Original Research

Impact of far-infrared ray exposure on the mechanical properties of unwounded skin of rats

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Abstract

Far-infrared ray (FIR) has been applied to promote growth, modulate sleep, speed up the healing of wounds and accelerate microcirculation. The action of FIR on wounds has been well established in previous studies. However, whether the same action also works on normal skin is unclear. Therefore, the aim of this study was to evaluate the impact of FIR exposure on the tensile strength (TS) and composition of unwounded skin. In this study, 84 Sprague–Dawley (SD) rats were randomly divided into control and FIR groups. The dorsum samples were harvested for mechanical testing and histological observation at one, two, four and six weeks. The TS in the control group had no significant difference for all durations. However, a steep increase in the TS occurred between one and two weeks ($P = 0.033$) in the FIR group. The TS in the FIR group was found to be significantly higher than the TS in the control group at two weeks ($P = 0.049$). From histological observation, capillary dilation and increased inflammatory cells around the capillaries were observed at one week in FIR-treated groups. However, the mild inflammatory changes vanished after two weeks. In conclusion, our results showed that FIR may induce inflammatory changes and enhance skin TS in the short term, but the effect diminished with time.

Keywords: far-infrared ray, normal skin, tensile strength, histology, short-term effect

Experimental Biology and Medicine 2010; **235**: 952–956. DOI: 10.1258/ebm.2010.009372

Introduction

Far-infrared ray (FIR) is a natural source from the sun and is one of three infrared radiation categories: near-infrared (0.76–1.5 μm), middle-infrared (1.5–4 μm) and FIR radiation (4–1000 μm).[1] FIR has been applied in clinical practice for many years. A variety of applications include growth promotion,[2] sleep modulation,[3] speeding up the healing of wounds[4,5] and acceleration of microcirculation.[6,7] In a review of the literature, it was found that there had been physiological examinations and pathological observations of wounds exposed to FIR. Some studies revealed that wounds treated with FIR healed more rapidly, with greater collagen regeneration and more fibroblast proliferation, than wounds that were not treated with FIR.[4] When normal skin was exposed to FIR, some beneficial biological activities which were proposed to be driven by an increase in skin microcirculation on the human body were found.[6]

So far, most FIR research has focused on wound treatment and physiological reactions. The effect of FIR exposure on wounds is therefore well established. However, whether the same action also works on the normal skin is not known. Until now, no study has discussed the influence of FIR exposure on normal skin. The aim of this study is to find the impact of FIR exposure on the mechanical performances and composition of normal skin.

Materials and methods

Animal study setup

Eighty-four male Sprague–Dawley (SD) rats of 6–8 weeks of age were used in this study. The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Laboratory Animal Center at National Cheng Kung University, Taiwan. The rats were randomly divided into two groups by sequence, a control group and an experimental FIR group, and then sacrificed at one, two, four and six weeks to evaluate the differences in mechanical property and histology of the skin.

The rats were anesthetized with 400 mg/kg of chloral hydrate (Sigma-Aldrich, St Louis, MO, USA). After anesthetization, the dorsum of each rat was shaved with electric clippers and then sterilized with 70% alcohol solution. In the FIR groups, the rats were fixed in the jig with adhesive tape to maintain similar posture and exposed uniformly to FIR (CH-8222 FIR, 200 W, Chung Cheng Electric Heating Co, Ltd, Tainan, Taiwan) with a wavelength of 4–14 μm for 10 min/d, 5 d/week, until they were sacrificed. The rats in the experimental group were placed inside a $20 \times 20 \times 20\,cm^3$ aluminum film enclosed box, and an FIR emitter was placed 20 cm above the top of the dorsal skin. However, in the control group, the rats were not restrained on fixing jig and only given regular care without FIR exposure. In addition, the skin temperatures in both groups were monitored. At one, two, four and six weeks, the rats were euthanized with carbon dioxide and the skin samples were harvested and placed in a phosphate-buffered saline solution for later tensile strength (TS) testing and pathological observation.

TS testing

The soft tissue Microforce Testing System (MTS Tytron 250, MTS Systems Corp, Eden Prairie, MN, USA) with soft tissue clamps was used to measure the TS of the skin. Biopsy specimens were flattened with a preload force of 0.2 N and punched into dumbbell shapes (Figure 1a). After being clamped to the MTS, each specimen was subjected to a 10 mm/min displacement rate at room temperature until failure (Figure 1b). The data were recorded at a sampling rate of 5 Hz. The force–elongation curve for each specimen was recorded, and the maximum force was identified as the breaking strength of the specimen. The TS was calculated from the maximum breaking strength divided by the cross-sectional area of each specimen (Figure 1c).[8,9]

Pathological evaluation

A slice of skin tissue adjacent to the mechanical testing specimen was harvested for pathological testing. The sectioned tissue underwent classic specimen processing, including fixation, dehydration, infiltration and embedding. The paraffin-embedded tissue block was sectioned and stained with hematoxylin and eosin (Sigma Pharmaceutical Pty Ltd, Clayton, Australia). The histological slides were observed with conventional optical microscopy.

Statistical analysis

Results were presented as means $\pm$ standard deviations, and comparisons were made using the Wilcoxon rank sum test. All statistical tests were conducted using the Statistical Package for the Social Sciences (SPSS) 15.0 for Windows at the two-tailed significance level of 0.05.

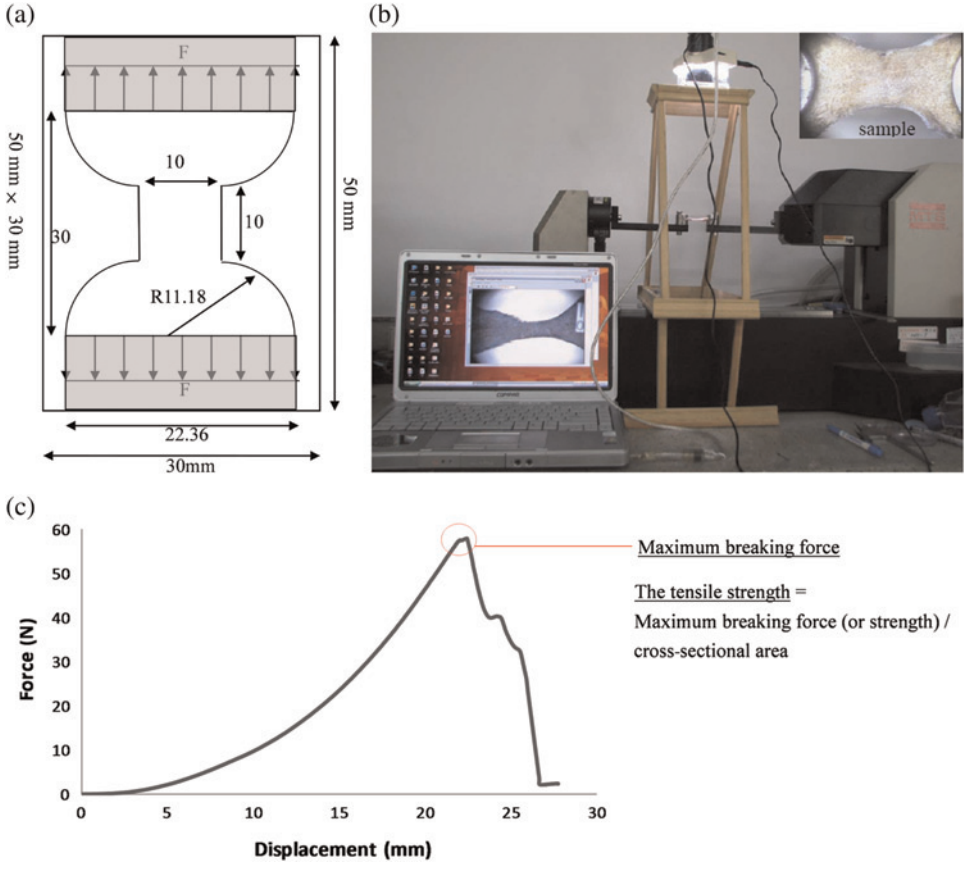

Figure 1 (a) Dimension and bell shape of the test sample; (b) representation of experimental setup for tensile test; and (c) the diagram to demonstrate the force–displacement curve and the method of calculated tensile strength (TS) (A color version of this figure is available in the online journal)

Results

Characteristics of rats

Of the 84 rats selected for the experiment, three died during FIR therapy, five died due to acute peritonitis and two were injured during routine experimental procedures. Therefore, a total of 74 samples were analyzed in the study. The sample sizes at one, two, four and six weeks were 12, 10, 9 and 9 in the control group, respectively, and were 8, 8, 10 and 8 in the FIR group, respectively. Rats used in the beginning of the study aged 6–8 weeks, and records of the rats' weights and skin temperatures for the experiment were kept. The average weights at week 0 were 313.7 ± 44.1 and 224.5 ± 38.0 g in control and FIR groups, respectively. The average weights of the rats in the control group were 302.0 ± 34.4, 303.6 ± 50.6, 394.5 ± 35.1 and 398.6 ± 41.2 g at one, two, four and six weeks, respectively. For rats in the FIR group, the average weights were 260.8 ± 24.6, 241.4 ± 28.0, 267.0 ± 46.9 and 315.8 ± 26.8 g at one, two, four and six weeks, respectively. In general, the weights of the rats increased with the growth of the rats, except for those in the FIR group at two weeks. The average skin temperatures in the experimental group after exposure to FIR and in the control group were 35.8 ± 1.4 and $31.6 \pm 1.0^{\circ}$C, respectively.

Tensile strength

The average TSs of the dorsal skin of rats at one, two, four and six weeks were 5.2 ± 1.0, 7.4 ± 2.4, 6.6 ± 1.5 and 6.3 ± 1.5 MPa in the FIR group and 5.1 ± 1.3, 5.4 ± 1.2, 6.3 ± 1.8 and 7.2 ± 2.0 MPa in the control group, respectively (Table 1). Changes in the TSs developed in distinct ways over time (Figure 2). In the control group, the TS increased slightly over time, and no significant differences were found. However, a steep increase in the TS occurring between one and two weeks ($P = 0.033$) was found in the FIR group. We further compared the TS between the two groups at one, two, four and six weeks and found that the TS at two weeks was statistically different ($P = 0.049$). Overall, these results imply that FIR exposure can enhance the TS of skin in the short term, but that the beneficial effect do not last after four weeks under the given exposure dosages in this study.

Histology

Figure 3 shows the histology of the FIR-treated and -untreated groups. At all time points, no significant morphological changes were found between the two groups, except capillary dilation and increased inflammatory cells, predominant lymphocytes and histiocytes, around the capillaries (up to 37 inflammatory cells versus 2 inflammatory cells per high power field, ×400) were observed at one week in FIR-treated groups compared with non-FIR-treated groups. However, the mild inflammatory changes vanished after two weeks. The composition and orientation of collagen fibers in dermis were not different under microscope.

Table 1 Average tensile strengths of the dorsal skins in the control group and in the far-infrared ray (FIR)-exposed group at one, two, four and six weeks

Tensile strength (MPa)	1 week	2 weeks	4 weeks	6 weeks
Control group	5.1 ± 1.3	5.4 ± 1.2	6.3 ± 1.8	7.2 ± 2.0
FIR group	5.2 ± 1.0	7.4 ± 2.4	6.6 ± 1.5	6.3 ± 1.5
P value	0.700	0.049	1.000	0.386

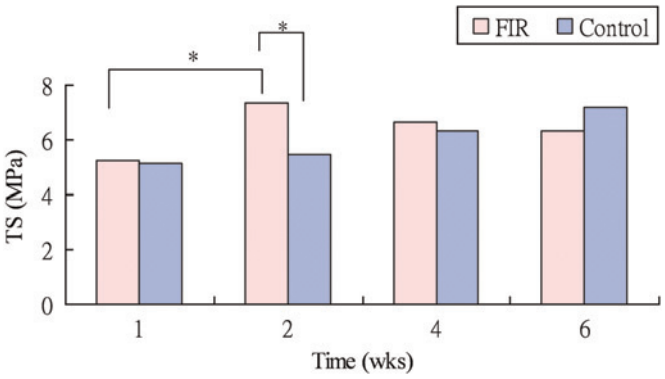

Figure 2 Tensile strength (TS) of dorsal skin at different times; $^{*}P < 0.05$ (A color version of this figure is available in the online journal)

Discussion

The effect of FIR exposure on wounds has been well established in previous studies. However, whether the same action also works on normal skin is unclear. When normal skin TS in our study was compared with wound TS reported in previous studies, we found that FIR exposure led to similar improvement in TS for both conditions. In situations with simple incisions, Schramm *et al.*[10] showed that a group treated with FIR for 15–18 d had 36.4% TS improvement compared with untreated groups (2.36 MPa for FIR groups and 1.73 MPa for untreated groups). However, in situations where there were no wounds in our study, there was 37.0% TS improvement (7.4 MPa for FIR groups and 5.4 MPa for untreated groups at 2 weeks).

The TS of skin represents the functional performance, which may relate to the tissue composition and fiber orientation. In this study, we attempted to combine TS and histological results to find the effect of FIR exposure. In the TS results, we found that in the FIR group, the TS of skin was enhanced in the short term, but not in the long term. Nevertheless, only mild vasodilation and inflammatory cells infiltration were observed in the group exposed to short-term FIR at histological sections. The orientation and composition of dermal collagen fibers were not different at morphological level. As Yu *et al.*[6] pointed out in a previous study, FIR therapy on normal skin increases skin microcirculation, which was also observed in our early FIR group. This may be the reason why more infiltrated inflammatory cells were found in our study.

The effect of FIR therapy on wounds has been studied and was found to involve complex interactions between healing cascade and FIR stimulation, but the side-effect of FIR therapy on normal skin has rarely been discussed. Our results found that the effects of FIR therapy on

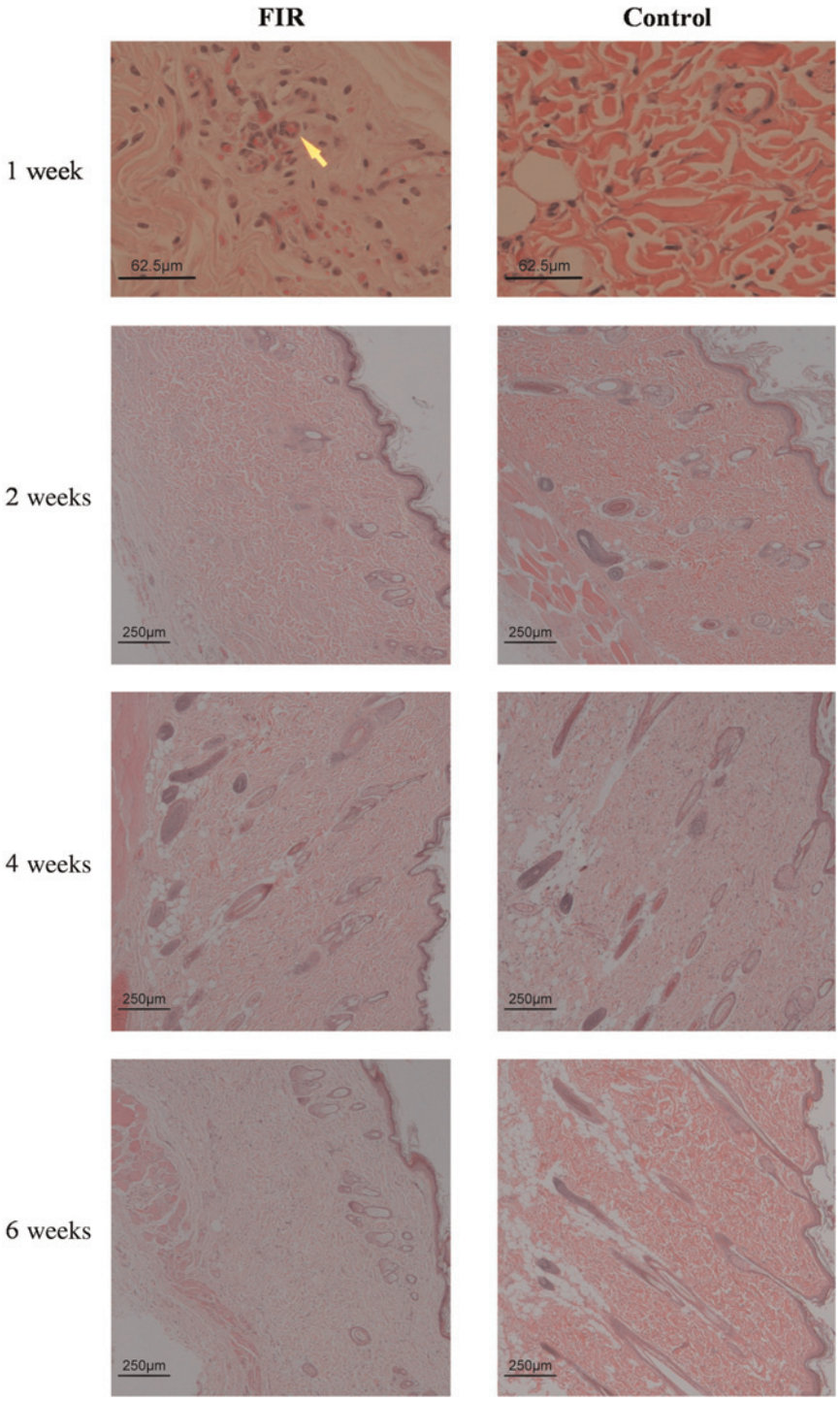

Figure 3 Histology hematoxylin and eosin (H&E stain) of the far-infrared ray (FIR) and control groups at one (×400), two, four and six weeks (×40). Arrow indicates increased dilated capillaries. Many inflammatory cells around them were seen

normal skin may induce inflammatory cells, re-organize collagen fibers and change the mechanical property of skin within one week. However, in the long run, the effect vanished and there was no difference with the non-treated group. According to the wound healing model study conducted by Levenson *et al.*[9] the effect of FIR therapy on wounds was found to involve complex interactions between healing cascade and FIR stimulation. Besides, a strong relationship between the orientation of collagen fibers and TS was found. We speculate that these transient inflammatory changes may induce cytokine release which may result in conformation or composition changes of collagen fibers and consequently affect TS. This hypothesis warrants further investigation.

Because the specimen in the experiment group is intake skin, the procedure to re-train the rats on the fixing jig should not affect the biological behavior of skin. According to the Laboratory Animal Center at NCKU, the

weight of an SD rat is 311 ± 16, 334 ± 26 and 400 ± 27 g at eight, nine and 11 weeks old, respectively, which corresponds to the evaluation time point one, two and four weeks (the data at 6 weeks was not shown since it was not within the estimated range). These weights standards are similar to our results in the control group, but not in the FIR group. Overall, the weights of the rats in the FIR group were less than those in the control group. The weight loss effect in this study conflicts with past studies, which reported that FIR caused a weight gain in growing rats of the SD strain.[3] Further investigation is needed to verify the effect of FIR on weight loss. In addition, the relation between rat weight and TS was poorly correlated (Spearman's correlation coefficient are 0.235 and 0.150 in the control and FIR groups, respectively), which is coincident with the findings of previous results.[9]

In conclusion, our results showed that FIR may change the composition of skin and influence its performance in the first two weeks. However, this effect may diminish in the longer term.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of Chia-Yi Christian Hospital in Taiwan (Grant no. R97-4). We also appreciate Professor Su, Fong-Chin at Institute of Biomedical Engineering of National Cheng Kung University for providing us the MTS.

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(Received December 5, 2009, Accepted April 27, 2010)