QUALITATIVE AND SEMI QUANTITATIVE ANALYSIS IN THE HEALING AREA OF ATHYMIC NUDE MICE SKIN ENGRAFTED WITH HUMAN SKIN STERILIZED WITH GAMMA RADIATION

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ABSTRACT

In recent decades there has been a great interest in the radio-sterilized grafts for human skin grafts. This tissue is taken from a cadaver or multi-organ donor and samples are processed and stored in glycerol at concentrations above 85%. Although this procedure is carried out under aseptic conditions, after the final packaging one can sterilize the tissues with ionizing radiation in order to increase the safety level of sterility. The purpose of this study was to evaluate the behavior of the healing repair process that occurs between the graft and the skin of athymic NUDE mice. The samples of human skin treated with glycerol were divided into three groups: the control group 1 (non-irradiated), irradiated group 2 at 25 kGy and irradiated group 3, at 50 kGy. These tissues were grafted onto athymic NUDE mice which were sacrificed after 3, 7 and 21 days. After the sacrifice, part of the back fur of the animals containing human skin graft was removed with hematoxylin and eosin (H/E). The histological sections were analyzed for the integrity of tissue, presence and location of keratinocytes, fibroblasts, defense cells and blood vessels. Thus it was examined whether over time the graft was incorporated into the body or if there was a process of healing by secondary intention.

1. INTRODUCTION

The International Atomic Energy Agency (IAEA) promotes and encourages the use of ionizing radiation for sterilization purposes. The support of this agency has resulted in the development of national and international programs in tissue banks in order to use ionizing radiation for the sterilization of tissues to allogenic (individuals who are genetically different but of the same species) transplants [1, 2]. The graft is part of a tissue transplanted from one area to another without nutritional relationship between the donor and the receptor area at the same organism or different organisms [3].

Chemical methods such as Ethylene Oxide (EtO), hydrogen peroxide, peracetic acid and glutaraldehyde, leave toxic residues on the material [4]. On the other hand, the sterilization by ionizing radiation is a method in which there is a minimum increase in temperature and leaves no toxic residues, which makes it suitable to be used, in addition to being a final sterilization. The effectiveness of the use of gamma radiation as a sterilization method is due to its great ability to penetrate the matter and its high efficiency in inactivating micro-organisms, in addition to allowing the sterilization of materials that are in previously unopened containers, thus preventing a posterior recontamination [2].

The aim of this study was to assess the quality and semi-quantitative characteristics of the human skin graft in healing areas of athymic NUDE mice, by the analysis of cells and blood vessels in tissue sections stained with hematoxylin and eosin (H/E).

2. MATERIALS AND METHODS

2.1. Preparation of the material for histological examinations

The samples were subjected to morphofunctional evaluation methodology of human skin preserved in glycerol at concentrations greater than or equal to 85%, and subjected to gamma radiation. They were divided into three groups, the non-irradiated (control), irradiated at 25 kGy and at 50 kGy, to evaluate the healing behavior of grafts in NUDE athymic mice. Euthanasia was performed 3, 7 and 21 days after grafting [5].
Tissues from animals were embedded in paraffin blocks, which were cut on a microtome (Leica Microsystens®) with a thickness of 4 micrometers, the slides were stained with hematoxylin and eosin (Merck, São Paulo, Brazil) (H/E).

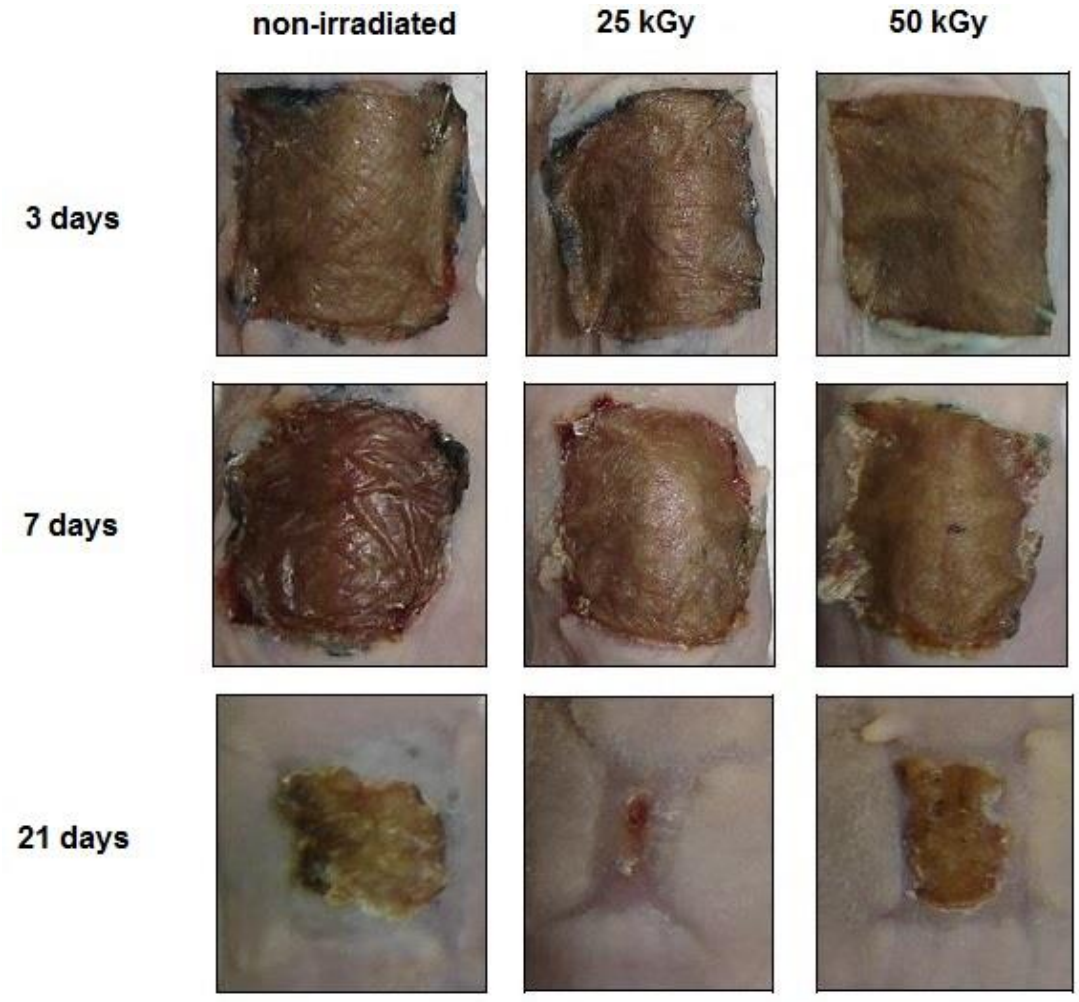


Figure 1: Area grafted with human skin, showing the repair of the tissue of animals in experimental groups 3, 7 and 21 days post-grafting, using non-irradiated human skin (control), skin irradiated at 25 kGy and at 50kGy [5].

2.2. Cell count method

Keratinocyte cells, fibroblasts, immune cells and blood vessels were counted. Analyzed by total area of the H/E slides, the standard used was 0 (no visualization of cells), +1 (for the visualization of up to 75 cells per slide), +2 (for the visualization of up to 150 cells per slide), +3 (for the visualization of up to 225 cells per slide), +4 (for the visualization of 350 or more cells per slide) (Fig. 2). The presence of the cells was placed as edge (for the edges of the tissue) and the center (for the central region of the tissue).

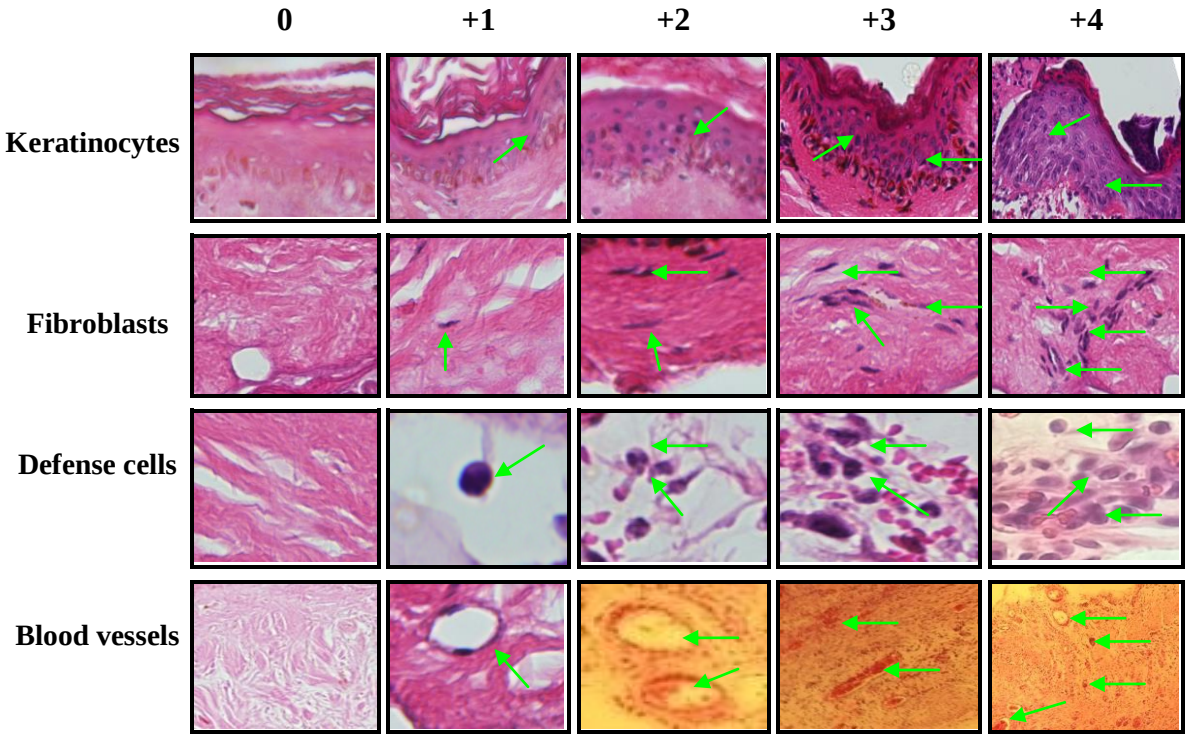


Figure 2: Histology of the H/E slides, showing the area grafted with human skin in the athymic mice, and the repair of the tissue of the animals in the experimental groups 3, 7, and 21 days after grafting, using non-irradiated human skin (control), irradiated at 25 kGy and 50 kGy, differentiating the quantity of cells on o, +1, +2, +3 and +4. The arrows (→) are indicating the localization of cell structures.

3. RESULTS AND DISCUSSION

3. 1. Cell Quantification

The comparison between the percentage of cell growth on the tissue, due to the moment in which the sacrifices were performed, and the doses applied at 25 kGy and 50 kGy and the non-irradiated group, in human skin tissues grafted onto nude mice, may indicate the level of tissue repair for each group. These data are shown in Fig. 3-A, B, C; 4-A, B, C; 5-A, B, C; 6-A, B, C; 7-A, B, C.

3.1.1. Keratinocytes

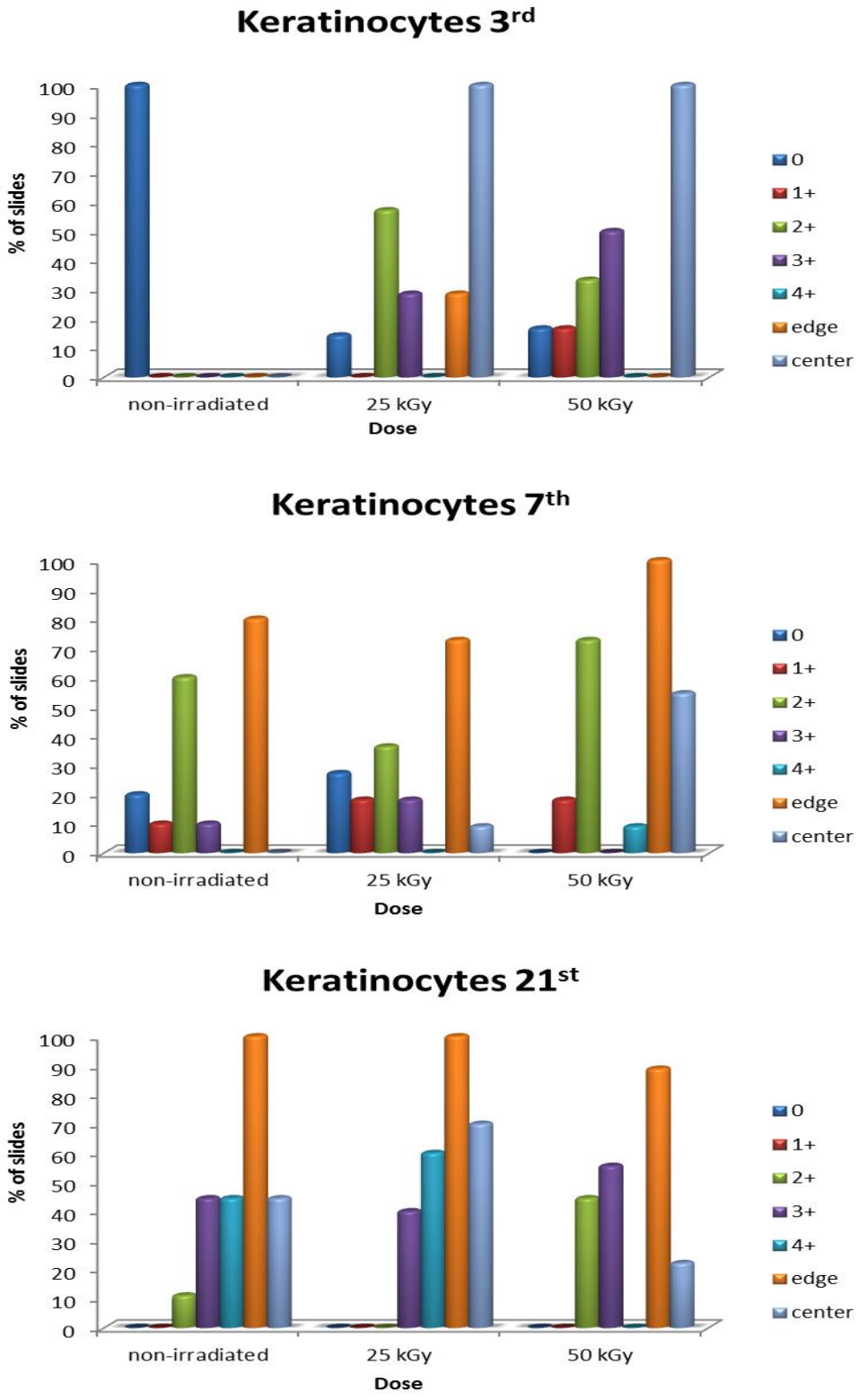
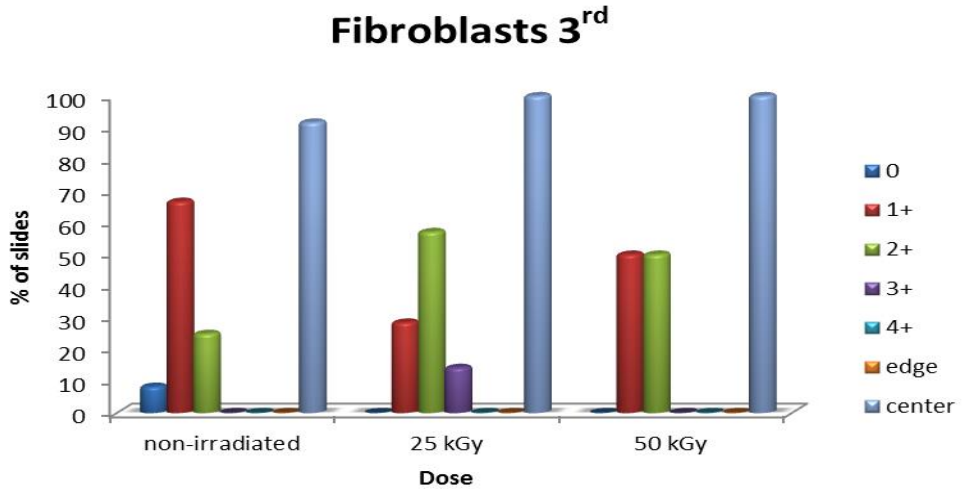
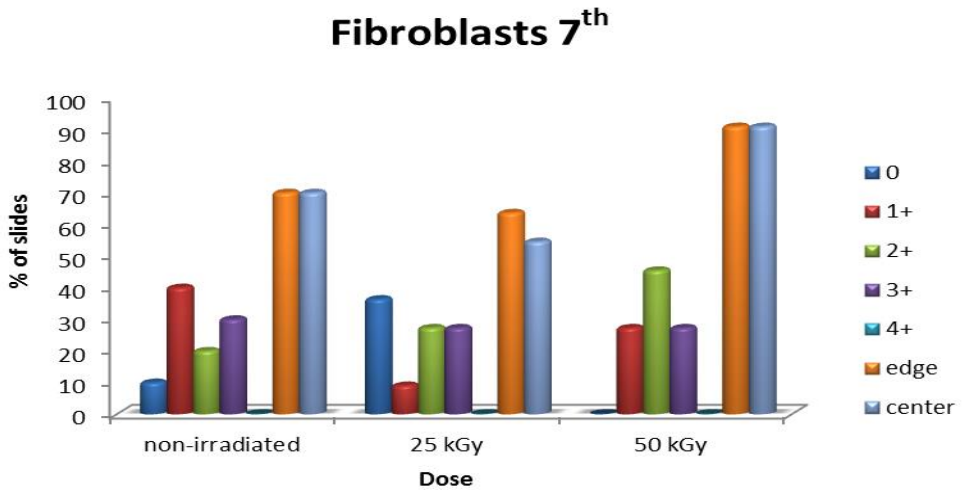


Figure 3 – Graph of the variation of the quantity keratinocytes in percentage (%) of samples slides (H/E) in non-irradiated group, irradiated at 25 kGy and 50 kGy. The representation of the quantity of cells is divided into 0 (■), +1 (■), +2 (■), +3 (■), +4 (■), edge (■) and center (■). A) sacrifice on the third day; B) sacrifice on the seventh day and C) sacrifice on the twenty-first day after grafting.

Based on Fig. 3-A, 3-B and 3-C can be observed, for the moment of the sacrifice, on the third, seventh and twenty-first days, that the quantities of keratinocytes gradually increase over time.
For the experimental period of the third day (Fig. 3-A), no keratinocytes was found in the non-irradiated group, only in the groups irradiated at 25 kGy and 50 kGy, viewing a minimum variation among them as for the amounts of cells, and increased proliferation of cells in the center of the tissue.
On the 7th day (Fig. 3-B) we can see a proliferation in the non-irradiated group. Groups irradiated at 25 kGy and 50 kGy had higher production of keratinocytes. The largest quantity of keratinocytes was found by the edges of the tissue, but the 50 kGy group had proliferation by the edges as much as by the center.
On the 21st day (Fig. 3-C) we can note that the production of keratinocytes in non-irradiated group was lower than in the group of 25 kGy. In the group of 25 kGy, the cell production is the highest compared to the other groups and its distribution covers the largest area both by the edges and by the center of the tissue. The 50 kGy group had smaller presence than the other two groups.

3.1.2. Fibroblasts





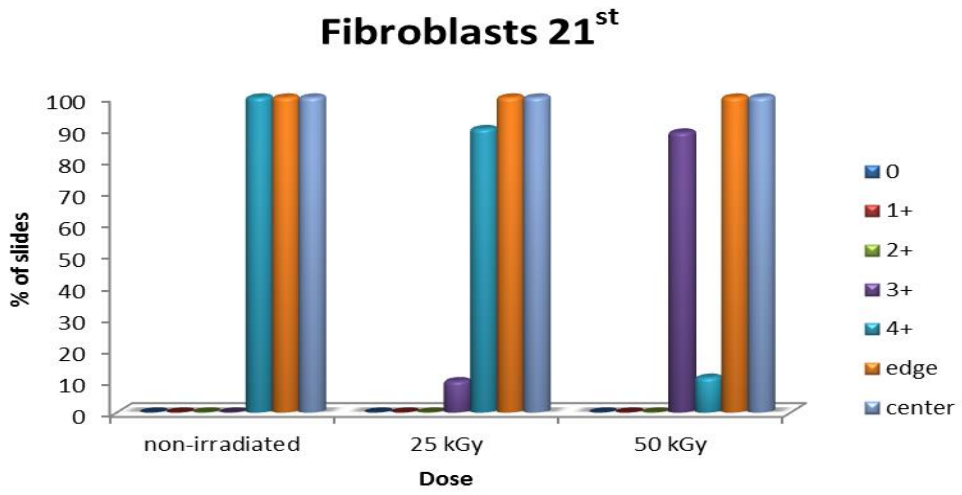


Figure 4 – Graph of the variation of the quantity fibroblasts in percentage (%) of samples slides (H/E) in non-irradiated group, irradiated at 25 kGy and 50 kGy. The representation of the quantity of cells is divided into 0 (■), +1 (■), +2 (■), +3 (■), +4 (■), edge (■) and center (■). A) sacrifice on the third day; B) sacrifice on the seventh day and C) sacrifice on the twenty-first day after grafting.

Regarding fibroblasts (Fig. 4-A, 4-B and 4-C) over time there is an increase in the quantity of fibroblasts, and on day 3 (Fig. 4-A) the proliferation occurs over the center of the tissue, and after 7 days (Fig. 4-B) it starts to appear both by edges and by the center of the tissue. The same is noted on day 21 (Fig. 4-C).

Analyzing the 3rd day (Fig. 4-A) the non-irradiated group presents smaller number of fibroblasts when compared to the other two groups. The group of 25 kGy presents a slightly higher percentage of fibroblasts when compared to the other groups. In all groups the fibroblasts were located only in the center of the tissue.

On the 7th day (Fig. 4-B) the amount of fibroblasts in the non-irradiated group, when compared to the other two groups, is only below 50 kGy group. The group of 25 kGy, has the lowest percentage of fibroblasts, so the group of 50 kGy presented the highest amount of fibroblasts. The non-irradiated group and the 50 kGy have an area of homogeneous distribution between edge and center.

On the 21st day (Fig. 4-C) on the non-irradiated group and on the group of 25 kGy we can not a massive presence of fibroblasts and on the group of 50 kGy, despite a large amount of cells, it is lower when compared to the other groups. The distribution of the fibroblasts area is homogeneous in all groups.

In time course, it can be noted an initial migration of fibroblasts, which over time were uniformly displaying themselves through the tissue, facilitating their proliferation.

3.1.3. Defense Cells

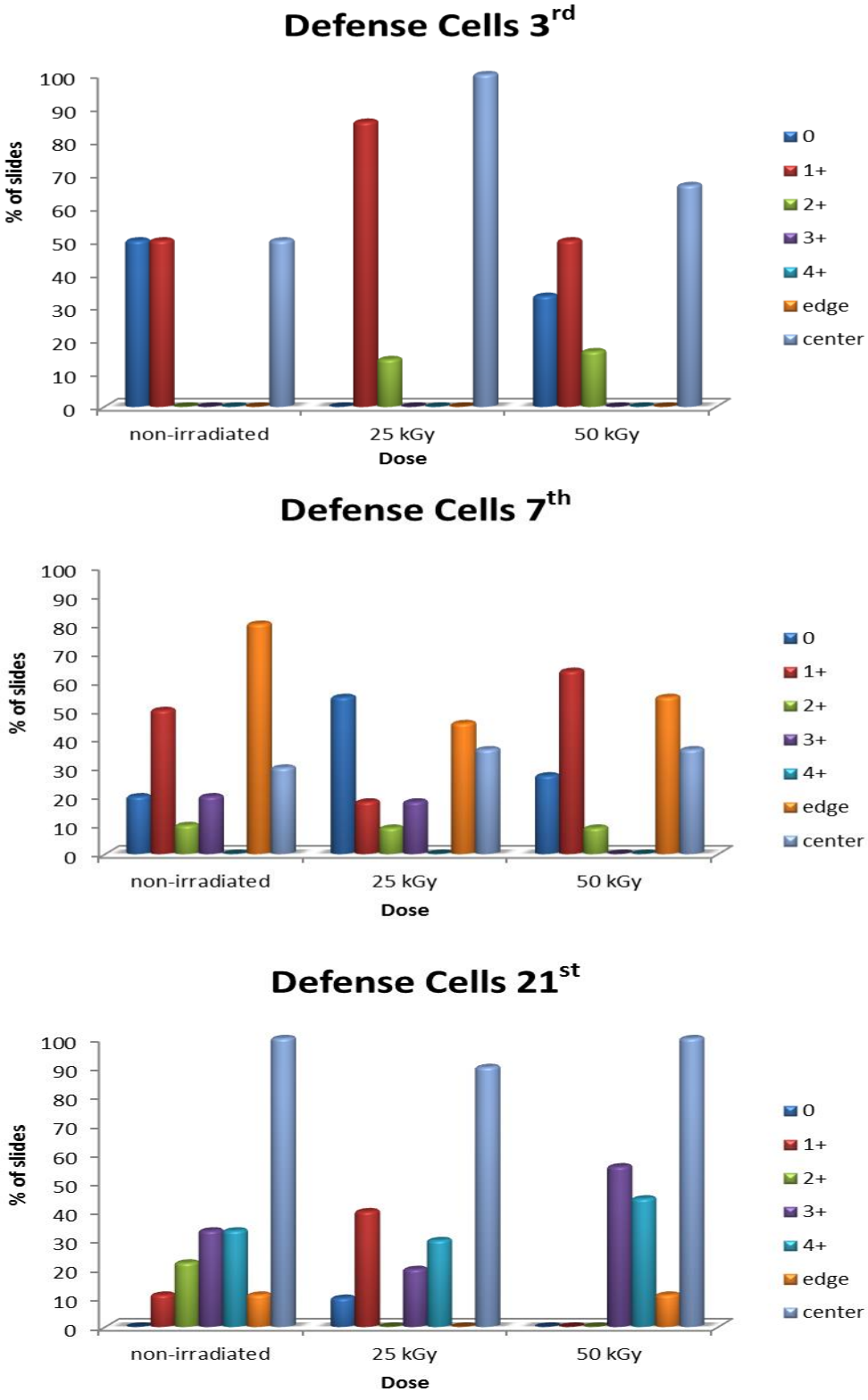
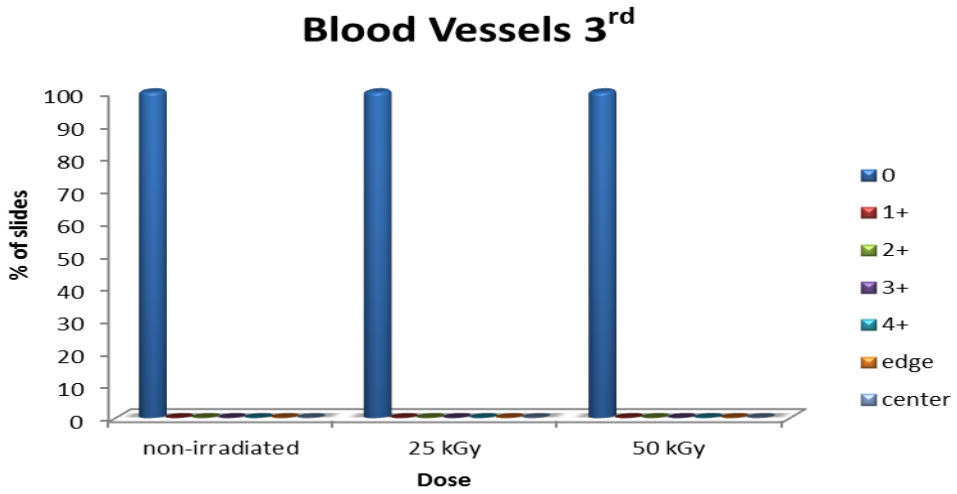
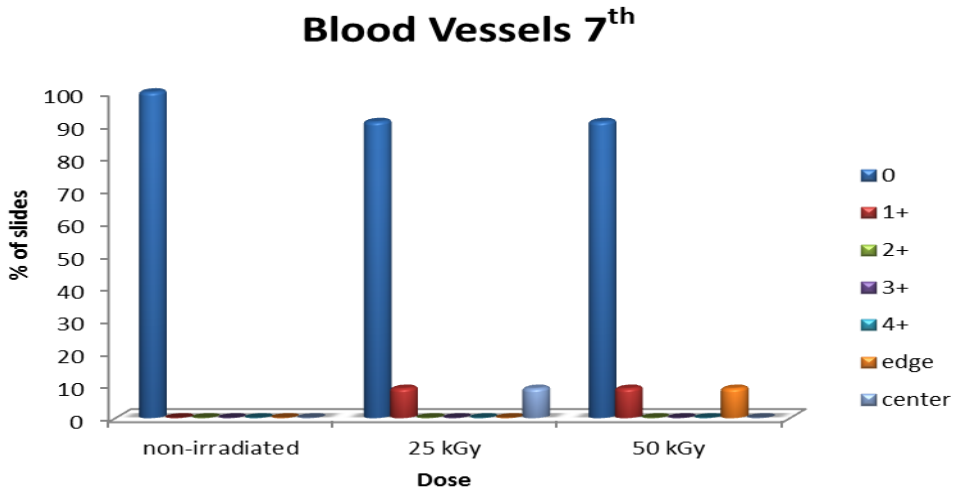


Figure 5 – Graph of the variation of the quantity defense cells in percentage (%) of samples slides (H/E) in non-irradiated group, irradiated at 25 kGy and 50 kGy. The representation of the quantity of cells is divided into 0 (■), +1 (■), +2 (■), +3 (■), +4 (■), edge (■) and center (■). A) sacrifice on the third day; B) sacrifice on the seventh day and C) sacrifice on the twenty-first day after grafting.

Observing the defense cells (Fig. 5-A, 5-B, 5-C) can be noted that the amount of cells between days and groups are different, but the region where these cells appear keeps a standard, in 3rd day (Fig. 5-A) the three groups present these cells in the center of the tissue, on the 7th day (Fig. 5-B), the cells are more concentrated on the ends but also appear in the center of the tissue, on the 21st day (Fig. 5-C) the number of cells on the edges is too low and the greater amount appears in the center of the tissue. This is due to the contraction of the wound and the formation of the crust, which on the 21st day appears more by the center of the tissue, as can be seen in Fig. 1.
Data from the 3rd day (Fig. 5-A) show that the non-irradiated group has the least amount of defense cells, while the groups irradiated at 25 kGy and 50 kGy showed a greater production of them, and the three groups showed a predominance of defense cells in the region closer to the center of the tissue.
On the 7th day (Fig. 5-B) the non-irradiated group has increased production of defense cells when compared to groups 25 kGy and 50 kGy, a pattern that appears between the three groups is the location of the defense cells in the tissue, which appear more by the edges, but also by the center.
On the 21st day (Fig. 5-C) the group 25 kGy has the smallest amount of cells when compared to the other two groups. The group of 50 kGy has the highest concentration of these cells and the pattern of location of these cells remains the same for the three groups, being almost entirely by the center of the tissue.

3.1.4. Blood Vessels





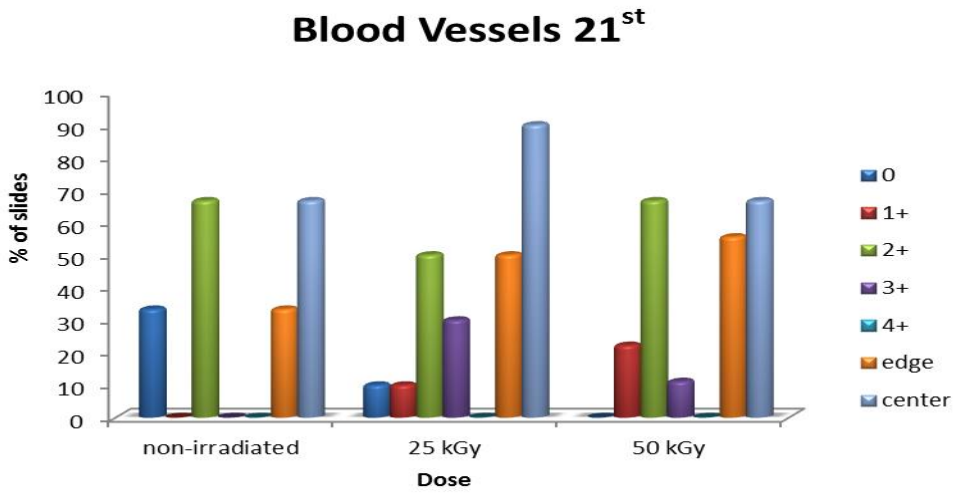


Figure 6 – Graph of the variation of the quantity blood vessels in percentage (%) of samples slides (H/E) in non-irradiated group, irradiated at 25 kGy and 50 kGy. The representation of the quantity of cells is divided into 0 (■), +1 (■), +2 (■), +3 (■), +4 (■), edge (■) and center (■). A) sacrifice on the third day; B) sacrifice on the seventh day and C) sacrifice on the twenty-first day after grafting.

Analyzing the data of the blood vessels (Fig. 6-A, 6-B, 6-C) we can note that on day 3 (Fig. 6-A) there is no structure of blood vessels.
On the 7th day (Fig. 6-B) only the irradiated groups showed a small amount of these structures and in the group irradiated at 25 kGy the structures were found by the center of the tissue and in group of 50 kGy, by the edges of the tissue.
An increase in the number of blood vessels occurred only on day 21 (Fig. 6-C) and non-irradiated group had the least amount of vessel structures. The slides analyzed of the group 25 kGy showed the greatest development among the three groups. All three groups showed an increase by the ends of the tissue, but its greatest presence occurred by the center. The blood vessels are responsible for the oxygenation of the wound and the cells transport [6].

4. CONCLUSION

With the analysis of several cell types and the formation of blood vessels (Figures 3, 4, 5 and 6) evaluated at different times, it can be concluded that the tissue irradiated at 25 kGy had better repair and less healing time when compared to the other two groups, and greater contraction of the grafted area, as seen in Figure 1. The second best group was the group irradiated at 50 kGy. The non-irradiated group showed, over time, the least amount of cells and a slower healing time.

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