation system in lymphedema tissue [11,20–22]. Hu et al. also demonstrated that local hyperthermia improves microcirculation, reduces chronic inflammation, and promotes tissue repair [23–25].

Compression bandaging treatment has been applied in post-mastectomy lymphedema patients, and the resulting compression of the affected limb may lead to the formation of new tissue channels that helps in reducing the accumulation of lymph fluid [19]. Bandage treatment has been shown to be a safe conservative treatment for post mastectomy lymphedema and is widely utilized [26].

Our previous studies have demonstrated that the circumference and water content of lymphedema limbs may be reduced after FIR treatment and FIR decreases the frequency of DLA [17,19]. However, it remains unknown whether FIR treatment influences breast cancer recurrence rates or increases its metastatic risk. Metastasis is considered the most important predictor of a patient's prognosis, and the current focus of treatment is therefore directed toward the unequivocal determination of the presence of metastatic tumors in the adjacent epidermis, sentinel lymph nodes, circulation, and distant sites [27,28]. Serum Cancer antigen 125 and Cancer antigen 153 are considered specific biomarkers for breast cancer recurrence and metastasis [29]. Cancer antigen 153 (CA153) is used in the management of the prognoses, metastases and recurrences of breast cancer patients [30–32]. The preoperative levels of CEA and CA153 in the serum are well known to significantly influence the prognosis of breast cancer

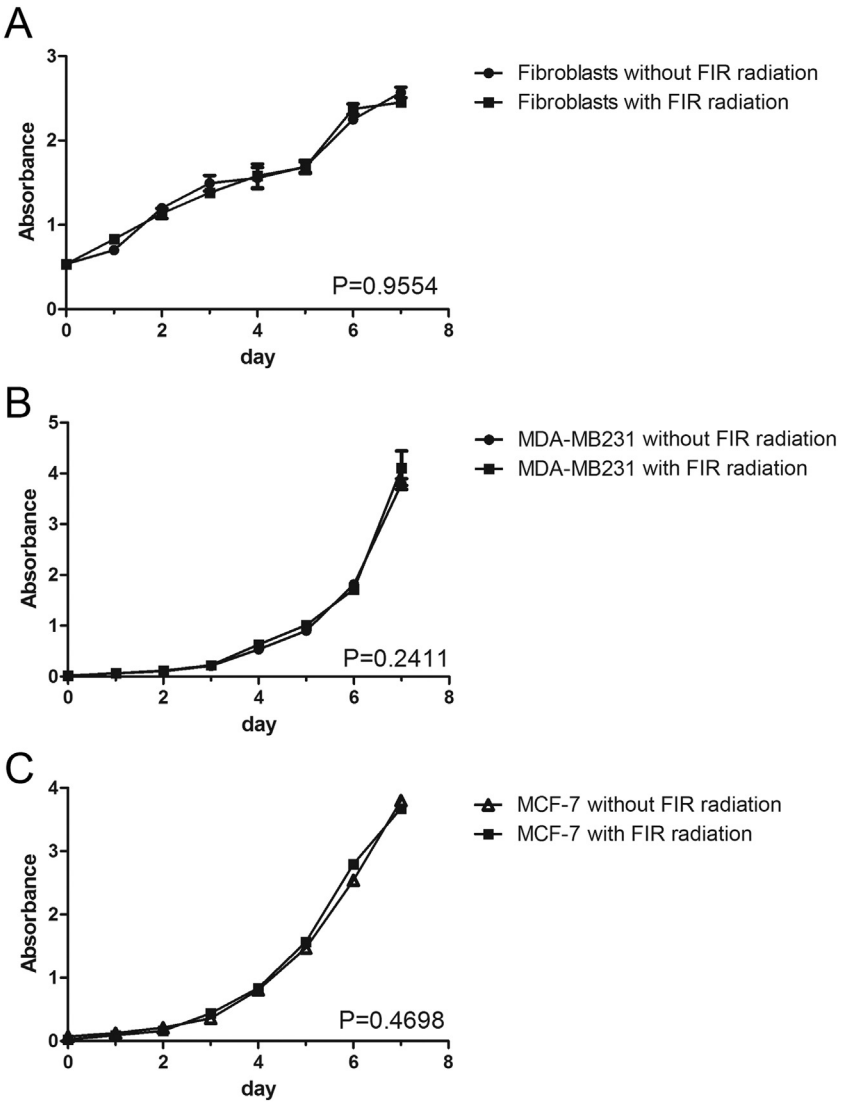


**Fig. 2.** Cell viability and proliferation ability were assessed in far infrared ray (FIR) radiated groups and unirradiated groups of fibroblasts, MBA-MD231 and MCF-7 cell lines. Cells were incubated in 96-well plates with 4000 cells per well.

**Table 3**
Cell cycle analysis. Analysis of cell cycle in FIR radiated and non-rradiated human fibroblasts, MDA-MB231 and MCF-7 cells. Data represents group means ± SD for three independent experiments.

| | | G1 (%) | P | S (%) | P | G2/M (%) | P |
|---|---|---|---|---|---|---|---|
| Fibroblasts | Unirradiated | 72.96 ± 5.49 | 0.959 | 7.97 ± 1.45 | 0.775 | 19.07 ± 4.45 | 0.762 |
| | Radiated | 74.57 ± 4.96 | | 8.63 ± 1.27 | 16.80 ± 3.80 | | |
| MDA-MB231 | Unirradiated | 31.08 ± 1.36 | 0.810 | 48.40 ± 6.91 | 0.867 | 20.52 ± 5.56 | 0.797 |
| | Radiated | 31.64 ± 1.68 | | 50.41 ± 8.87 | 17.96 ± 7.47 | | |
| MCF-7 | Unirradiated | 66.45 ± 2.73 | 0.893 | 24.80 ± 3.29 | 0.642 | 8.83 ± 0.66 | 0.051 |
| | Radiated | 66.99 ± 2.67 | | 26.89 ± 2.53 | 6.11 ± 0.71 | | |

[33]. Cancer antigen 125 (CA125) has been found to be up-regulated in breast cancer tissues and not expressed in non-neoplastic ducts [34]. In our study, 63 patients were evaluated for the detection of serum CA125 and CA153 after FIR or bandage treatment, and no patient in either treatment group demonstrated CA125 or CA153 positivity. We suggest that FIR treatment is a safe and effective method for treating BCRL that is unlikely to induce tumor recurrence or systematic metastases.

Ultrasonography is a simple, inexpensive, and highly accurate detection method widely used in the early detection of breast cancer or metastases [35,36]. In our study, after one year of follow-up, no patients treated by FIR or compression bandages were found with a new pathology on liver, spleen, kidney or breast, and no patients showed new evidence of lymphadenectasis. This seems to further confirm the safety and reliability of FIR as a treatment for BCRL.

During clinical follow-up, we monitored the adverse reactions that may occur after FIR therapy. No patients were diagnosed with burns, local infection, pyrexia, discomfort or pain in the treated limbs, indicating a good tolerance for FIR in BCRL treatment.

Although the majority of patients with a history of breast carcinoma have no signs of clinically detectable residual tumor after surgical removal, radiation and/or chemotherapy, individual residual tumor cells may exist [37,38]. These tumor cells may not be detected clinically

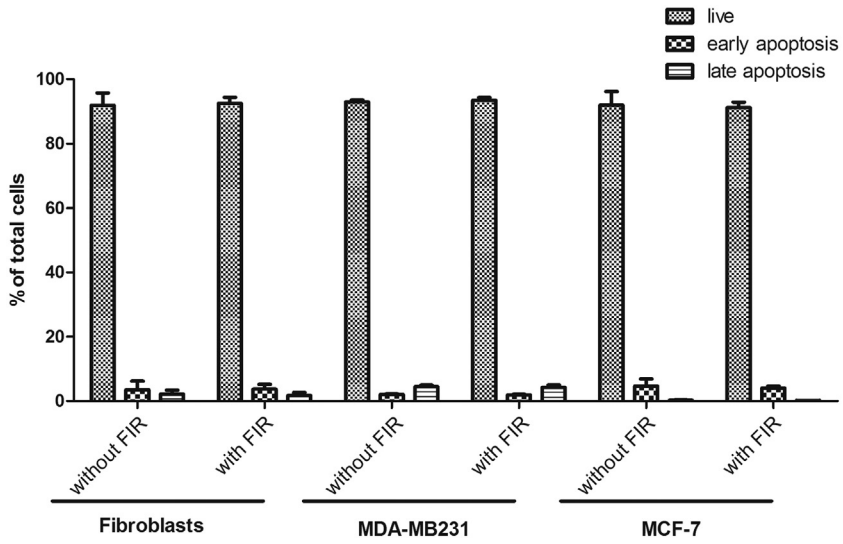


**Fig. 3.** Number of living, early and late apoptotic human fibroblasts, MCF-7 and MDA-MB231 cells in both far infrared ray (FIR) exposed and unexposed groups. Bar graphs show the compiled mean value ± SD of three independent experiments. The results did not show significant differences between radiated groups and unirradiated groups.

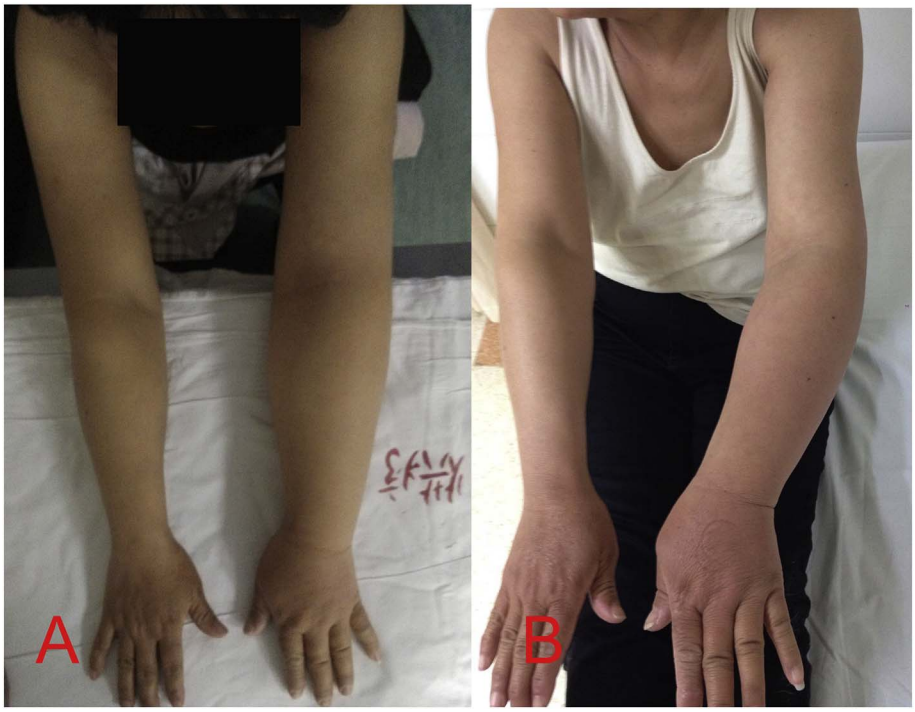


**Fig. 4.** A. Preoperative patient B. Postoperative patient.

as the cell numbers can be too small or the cells are in a latency period. One of the aims of this study was to evaluate whether FIR treatment may activate residual tumor cells in postmastectomy lymphedema patients.

In our research, FIR was applied to cells for 1 h daily over 7 days. The cells were maintained at a temperature of 37 ± 0.5 °C in an attempt to simulate the *in vivo* environment during FIR treatment. Cell proliferation and the viability of fibroblasts, MDA-MB231 and MCF-7 cell lines were examined after each radiation treatment. No significant differences were detected between the experimental group and control groups ($p > 0.05$) indicating that FIR is unlikely to promote proliferation of breast cancer cells and increase the risk of relapse.

Meanwhile, cell cycle tests were performed on the FIR treated cells for all cell lines. The results demonstrated no difference in cell proliferation ($p > 0.05$) strongly suggesting that FIR does not shorten the cell cycle of breast cancer cells, a further confirmation that breast cancer cells are not activated by FIR therapy.

Chen et al. [39] reported that FIR irradiation attenuates apoptosis and cell death of cultured keratinocytes. In our research, we found that the irradiated groups of fibroblasts,MDA-MB231 and MCF-7 had the same apoptosis status compared to the non-irradiated groups. FIR treatment does not seem to induce or delay apoptosis in breast cancer cells.

There are limitations of this study: all patients with postmastectomy lymphedema underwent mastectomy more than 5 years ago, and any residual breast cancer cells in these patients may have been relatively stable and difficult to activate. It remains to be determined whether FIR treatment is safe in patients following mastectomies performed less than 5 years prior to treatment (Fig. 4). Moreover, new bio factors can be used as novel biomarkers for the diagnosis and prognosis of breast

cancer [40].

Despite our promising results, further research is needed to validate the safety of FIR in treating early postmastectomy lymphedema.

## 5. Conclusions

To summarize, FIR is feasible and safe for the treatment of BCRL patients who underwent mastectomy and cancer treatment 5 years or more prior to initiating FIR therapy. FIR does not promote recurrence or metastasis of breast cancer and has no adverse reactions in patients. *In vitro* cell research demonstrated that FIR does not promote cell proliferation or shorten the cell cycles of breast cancer cells. Moreover, it does not attenuate apoptosis, confirming the oncological safety of FIR treatment for treating postmastectomy lymphedema.

## Funding

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## Conflict of Interest

The authors have no conflicts of interest.

## Ethical approval

All procedures performed on patients were in accordance with the ethical standards set by the local and national research committees and in accordance with the 1964 Helsinki declaration and its latest amendments.

## Informed consent

Informed consent was obtained from all individual participants included in the study.

## References

[1] M. Golshan, B. Smith, Prevention and management of arm lymphedema in the patient with breast cancer, J. Support. Oncol. 4 (2006) 381–386.
[2] F. Cialdai, I. Landini, S. Capaccioli, et al., In vitro study on the safety of near infrared laser therapy in its potential application as postmastectomy lymphedema treatment, J. Photochem. Photobiol. B 151 (2015) 285–296.
[3] R.A. Vieira, A.M. da Costa, J.L. de Souza, et al., Risk Factors for Arm Lymphedema in a Cohort of Breast Cancer Patients Followed up for 10 Years, Breast care (Basel, Switzerland) 11 (2016) 45–50.
[4] E.S. Dylke, L.C. Ward, J.D. Meerkin, L. Nery, S.L. Kilbreath, Tissue composition changes and secondary lymphedema, Lymphat. Res. Biol. 11 (2013) 211–218.
[5] C.E. Markhus, T.V. Karlsen, M. Wagner, et al., Increased interstitial protein because of impaired lymph drainage does not induce fibrosis and inflammation in lymphedema, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 266–274.
[6] W.L. Olszewski, Episodic dermatolymphangioadenitis (DLA) in patients with lymphedema of the lower extremities before and after administration of benzathine penicillin: a preliminary study, Lymphology 29 (1996) 126–131.
[7] W. Döller, Lymphedema: anatomy, physiology and pathophysiology of lymphedema, definition and classification of lymphedema and lymphatic vascular malformations, Wien. Med. Wochenschr. 163 (2013) 155–161.
[8] J. McWayne, S.P. Heiney, Psychologic and social sequelae of secondary lymphedema: a review, Cancer 104 (2005) 457–466.
[9] The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology, Lymphology 46 (2013) 1–11.
[10] S. Lin, J. Kim, M.J. Lee, et al., Prospective transcriptomic pathway analysis of human lymphatic vascular insufficiency: identification and validation of a circulating biomarker panel, PLoS One 7 (2012) e52021.
[11] F. Bruns, O. Micke, M. Bremer, Current status of selenium and other treatments for secondary lymphedema, J. Support. Oncol. 1 (2003) 121–130.
[12] N.F. Liu, W. Olszewski, The influence of local hyperthermia on lymphedema and lymphedematous skin of the human leg, Lymphology 26 (1993) 28–37.
[13] W.T. Hwang, S.H. Chung, J.S. Lee, Complex decongestive physical therapy and low-level laser therapy for the treatment of pediatric congenital lymphedema: a case report, J. Phys. Ther. Sci. 27 (2015) 2021–2022.
[14] C.H. Wong, L.C. Lin, H.H. Lee, C.F. Liu, The analgesic effect of thermal therapy after total knee arthroplasty, J. Altern. Complement. Med. 18 (2012) 175–179 (New York, NY).
[15] T.K. Leung, In vitro and in vivo studies of the biological effects of bioceramic (a material of emitting high performance far-infrared ray) irradiation, Chin. J. Physiol. 58 (2015) 147–155.
[16] A. Dominguez-Vidal, N. Kaun, M.J. Ayora-Cañada, B. Lendl, Probing intermolecular interactions in water/ionic liquid mixtures by far-infrared spectroscopy, J. Phys. Chem. B 3 (111) (2007) 4446–4452.
[17] K. Li, Z. Zhang, N.F. Liu, S.Q. Feng, Y. Tong, J.F. Zhang, J. Constantinides, D. Lazzeri, L. Grassetti, F. Nicoli, Y.X. Zhang, Efficacy and safety of far infrared radiation in lymphedema treatment: clinical evaluation and laboratory analysis, Lasers Med. Sci. 32 (2017) 485–494.
[18] S. Hwang, D.H. Lee, I.K. Lee, Y.M. Park, I. Jo, Far-infrared radiation inhibits proliferation, migration, and angiogenesis of human umbilical vein endothelial cells by suppressing secretory clusterin levels, Cancer Lett. 346 (2014) 74–83.
[19] K. Li, N. Liu, Z. Yu, P. Sadigh, D. Lazzeri, Y.X. Zhang, Heating and compression bandage treatment is effective for chronic lymphedema with Dermatolymphangioadenitis-a case-controlled study, Lymphat. Res. Biol. (2016).
[20] W. Cao, D. Zhang, J. Gan, Effects of microwave baking on the immunological cells in primary lymphedema patients, Zhonghua zheng xing shao shang wai ke za zhi = Zhonghua zheng xing shao shang waikf [ie waike] zazhi, Chinese journal of plastic surgery and burns 15 (1999) 357–359 [Chung-hua cheng hsing shao shang wai k'o tsa chih pien].
[21] W. Cao, D. Zhang, J. Gan, [microwave effect on immunological response of chronic limb lymphedema]. Zhongguo xiu fu chong jian wai ke za zhi = Zhongguo xiufu chongjian waike zazhi, Chinese J. Reparative Reconst. Surg. 14 (2000) 105–109.
[22] S.Y. Yu, J.H. Chiu, S.D. Yang, Y.C. Hsu, W.Y. Lui, C.W. Wu, Biological effect of far-infrared therapy on increasing skin microcirculation in rats, Photodermatol. Photoimmunol. Photomed. 22 (2006) 78–86.
[23] J.H. Park, S. Lee, D.H. Cho, Y.M. Park, D.H. Kang, I. Jo, Far-infrared radiation acutely increases nitric oxide production by increasing ca(2 +) mobilization and ca (2 +)/calmodulin-dependent protein kinase II-mediated phosphorylation of endothelial nitric oxide synthase at serine 1179, Biochem. Biophys. Res. Commun. 436 (2013) 601–606.
[24] A. Masuda, M. Nakazato, T. Kihara, S. Minagoe, C. Tei, Repeated thermal therapy diminishes appetite loss and subjective complaints in mildly depressed patients, Psychosom. Med. 67 (2005) 643–647.
[25] K.H. Hu, W.T. Li, Clinical effects of far-infrared therapy in patients with allergic rhinitis, Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference, 2007 2007, pp. 1479–1482.
[26] I.S. Dayes, T.J. Whelan, J.A. Julian, et al., Randomized trial of decongestive lymphatic therapy for the treatment of lymphedema in women with breast cancer, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 31 (2013) 3758–3763.
[27] F. Nicoli, P. Ciudad, S.Y. Lim, D. Lazzeri, C. D'Ambrosia, K. Kiranantawat, R.M. Chilgar, S. Sapountzis, B. Sacak, H.C. Chen, Potential use of transferred lymph nodes as metastasis detectors after tumor excision, Arch Plast Surg. 42 (2015) 478–483.
[28] S. Zhao, Y. Mei, J. Wang, K. Zhang, R. Ma, Different levels of CEA, CA153 and CA125 in milk and benign and malignant nipple discharge, PLoS One 21 (2016) 11(6).
[29] L. Ju, Y. Wang, Q. Xie, et al., Elevated level of serum glycoprotein bifucosylation and prognostic value in Chinese breast cancer, Glycobiology 26 (2016) 460–471.
[30] C.M. Sturgeon, M.J. Duffy, U.H. Stenman, H. Lilja, N. Brunner, D.W. Chan, et al., National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers, Clin. Chem. 54 (12) (2008).
[31] M.J. Duffy, D. Evoy, E.W. McDermott, CA 15–3: uses and limitation as a biomarker for breast cancer, Clin. Chim. Acta; Int. J. Clin. Chem. 411 (23–24) (2010) 1869–1874.
[32] M.J. Kim, B.W. Park, J.B. Lim, H.S. Kim, J.Y. Kwak, S.J. Kim, et al., Axillary lymph node metastasis: CA-15-3 and carcinoembryonic antigen concentrations in fine-needle aspirates for preoperative diagnosis in patients with breast cancer, Radiology 254 (3) (2010) 691–697.
[33] S.G. Wu, Z.Y. He, J. Zhou, J.Y. Sun, F.Y. Li, Q. Lin, et al., Serum levels of CEA and CA15-3 in different molecular subtypes and prognostic value in Chinese breast cancer, Breast 23 (1) (2014) 88–93.
[34] I. Lakshmanan, M.P. Ponnusamy, S. Das, S. Chakraborty, D. Haridas, P. Mukhopadhyay, et al., MUC16 induced rapid G2/M transition via interactions with JAK2 for increased proliferation and anti-apoptosis in breast cancer cells, Oncogene 31 (7) (2012) 805–817.
[35] F. Langlands, E. Cornford, E. Rakha, et al., Imaging overview of metaplastic carcinomas of the breast: a large study of 71 cases, Br. J. Radiol. (2016) 201406e44.
[36] W.C. Tsai, H.K. Wei, C.F. Hung, et al., Better overall survival for breast cancer patients by adding breast ultrasound to follow-up Examinations for Early Detection of Locoregional recurrence-a survival impact study, Ultrasound Med. Biol. (2016).
[37] B. Paquette, C. Baptiste, H. Therriault, G. Arguin, B. Plouffe, R. Lemay, In vitro irradiation of basement membrane enhances the invasiveness of breast cancer cells, Br. J. Cancer 97 (2007) 1505–1512.
[38] H. Iinuma, T. Watanabe, Development of minimal residual cancer cell research, Nihon Geka Gakkai zasshi 113 (2012) 177–184.
[39] Y.C. Chen, L.C. Lai, Y.P. Tu, S.D. Wu, C.F. Chen, B. Li, Far infrared ray irradiation attenuates apoptosis and cell death of cultured keratinocytes stressed by dehydration, J. Photochem. Photobiol. B 106 (2012) 61–68.
[40] G. Wang, Y. Qin, J. Zhang, J. Zhao, Y. Liang, Z. Zhang, M. Qin, Y. Sun, Nipple discharge of CA15-3, CA125, CEA and TSGF as a new biomarker panel for breast cancer, Int. J. Mol. Sci. 28 (15) (2014) 9546–9565.