# Protective Effect of Non-Ionizing Radiation from Ceramic Far Infrared (cFIR)-Emitting Material Against Oxidative Stress on Human Breast Epithelial Cells

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**Abstract**

Previous research has identified that ceramic far infrared (cFIR)-emitting material can modulate various biological processes, particularly those involving hydrogen peroxide scavenging and antioxidant activities. The present study treated MCF-10A cells with 50 and 100 μM hydrogen peroxide before incubating them for 24 h on the top of The present study treated MCF-10A cells with 50 and 100 μM hydrogen peroxide before incubating them for 24 h on the top of cFIR or control powder. cFIR or control powder. Cells were also treated with ionizing radiation from a fluoroscopic X-ray source to induce cell damage and cultured for 48 h beneath cFIR or control powder. The effects of cFIR on cell survival were evaluated using XTT and MTT assays. A total accumulated radiation dose of 1 Gy to 2 Gy was sufficient to cause cell damage and reduce cell viability. In both the hydrogen peroxide toxicity and radiation exposure experimental models, the cFIR groups demonstrated significantly higher cell survival rates than those of the control groups ($p < 0.05$). Considering the relationship between indirect-ionizing-radiation- and oxidative-stress- induced cell damage and the accumulation of free radicals, these results indicate that the protection of cFIR against ionizing radiation is predominantly through an antioxidant mechanism. cFIR-emitting material has potential use in reducing radiation damage caused by medical instruments and radiation pollution.

***Keywords***: Far-infrared rays, Bioceramic, Low-dose radiation, Breast epithelial cells, Antioxidants, Oxidative stress

## 1. Introduction

Ionizing radiation is a form of electromagnetic radiation (such as X-rays, alpha rays, gamma rays, and universal rays). In a clinical setting, electromagnetic radiation and particulate ionizing radiation (mostly electrons and, to a lesser extent, neutrons and protons) are used for radiation diagnosis and oncology purposes [1,2]. Ionizing radiation has sufficient energy to remove tightly bound electrons from their orbits, causing the atom to become charged or ionized. It deposits

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energy at the molecular level, leading to chemical changes that induce biological changes. These changes include cell death, cell transformation, and irreparable cell damage. The effects of this radiation cannot be attributed to heating. The chemical and biological effects of ionizing radiation arise from two basic types of interaction. In its direct action, the radiation energy is deposited directly into its targets. In its indirect action, the external medium absorbs the radiation energy, leading to the production of diffusive intermediates that attack the targets. Therefore, radiation damages cells directly through the ionization of DNA (Fig. 1) and other cellular targets, and indirectly through reactive oxygen species (ROS), causing oxidative stress via free radical cellular damage.

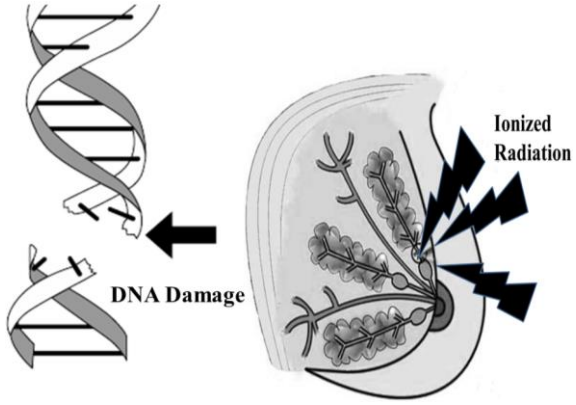


Figure 1. Breast epithelial cell DNA damaged directly by ionizing radiation.

The exposure of eukaryotic cells to ionizing radiation results in the immediate formation of free radicals. Molecular changes are associated with the initial production of free radicals at the time of irradiation, which induce perturbations in the metabolic processes of biological tissues [2]. Exposure to ionizing radiation leads to production of oxygen-derived free radicals in the tissue environment, including hydroxyl radicals (the most damaging), superoxide anion radicals, and other oxidants such as hydrogen peroxide [3-6].

Non-ionizing radiation and far-infrared (FIR) rays differ from ionizing radiation. FIR irradiation belongs to the portion of the wavelength spectrum that produces no ionizing effects and does not emit high-speed free electrons. Ionizing radiation has a higher frequency and shorter wavelength than those of visible light (400 nm to 750 nm). It has sufficient energy to break chemical bonds. High-energy ionizing radiation can remove electrons or break up the nuclei of atoms [7-9]. Non-ionizing radiation has lower frequencies and longer wavelengths than those of the visible light spectrum. Non-ionizing radiation You don't use this abbreviation below.has insufficient energy to break chemical bonds. The spectrum of FIR is defined as wavelengths ranging from 4 to 16 μm. In the present study, ceramic FIR (cFIR)-emitting material was controlled and stabilized to emit in the wavelength range of 8 to 14 μm [7-9].

Our group's previous studies on cFIR found that cFIR promotes microcirculation and induces other effects in various cell lines by upregulating calcium-dependent nitric oxide and calmodulin [10-13]. Results also demonstrated that cFIR induces antioxidant effects by increasing the hydrogen peroxide scavenging abilities of various cells, including murine macrophages (RAW264.7) [14], murine calvaria-derived osteoblast-like cells (MC3T3-E1) [15,16], NIH3T3 fibroblast cells [15], and murine myoblast cells (C2C12) [17].

In the study of Leung et al., cFIR irradiation treatment decreased melanoma cell proliferation and reduced intracellular heat shock protein (HSP-70) and intracellular nitric oxide (iNO) content [18]. This suggests a potential future use of cFIR in antitumor applications. Results from the study of Leung et al. also suggest that cFIR might have beneficial effects on the heart during oxidative stress by suppressing contractility and potentially ameliorating long-term oxidative stress, thus reducing the likelihood of cardiac arrest and ischemic myocardial injury [19].

The present study investigates the possible inhibitory effects of X-ray ionizing radiation on normal human cell lines using a diagnostic medical instrument (fluoroscope) as the radiation source, and the possible mechanism was investigated.

## 2. Materials and methods

### *2.1 Far-infrared-ray-emitting ceramic powder*

cFIR powder was obtained from the Department of Radiology, Taipei Medical University Hospital It was composed of microsized particles of several components, namely calcium (Ca), zirconium (Zr), sulphur (S), silicon (Si), aluminum (Al), magnesium (Mg), iron (Fe), oxygen (O), and carbon (C). The average emissivity of the ceramic powder, at wavelengths of 6 to 14 μm, was 0.98, as determined using a spectroradiometer (SR5000, CI Systems, israel) This represents an extremely high FIR intensity ratio. The ceramic powder can induce several physical, chemical, and biological effects at room temperature without direct contact. Equal amounts (100 g) of cFIR powder were enclosed in plastic bags (10 cm × 20 cm) and served as the irradiation source. These were inserted beneath dishes containing human breast epithelial (MCF-10A) cell culture discs without direct contact.

### *2.2 Control group*

Equal amounts (100 g) of milk powder were enclosed in plastic bags (10 cm × 20 cm) and inserted beneath the dishes containing MCF-10A cell culture discs without direct contact.

### *2.3 Cell culture*

Cultures of MCF-10A cells were maintained in Dulbecco's minimum essential medium (Cellgro, Herndon, VA, USA), supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 1% L-glutamine, penicillin (100 units/ml), and streptomycin (100 g/ml), in a humidified incubator (5% $CO_2$) at 37 ℃.

*2.4 Determination of cFIR effects on hydrogen-peroxide-mediated oxidative stress using XTT assay*

Cell viability was determined using the XTT assay [20] according to its mitochondrial-dependent reduction to formazone. Cells were plated at a density of $4 \times 10^5$ cells/well into 24-well plates for 24 h, then treated with two concentrations (50 and 100 μM) of hydrogen peroxide ($H_2O_2$) [21]. Cells were treated for a further 24 h with cFIR powder and control powder as described previously. Cells were washed 3 times with phosphate-buffered saline (PBS) (Gibco). XTT (1 mg/ml) was then added to the medium. After 3 h, the supernatant was collected. The absorbance was read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) analyzer (Gemini XPS Molecular Devices, Sunnyvale, CA, USA).

*2.5 Source of X-ray ionizing radiation*

Culture discs of cells were vertically irradiated using a fluoroscopic X-ray instrument (GE Medical system, Prestige SI, Italy). The exposure field was 26 cm × 26 cm. To ensure homogenous and equal dose exposure, the height of the table and X-ray source were all controlled at 95 cm. During X-ray irradiation, cell culture discs (control and experimental groups) were placed at the isocenter of the exposure field (Fig. 2).

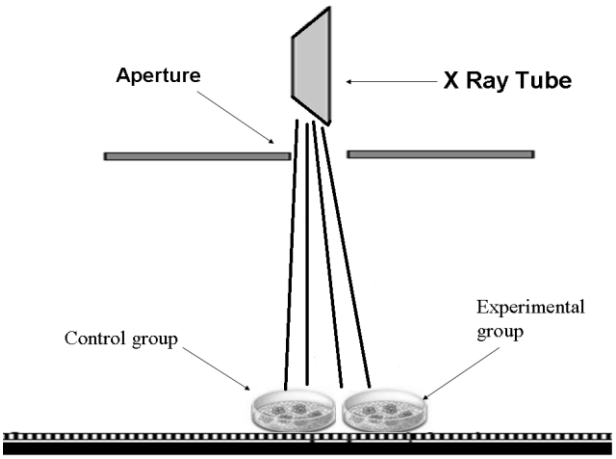


Figure 2. Setup for X-ray exposure of cell culture discs with the ionization source at a fixed distance from the surfaces of the discs.

*2.6 Determination of cell growth inhibitory effects induced by 1- and 2-Gy X-ray radiation using MTT assay*

The MCF-10A cell culture plates, with uniform distribution and equal amounts of cells, were divided into control (non-ionizing radiation) and experimental (ionizing radiation) groups. Only the cell samples of the experimental group were exposed to radiation. The two groups were compared using the MTT assay [25], which evaluates mitochondrial activity in viable cells. In brief, MTT was freshly prepared at 1 mg/ml in PBS, and then 800 μl was added to each well, which was incubated at 37 ℃ for 4 h. An equal volume (800 μl) of dimethyl sulfoxide (DMSO) was added to each well to dissolve the MTT-formazan crystals. After incubation at 37 ℃ for 10 min, the solution was transferred to a 96-well ELISA plate, and the absorbance was measured using a spectrophotometer at 540 nm. The optical density (OD) of the control cells was considered to be 100%. The absorbance of each well was also recorded using a microplate spectrophotometer at 595 nm. Cell proliferation in the control and experimental groups was then compared.

*2.7 Determination of cFIRs effects on ionizing-radiation-induced cell damage using MTT assay*

The MCF-10A cell culture plates, with uniform distribution and equal amounts of cells, were divided into control (milk powder beneath the discs) and cFIR (cFIR powder beneath the discs) groups. All cell culture discs of the control and cFIR groups were exposed to radiation using the dosages and exposure times described previously. Cell counting was performed immediately after X-ray exposure and after culture for a further 48 h with milk powder and cFIR powder placed beneath the discs.

*2.8 Determination of COX-2 production in MCF- 10A cells with and without 2-Gy X-ray radiation*

To investigate the effects of cFIR on cyclo-oxygenase-2 (COX-2) production in cells exposed to radiation, cells were divided into 3 groups: treatment without X-ray (blank group), treatment with X-ray (2-Gy dose) (control group), and treatment with X-ray (2-Gy dose) and cFIR material placed beneath the culture medium discs (cFIR group). Equal amounts of whole-cell extracts were analyzed using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electropheresis, the protein was transferred to polyvinylidene difluoride (PVDF)-nylon membranes (1.5 h). The membranes were then blocked with PBS Tween-20 (PBST) containing 6% bovine serum albumin at 4 ℃ overnight. After blocking, the membranes were incubated with the primary antibodies anti-COX-2 (Cell Signaling Technology, Beverly, MA, USA) (1:1000) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1 000) diluted in PBST at 4 ℃ for 20 h. They were then washed 4 times with PBST for 10 min each time. The membranes were then incubated with the secondary antibodies (anti-COX-2 antibodies) (The Jackson Laboratory, Bar Harbor, ME, USA), diluted to 1:1000 in PBST at room temperature for 2 h, and then washed 4 times with PBST for 15 min each time. After washing, the membranes were visualized using electrochemiluminescence (ECL) detection reagents and autoradiographic film (Amersham Pharmacia Biotech,USA).

*2.9 Statistical analysis*

Data are presented as means ± standard deviation (SD) of three independent experiments. The paired *t*-test was used to evaluate the significance of differences between groups. A $p$-value < 0.05 was considered statistically significant.

## 3. Results

### *3.1 Effects of cFIR on hydrogen-peroxide-mediated oxidative stress*

Results from XTT assay indicate that cFIR can prevent MCF-10A cell toxicity resulting from $H_2O_2$-induced oxidative stress at $H_2O_2$ concentrations of 50 and 100 μM (Fig. 3). At both of the tested $H_2O_2$ concentrations, cell proliferation (%) significantly differed between cFIR and control groups ($p < 0.05$).

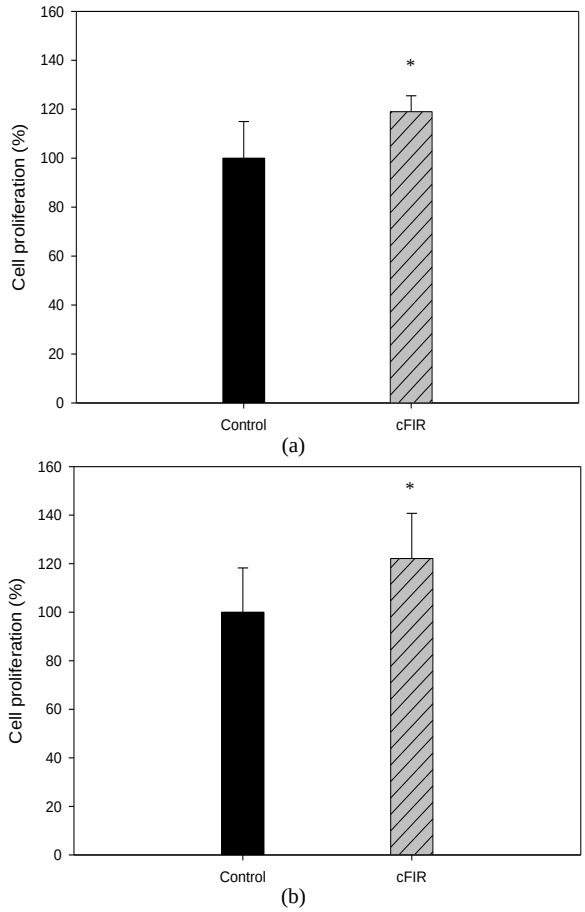


Figure 3. In MCF-10A cells subjected to $H_2O_2$-induced toxicity, at 50 μM (a) and 100 μM (b) $H_2O_2$ concentrations were significantly higher cell proliferation rates(%)for Cfir (right) groups observed than in cell proliferation rates in control groups(left) (*$p < 0.05$).

### *3.2 Cell growth inhibitory effects of 1- and 2-Gy X-ray radiation as determined using MTT assay*

After 12 min of ionizing radiation, an accumulated X-ray radiation dose of 1 Gy significantly suppressed MCF-10A cell proliferation (Fig. 4; $p < 0.05$). A 24-min X-ray accumulated irradiation dose of 2 Gy also significantly suppressed MCF-10A cell proliferation (Fig. 4; $p < 0.01$).

### *3.3 Effects of cFIR on ionizing-radiation-induced cell damage*

As shown in Fig. 5, in response to a 2-Gy X-ray radiation dose, the immediate viability of cells treated with cFIRs was

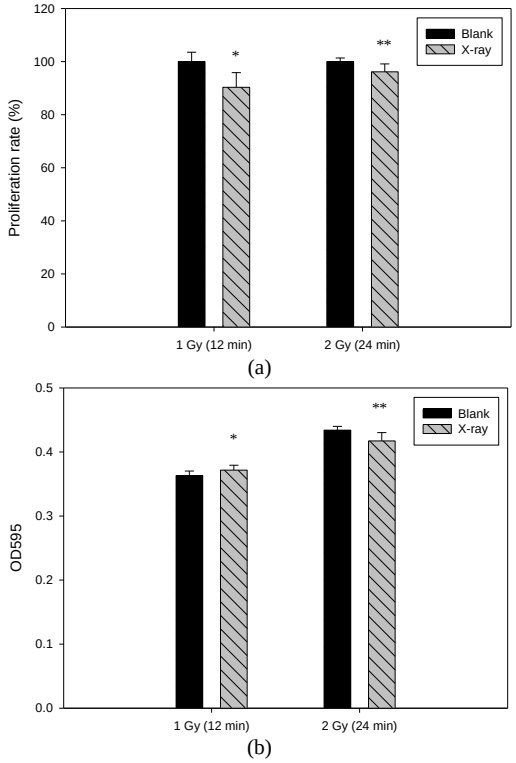


Figure 4. (a)Optical densities (OD) and (b) proliferation rates ofblank cell group (without radiation exposure) and X-ray cell groups in response to 1- and 2-Gy X-ray radiation doses. A significant inhibitory effect was observed (*$p < 0.05$; ** $p < 0.01$).

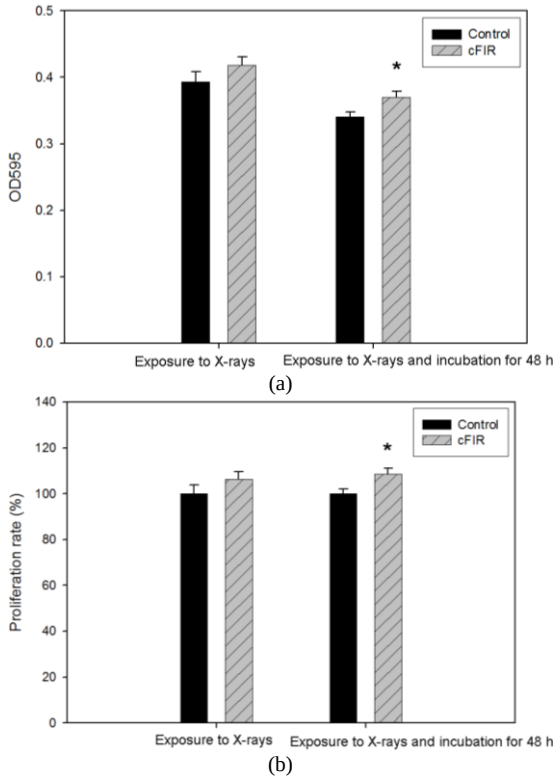


Figure 5. Proliferation rates represented by optical densities for control and cFIR groups immediately after exposure to 2-Gy X-ray radiation and after incubation for a further 48 h. cFIR group shows more proliferation of cells with significant difference (*$p < 0.05$).

greater than that of the control cells. After 48 h of incubation and additional cFIR treatment, a significant increase in MCF-10A cell viability was observed ($p < 0.05$).

### *3.4 Effects of cFIR on ionizing-radiation-induced COX-2 production*

As shown in Fig. 6, following a total X-ray irradiation dose of 2 Gy, COX-2 production significantly increased in the control and cFIR groups compared to that in the blanks (without X-ray irradiation). COX-2 production in the cFIR group was lower than that in the control group.

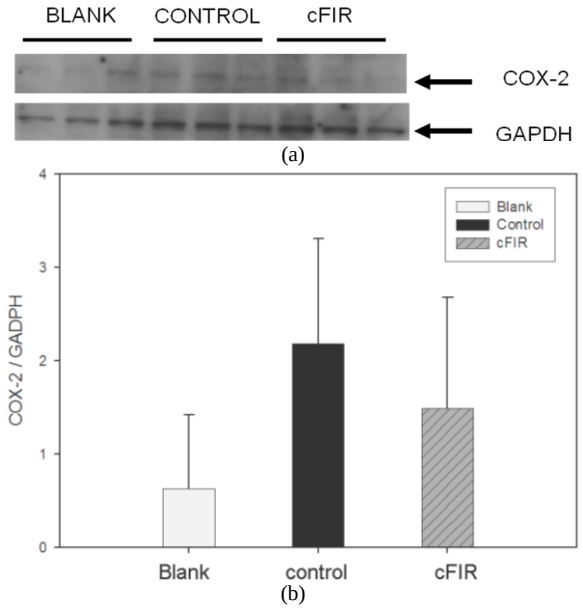


Figure 6. Increased COX-2/GADPH protein production was observed in control and cFIR groups receiving 2-Gy X-ray irradiation compared to the blank group (without X ray irradiation). COX-2 production occurred to a lesser extent in the cFIR group than in the control group.

## 4. Discussion

In previous research, a linear no-threshold (LNT) model has been used to estimate the cancer risk to humans from exposure to low-level radiation[26]. The LNT model assumes that damage caused by ionizing radiation is directly proportional to the dose at all dose levels [26]. Radiation is harmful with no safety threshold, and the sum of several very small exposures has the same effect as one large exposure equal to the sum. It cannot exclude the possibility of a single particle of radiation interacting with a single DNA molecule can initiate cancer process. And the number of initiating events is proportional to the number of particles of radiation and, thus, to the radiation dose [27-30]. In the study of Mackenzie, 50 women with carcinoma of the breast and receiving treatment, including repeated fluoroscopic examination of the chest over different periods of time, were retrospectively evaluated [31]. The authors concluded that irradiation played a significant role in the patients' subsequent development of mammary cancers [30]. In addition, Dehen et al. found an induction of chronic radiodermatitis following cardiac catheterization with fluoroscopic X-ray exposures [32]. These two reports have raised concerns about the use of low-dose ionizing radiation in medical imaging procedures [33]. Although double-strand breaks are not necessarily lethal, a radiation dose can cause up to 50% cell death [23,34,35]. Exposure to ionizing radiation results in the formation of free radicals in living systems. These free radicals are believed to persist for milliseconds, resulting in oxidative damage to biomolecules such as DNA, proteins, and lipids. Such exposure causes cumulative increases in $H_2O_2$ and superoxide radicals, and can potentially augment mutation rates of living cells [14,15]. In the presence of $O_2$, the initially formed radiation-induced free radicals include the hydroxyl radical, superoxide, and organic radicals [36-38].

Free radical scavengers and antioxidants can prevent free radical damage. Free radical scavengers offer protection against hydroxyl radical attack by competing with the target molecule for that radical [37]. The most important target molecule in the body is thought to be DNA. Previous research has also identified that oxidative damage to proteins, as determined according to the presence of carbonyl derivatives, is a significant cause of ageing, and that proteins from older subjects are more susceptible to oxidative stress [36]. Radiation-induced oxidative damage can be evaluated by assessing protein oxidation and also by analyzing inactivation of antioxidant enzymes, such as superoxide dismutase (SOD) [4,5]. A large proportion of cancer patients take antioxidant vitamin and mineral supplements, with the hope of improving the outcomes of conventional therapies and of reducing the adverse effects of these treatments. However, few studies exist on the efficacy and safety of antioxidant vitamin supplementation as an adjuvant to radiation therapy [3-5]. As mentioned above, previous radiation studies on MCF-10A cells found that 2 Gy is the radiation dose that provides optimal effects without causing extensive rapid damage [20-24]. To provide an equal total exposure dose of 1 Gy in experiments, 8.8 cGy radiation was provided every min for an exposure period of 12 min [20,21]. To provide an equal total exposure dose of 2 Gy in experiments, 8.8 cGy radiation was provided every min for an exposure period of 24 min [22-24].

Results from the present study indicate that cFIR can increase the survival rates of MCF-10A cells subjected to hydrogen-peroxide-induced oxidative stress and to 1-Gy to 2-Gy X-ray radiation doses. According to the concept of indirect-radiation-induced cell damage through the oxidative-stress-inducing effects of ROS, it is likely that cFIR induces an antioxidant process to protect cells from damage caused by ionizing radiation. The up-regulation of COX-2 has an important role in inflammatory processes [39-40]. COX-2-derived prostaglandins, particularly prostaglandin E2, are responsible for symptoms such as pain, fever, and swelling due to vasodilatation, representing the classic triad of inflammation (41). Our results confirm previous studies that found that COX-2 is involved in early radiation effects in normal tissues [39-42]. Radiation-induced COX-2 is involved in the pathogenesis of radiation-induced tissue damage, especially in the acute stage. There is growing evidence from experimental as well as clinical studies demonstrating that

radiation side effects of living tissues can be protected by selective COX-2 inhibition. Selective COX-2 inhibition may regulate the radiation sensitivity of tissues, to reduce radiation-induced side effects [39-41]. This study indicates that cFIR may act as potential a radiation-induced COX-2 inhibitor.

## 5. Conclusion

The present study observed an increased survival of MCF-10A cells following exposure to ionizing radiation in the presence of cFIR. This suggests that cFIR provides cells with a defensive mechanism during radiation exposure, and promotes cell repair during postexposure periods, through hydrogen-peroxide-scavenging and COX-2-inhibiting activities. However, further investigation is needed to fully elucidate the biomolecular mechanisms involved. This pilot study was limited by a small amount of data. Future studies could include *in vivo* analysis of animals following exposure to radiation. cFIR-emitting materials could potentially facilitate recovery from radiation-induced oxidative stress after radiation therapy, and following exposure to ionizing radiation diagnostic medical devices (including X-ray, mammography, computed tomography, and positron-emission tomography equipment). In the future, cFIRs could also potentially contribute to public health by reducing radiation damage resulting from radiation pollution [43,44].

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