Bioactivity of PDGF-BB-loaded NLCs after sterilization with gamma or beta radiation

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1. Introduction

Growth factors are cytokines that stimulate cell proliferation, differentiation and/or activation. Despite this, they are very sensitive to chemical and enzymatic degradation [1]. To protect them, they can be encapsulated within drug delivery systems, such as nanostructured lipid carrier (NLCs), which also enable controlled pharmacokinetics [2]. As the final objective of this nanoparticle system is the administration to the patient, one of the main requirements is sterility. The use of gamma and beta radiation is considered one of the most efficient techniques for the reduction of microorganisms [3].

In the present study, PDGF-BB growth factor, which is related to cell proliferation and migration, was encapsulated in NLCs. Then, the nanoparticles were submitted to gamma or beta radiation at two different doses. Sterilization was verified by a sterility test and the changes that each of the radiations might provoke in the biologic effect of the released factor was studied.

2. Materials and methods

2.1. NLCs preparation

To obtain the NLCs, an o/w emulsion was formed using the hot melt homogenization technique. Subsequently, the final product was lyophilized.

2.2. NLCs sterilization

NLCs were irradiated with gamma or beta radiation. The doses to be irradiated were 12 kGy and 25 kGy in both cases but there are methodological differences. Gamma radiation is more penetrating than beta radiation, with a longer exposure time (up to hours). In contrast, with beta radiation it is a few minutes.

2.3. Sterility test

To verify that the nanoparticles were correctly sterilized, we followed the test from the Royal European Pharmacopoeia. Sterilized NLCs were added to two different media, Fluid Thioglycollate Medium and Tryptic Soy Broth. Next, they were cultured at 32±2°C and 22±2°C respectively, for 14 days.

2.4. Bioactivity assay

A wound healing assay was performed with human mesenchymal stem cells. Photos were taken every 20 minutes for 48 hours to observe the wound closure using a Cytation (BioTek, Winooski, United States). Images were subsequently analyzed.

3. Results and Discussion

The sterility test was performed to check if the different doses of the two types of radiation were sufficient for achieving sterility of the NLCs. The results collected in Figure 1 show that at a dose of 25 kGy, both gamma and beta radiation were able to assure sterility. In contrast, when doses of 12 kGy were applied, beta radiation was able to kill all microorganisms and spores. However, when gamma radiation was used, microbial growth was observed. In Tryptic Soy Broth, the appearance of turbidity in the medium was observed, which indicates the growth of aerobic bacteria and fungi. Therefore, the 12 kGy dose of gamma radiation is not sufficient for achieving sterile NLCs.

One of the possible risks of using radiation is that it might affect the bioactivity of the factor encapsulated within the NLCs. For this reason, a bioactivity study was carried out to verify that there was no loss of activity of the released factor. Figure 2 shows the images collected from the bioactivity assay were differences can be observed among the images obtained at 48 hours. The wound of non-irradiated PDGF-BB and the one of PDGF-BB released from irradiated NLCs at the lower dose (12 kGy, both types of radiations) was very similar, achieving a complete confluence and closure of the wound. In contrast, PDGF-BB released from NLCs irradiated with 25 kGy did not completely close the wound, although it achieves a greater effect when beta radiation is used.

To analyze these images, a graph showing percentage of wound closure as a function of time is plotted and shown in Figure 3. When gamma radiation was used (Figure 3A), the 25 kGy dose showed no improvement in wound closure compared to baseline. Indicating that the dose is too aggressive for the factor, losing its activity. The

12 kGy dose was able to close the wound, as was the factor released by non-irradiated NLCs, although there was slight factor damage, as the rate of closure was lower in the case of irradiated NLCs.

In the case of beta radiation (Figure 3B), both the factor released from non-irradiated NLCs, as well as those irradiated with 12 kGy and 25 kGy, showed a greater effect than that of the basal condition. With a dose of 12 kGy, the curve was the same as when the nanoparticles were not irradiated, suggesting that dose does not modify the activity of the factor. In contrast, when the 25 kGy dose is used, there is a slight decrease in confluence in the wound. This fact might indicate that the higher dose slightly affects the activity of PDGF-BB.

4. Conclusions

It was observed that in both types of radiation, when sterilizing with 25 kGy dose, the activity was always lower than with 12 kGy, since the treatment is more aggressive. In addition, the data reflect that gamma radiation causes a greater loss of activity. This could be due to longer irradiation times, which means a higher temperature rise.

Therefore, the best option in order to achieve sterilized encapsulated PDGF-BB in NLCs might be to irradiate them with 12 kGy of beta radiation. In this way, we manage to sterilize the nanoparticles without affecting their biological activity.

5. Acknowledgements

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Figures

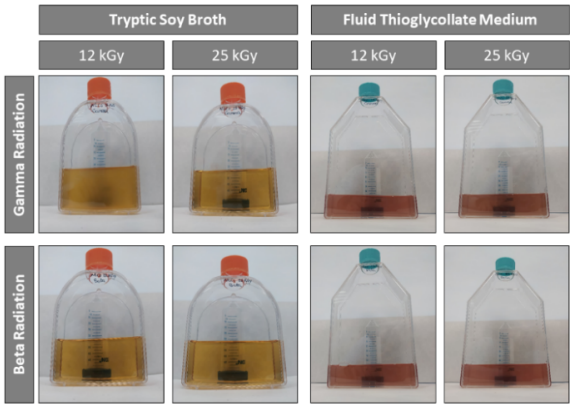

Figure 1. Sterility test, performed for 14 days.

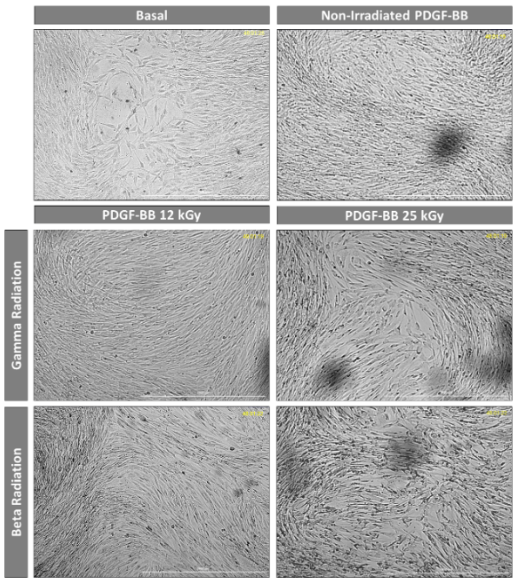

Figure 2. Images of the wound healing assay at 48 hours on human mesenchymal stem cells.

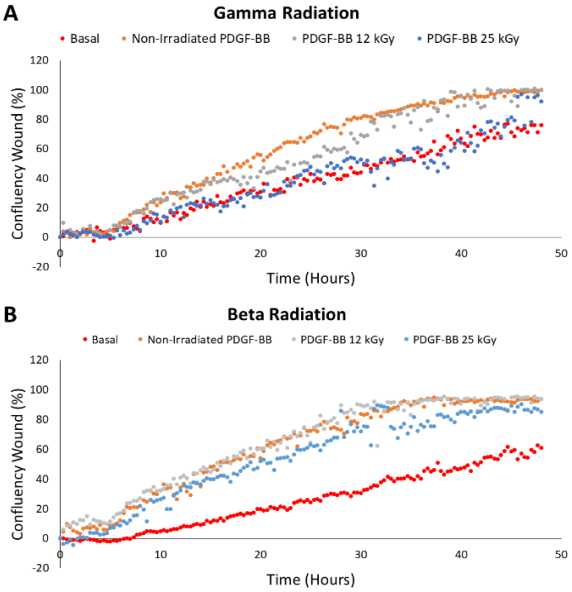

Figure 3. Wound healing assay plots, Confluency wound (%) vs. time. **A.** Gamma radiation. **B.** Beta radiation.