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A B S T R A C T

Background: External radiotherapy has become indispensable in oncological therapies. Unfortunately, radiation is responsible for serious side effects, such as radiodermatitis. The skin is weakened and ulcerated. Our study aimed to evaluate the subcutaneous transfer of microfat (MF) alone and two mixes: MF+Platelet-rich plasma (PRP) and MF+stromal vascular fraction (SVF) to treat radiation-induced skin lesions.
Method: We defined randomly five experimental groups of nine mice: 1 healthy control group and 4 irradiated (60 Grey) and treated groups. The skin lesions were treated 3 months after irradiation by MF, MF+PRP (50%–50%), MF+SVF (90%–10%) or Ringer-lactate subcutaneous injections. Wound healing was evaluated at 1, 2 and 3 months post-injection and histological wound analysis at 3 months, after euthanasia.
Results: All the irradiated mice presented with wounds. After sham-injection, the wound area increased by 91.1±71.1% versus a decrease of 15.9±23.1% after MF alone (NS), 27.3±23.8% after MF+SVF (NS) and 76.4±7.7% after MF+PRP ($P=0.032$). A significative reduction of skin thickness in wound periphery was measured for the three treated groups compared to sham-injection ($P<0.05$) but not in the healed wounds (NS). The most important subcutaneous neo-vessel density was shown after MF+SVF injection.

Introduction

External radiotherapy has become indispensable in oncology therapy, conferring many benefits to patients. Unfortunately, radiation is responsible for serious and frequent side effects, such as radiodermatitis [1]. Skin directly exposed to radiation becomes thickened and fibrotic, with diminished microvascularisation [2]. After an initial phase of vascular hyperpermeability, microvascular density decreases due to fibrotic and irregular capillaries, which are often occluded [3]. The epidermis is weakened and ulcerations appear a few months or years after radiation [4]. Healing of these ulcers is poor, and the wounds become chronic. A surgical procedure is frequently necessary to excise the damaged skin and replace it with a flap [5,6].

Since the fat transfer procedure was developed by Sydney Coleman in 1995 [7], many studies have shown a regenerative, as well as a volumizing, effect of the fat [8]. Injecting autologous fat under irradiated skin resulted in a procicatrizing and antifibrotic action in preclinical [9] and clinical studies [10,11]. The stromal vascular fraction (SVF) of the adipose tissue is the product remaining after enzymatic digestion of the mature adipocytes. This cell-based product seems to be the principal actor in the regenerative effects [12–15] of autologous fat grafting [16]. Platelet-rich plasma (PRP), a concentrate of blood platelets, is an attractive product to promote healing of chronic wounds [17,18]. PRP delivers various growth factors that stimulate neoangiogenesis in a paracrine manner [19]. These three autologous products (fat, SVF and PRP) are interesting, both separately and together, for the treatment of skin lesions induced by radiation. No study has compared the action of these different regenerative products on wound healing in the context of radiodermatitis.

The objective of our study was to evaluate and compare the efficacy of microfat (MF) alone, PRP mixed with MF, and SVF mixed with MF versus a control treatment in a mouse model of radiation-induced skin-lesions. A positive effect of these new cell-based products would demonstrate the potential for clinical application to treat or prevent radiation-induced skin lesions.

Materials and methods

2.1. *Animals*

This experimental study was approved by the National Animal Care and Ethics Committee (#00506.02). Based on previous experimental studies by our stem cell laboratory

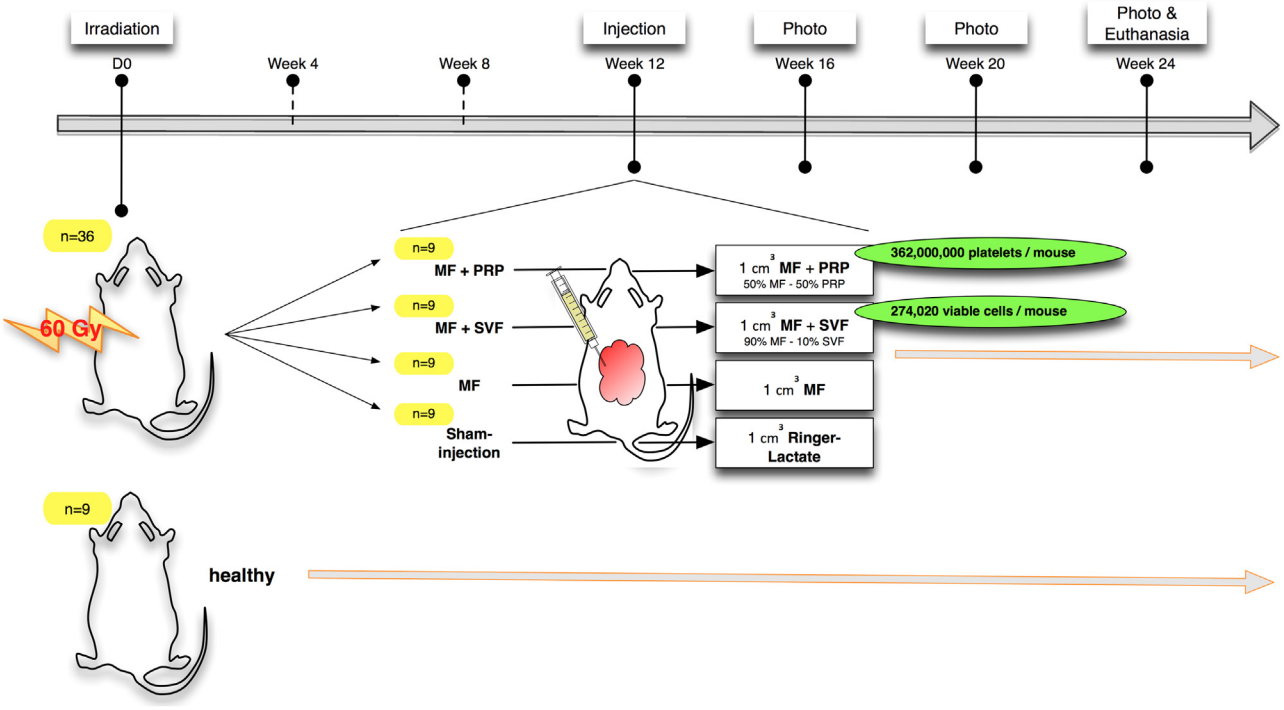

Fig. 1 – Design of the experimental study.

and by Sultan et al. [9] and Fogeron et al. [20] demonstrating the positive effect of fat grafting on radiation skin damage in murine models, we determined that nine mice per group would be sufficient to show a significant difference between the various products evaluated. We could not predict the number of deaths a priori because our model is the first murine model of skin ulceration after radiotherapy. To limit the effect of mortality on the decrease in power of the tests, we have planned non-parametric tests to take into account the lack of normality of groups by using all the test data. We also included more subjects than previously published studies to provide enough subjects for comparison. Thus, forty-five male or female nude NMRI-Foxnu1nu/nu mice (8 weeks old, 28 g) were purchased from the January Lab (Genest-Saint-Isle, France).The mice were randomly divided into two groups: nine control mice and 36 mice exposed to external radiotherapy.

2.2. *Experimental groups*

Nine mice each were randomly assigned to five experimental groups (Fig. 1): four treated groups (MF alone, MF+SVF, MF +PRP and sham injection see Table 1) exposed to radiation and one healthy control group. The mice were housed in individual cages after radiotherapy. Treatment was delayed for 3 months to reproduce the chronic radiodermatitis wounds and lesions. After treatment, the mice underwent a 3-month follow-up before euthanasia.

2.3. *Irradiation*

Targeted irradiation of the dorsal cutaneous lining was carried out simultaneously for mice of the four treated groups (n=36) after general anaesthesia with an intraperitoneal injection of ketamine/xylazine (200/10 mg/kg). The dorsal skin was retracted from the underlying bony skeleton, maintained by two separated sutures between two 20 cm × 20 cm fields of plexiglass. The two sutures were reproducibly spaced 3 cm apart, centred on the midline of the back of each mouse, so as to hold the dorsal skin of each mouse 1 cm under the plexiglass field. Four blocks of 8-cm-thick Cerrobend (Cerro Metal Products, Bellefonte, PA, USA) were placed on each edge of the Plexiglas above the mice to protect the body and spine from the diffusion of the radiation. (Fig. 2). The irradiation was delivered at a dose of 60 Gy in one targeted fraction in a 20 cm × 20 cm irradiation field, at the exact size of the plexiglass plates, using a linear accelerator Elekta synergy system (Elekta Beam Modulator; Elekta Oncology Systems, Crawley, UK) with 6 MV photons. The accelerator arm was set to 180°, and the collimator was set to 0° with a prescription at 1.5 cm and a skin source distance of 100 cm. The flow rate was 400 EU/min. Plexiglass is an homogeneous medium. Thus the irradiation delivered on the irradiation field was identical at all points under the plexiglass plate.

2.4. *Cell therapy products*

Cell therapy products were obtained from a healthy 35-year-old woman volunteer donor following provision of written informed consent. The lipoaspirate residue and a blood sample were recovered during the aesthetic liposuction procedure. Fat and MF were harvested from the lateral flank areas, and peripheral whole blood was taken during the intervention. The donor had no relevant diseases and was free of any drugs known to affect platelet function for 7 days before sampling. All cell therapy products were manufactured in a

Table 1 – Composition of the various products evaluated in this study.

	PRP		SVF		Microfat	Ringer-lactate
Sham injection (n=9 mice)	–		–		–	1 cm^3
MF (n=9 mice)			–		1 cm^3	
MF – PRP (n=9 mice)	0.5 cm^3				0.5 cm^3	
MF – SVF (n=9 mice)	–		0.1 cm^3		0.9 cm^3	
Characteristics and Composition	Injected cells/mouse		Viable injected cells/mouse		**Canula**St'RIM canula14 Gauges **Purification** Centrifugation 1200 × g – 3 min	–
	Platelets (×10^6)	36295.6%	Viability	80%		
	Red Blood Cells (×10^6)	15 3.9%	Leukocytes Including *Macrophages/Monocytes* *Lymphocytes* *Neutrophils*	46910 (17.6%) *7488 (2.8%)* 33388 (12.5%) *6034 (2.3%)*		
	Leukocytes (×10^6)	1.8 0.5%	Endothelial Progenitor Cells	106731 (40.2%)		
	Increase factor in platelets	2.26	Pericytes	48411 (18.2%)		
	Increase factor in leukocytes	0.5	Stromal Cells	63948 (24.0%)		

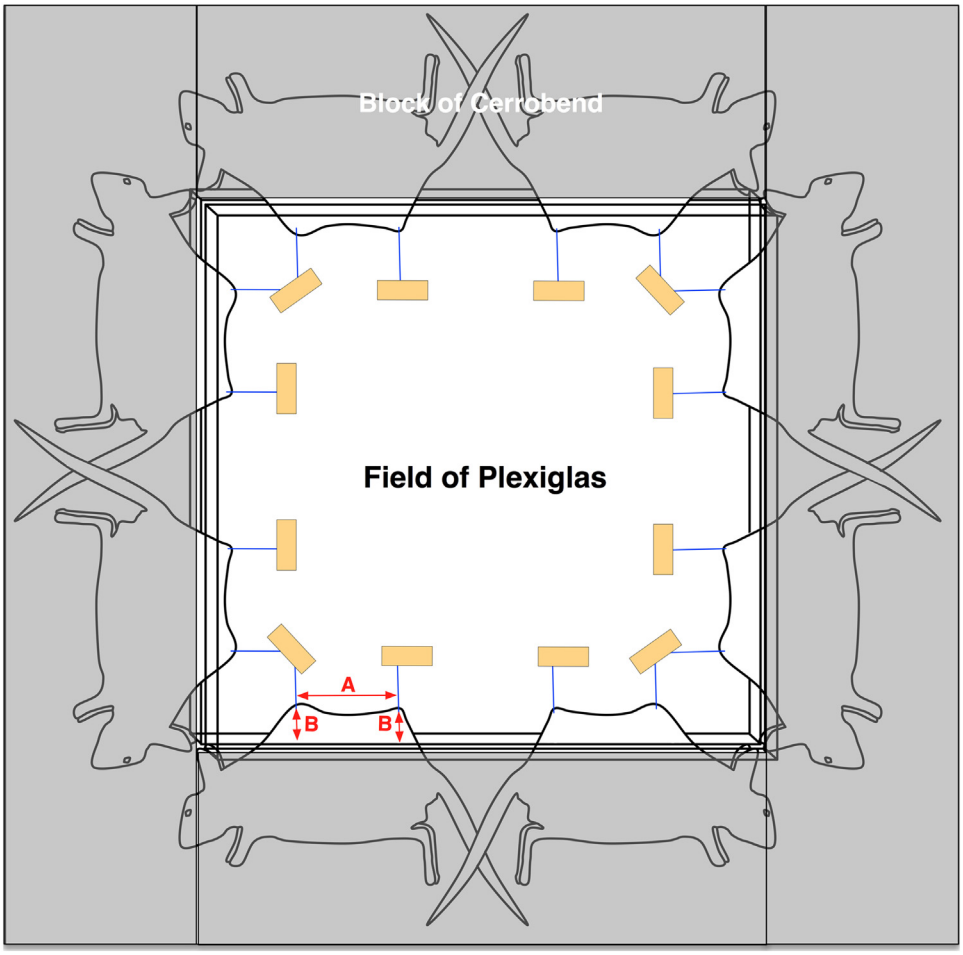

Fig. 2 – Experimental irradiation protocol. The dorsal skin was retracted from the underlying bony skeleton, maintained by two separated sutures between two fields of Plexiglass. The two sutures were reproducibly spaced 3 cm apart (A), centred on the midline of the back of each mouse, so as to hold the dorsal skin of each mouse 1 cm under the plexiglass field (B). Four blocks of 8-cm-thick Cerrobend (Cerro Metal Products, Bellefonte, PA, USA) were placed on each edge of the Plexiglas above the mice to protect the body and spine from the radiation. The irradiation was delivered at a dose of 60 Gy in one targeted fraction on the Plexiglas field by a linear accelerator Elekta synergy system (Elekta Beam Modulator; Elekta Oncology Systems, Crawley, UK) with 6 MV photons.

Class A microbiological safety facility located in the culture and cell therapy laboratory of our university hospital and injected on the same day, within 3 h of harvest.

2.5. *Microfat*

The MF (36 cm^3) was aspirated with a 14G cannula from the st'RIM procedure pack (Thiebaud Biomedical Devices, Margencel, France) for MF transfer. The MF was purified after centrifugation (1200 × *g* for 3 min) with a microcentrifuge (Medilite; Thermo Scientific, Waltham, MA, USA) to eliminate oily and bloody residue.

2.6. *Stromal vascular fraction*

Fat (184 cm^3) was aspirated with a 12 G, 12-hole harvesting cannula (Khouri Harvester, Koume, Plantation, FL, USA) mounted on a 10 cm^3 syringe. SVF was purified by enzymatic digestion of mature adipocytes during 45 min at 37° using a manual method and collagenase at the concentration of 0.25 U/mL (NB5, Heideberg, Germany). Total viable nucleated cell recovery and cell viability were determined using the Nucleocounter NC100 instrument (ChemoMetec, Denmark). Cellular components within isolated SVF were identified by a flow cytometry analysis (Beckman Navios instrument) using a panel of cell surface makers in agreement with International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) recommendations [21].

2.7. *Platelet-rich plasma*

PRP was produced according to a previously described cell therapy methodology [22,23]. Briefly, 18 cm^3 of whole blood was collected from the same healthy donor with 4 cm^3 adenosine citrate dextrose-acid solution (ACD-A, Ref.

BDB8651; Fenwal Inc., Portland, OR, USA) to make a 22 cm^3 solution. Another tube, coated with the EDTA anticoagulant, was used to determine the number and concentration of platelets with an automatic cell counter (ADVIA® 2120; Siemens Diagnostic Solutions, Malvern, PA, USA). The blood was centrifuged at 130 × *g* for 15 min and then again at 250 × *g* for 15 min in two 11 cm^3 conical tubes (NUNC®, Ref. 56423; Thermo Scientific) using a microcentrifuge (Medilite®, Ref. 448; Thermo Scientific). The supernatant or platelet-poor plasma (PPP) was removed by gentle aspiration (approximately 1 cm^3/tube). The PRP pellets were resuspended in the residual PPP and pooled. At the final measurement, we obtained a total of 5.6 cm^3 concentrated PRP. A 250 µl sample was used to determine the final PRP formulation with an automatic cell counter (ADVIA® 2120; Siemens Diagnostic Solutions, Erlangen, Germany).

2.8. *Mixtures*

MF, PRP and SVF were prepared in a cell therapy laboratory (Table 1). All products were packaged under sterile conditions in 1 cm^3 syringes for reinjection into the mice. Ringer's lactate was used as the medium to resuspend the SVF. The MF+SVF mix was in our initial hypothesis the most efficient product evaluated. Thus, Ringer-Lactate, as a medium for SVF resuspension, was chosen as the placebo for the sham-injection group.

2.9. *Injections*

After mixing when necessary, the injection procedure was performed blindly under general anaesthesia by inhalation of halogenated gas (sevoflurane; Baxter France, Maurepas, France). Two diametrically opposed 18 G punctures were made 5 mm from each wound to introduce the 21 G blunt cannula for reinjection into the st'RIM pack (Thiebaud Biomedical Devices). A 1 cm^3 syringe of MF, MF+PRP, MF +SVF or Ringer's lactate was injected using the conventional Coleman's procedure. The product was evenly distributed under the wound in each mouse.

2.10. *Animal examination*

The weight of each mouse and the size of the wounds were measured on the day the products were injected, and every month thereafter during the 3-month post-treatment period under general halogenated gas anaesthesia (sevoflurane; Baxter). Healing was evaluated by measuring standard digital photographs of the wounds using ImageJ 1.45s freeware (National Institutes of Health, Bethesda, MD, USA; http://imagej.net/ImageJ); a fixed landmark (millimetre ruler) was included in each photograph [24]. The percentage of healing was calculated by relating the measured wound area to the area of the initial wound (100 − ((wound area/initial wound area) × 100)).

2.11. *Histological examination*

Blinded histological analyses were performed by a skin pathologist. After the mice were euthanised, their skin was fixed in 10% formalin solution and embedded in paraffin. Then, 5-µm-thick sections were stained with haematoxylin and eosin, Masson's trichrome and orcein to evaluate the histopathological changes and detect collagen fibres. The sections were examined under a light microscope (Axiophot; Zeiss, Oberkochen, Germany) and photographed with a digital camera. A semi-quantitative evaluation was performed to assess the skin fat grafts (0, no trace of fat; +, trace of fat; ++, fat still present) [22]. Epidermal, dermal and total skin thicknesses were measured quantitatively in the skin exposed to radiation on the wound periphery and in the healed wound area. Skin thicknesses were calculated using GraphPad software (GraphPad Inc., La Jolla, CA, USA). Six measurements were made per mouse, and the means were compared for each experimental condition. A semi-quantitative evaluation was performed to evaluate subcutaneous vascularisation using the following scale: 0, capillaries present in normal numbers and showing normal morphology; +, increased number of congested capillaries; ++, numerous congested capillaries.

2.12. *Statistical analyses*

All statistical data are expressed as means ± standard error of the means (SEM). Difference between groups were assessed by Kruskal–Wallis and Mann–Whitney *U* tests or Nonparametric analysis of longitudinal data. A probability values of $P<0.05$ were considered significant. Bonferroni corrections were made for multiple testing. Data were analysed using nparLD package of R software (http://www.R-project.org/) and SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Results

3.1. *Clinical manifestations of the radiation*

Irradiation induced skin sclerosis and chronic wounds in the exposed dorsal skin. The irradiated dorsal skin appeared thicker and indurated to the touch. All treatment group mice presented with wounds (wound area was 144 ± 15 mm^2 on average, no statistical difference between the 4 groups ($P=0.563$)) at 3 months after irradiation and showed a loss of body weight compared to healthy mice (irradiated mice body weight=29.65 ± 0.41 g; healthy mice body weight=31.37 ± 0.31 g, $P=0.041$).

3.2. *Characterisation and injection of the different cell therapy products*

3.2.1. *Stromal vascular fraction*

We obtained 10.3 cm^3 of SVF from 184 cm^3 harvested fat, corresponding to 27.4 million of viable nucleated cells leading to 274000 viable nucleated cells injected/mouse. The cellular composition of the SVF is detailed in Table 1.

3.2.2. *Platelet-rich plasma*

We obtained 5.6 cm^3 PRP with a platelet concentration of 724 G/l, corresponding to 362 million of platelets injected/mouse. The cell composition of the PRP is shown in Table 1. This

formulation was similar to that previously reported for purified PRP [23,25].

3.3. *Animal evaluation*

We noted two deaths in the placebo group (at 1 and 2 months post-treatment), two deaths in the MF+SVF group (at 2 and 3 months post-treatment) one death in the MF+PRP group (at 2 months post-treatment) and one death in the MF alone group (at 1 month post-treatment) during the 3 month post-treatment period. Body weight in the placebo group was 30.39±0.55 g vs. 32.11±0.76 g in the MF+SVF group, 31.76 ±0.75 g in the MF+PRP group and 31.89±0.53 g in the MF group (NS, $P=0.231$) at 3 months post-treatment.

3.4. *Wound healing evaluation*

The wound area in the sham-injection group increased by 91.1 ±71.1% on average (−11.0±29.5 mm^2 of wound area healed on average, initial wound area: 129.2±27.2 mm^2) at 3 months post-treatment. In contrast, the wounds in all treated groups had partially healed at 3 months after treatment. We report that 15.9±23.1% on average of the wound area healed in the MF group (NS) (61.0±42.7 mm^2 of wound area healed on average, initial wound area: 147.6±33.0 mm^2 on average), 27.3±23.8% on average of the wound area healed in the MF+SVF group (NS) (39.5±24.2 mm^2 of wound area healed on average, initial wound area: 176.5±27.2 mm^2 on average)and 76.4±7.7% on average of the wound area healed in the MF+PRP group ($P=0.032$) (99.3±19.3 mm^2 of wound area healed on average, initial wound area: 121.3±21.5 mm^2) (Fig. 3).

3.5. *Histopathological changes after treatment*

Three months following treatment, the regenerative epidermis of the treated mice was thicker. In particular, the healing margins displayed more hyperplasia compared to the skin of the mice that had received sham injection. In the dermal granulation tissue, in which small clusters of adipocytes were stated in the mice treated by MF+PRP, higher cellularity was

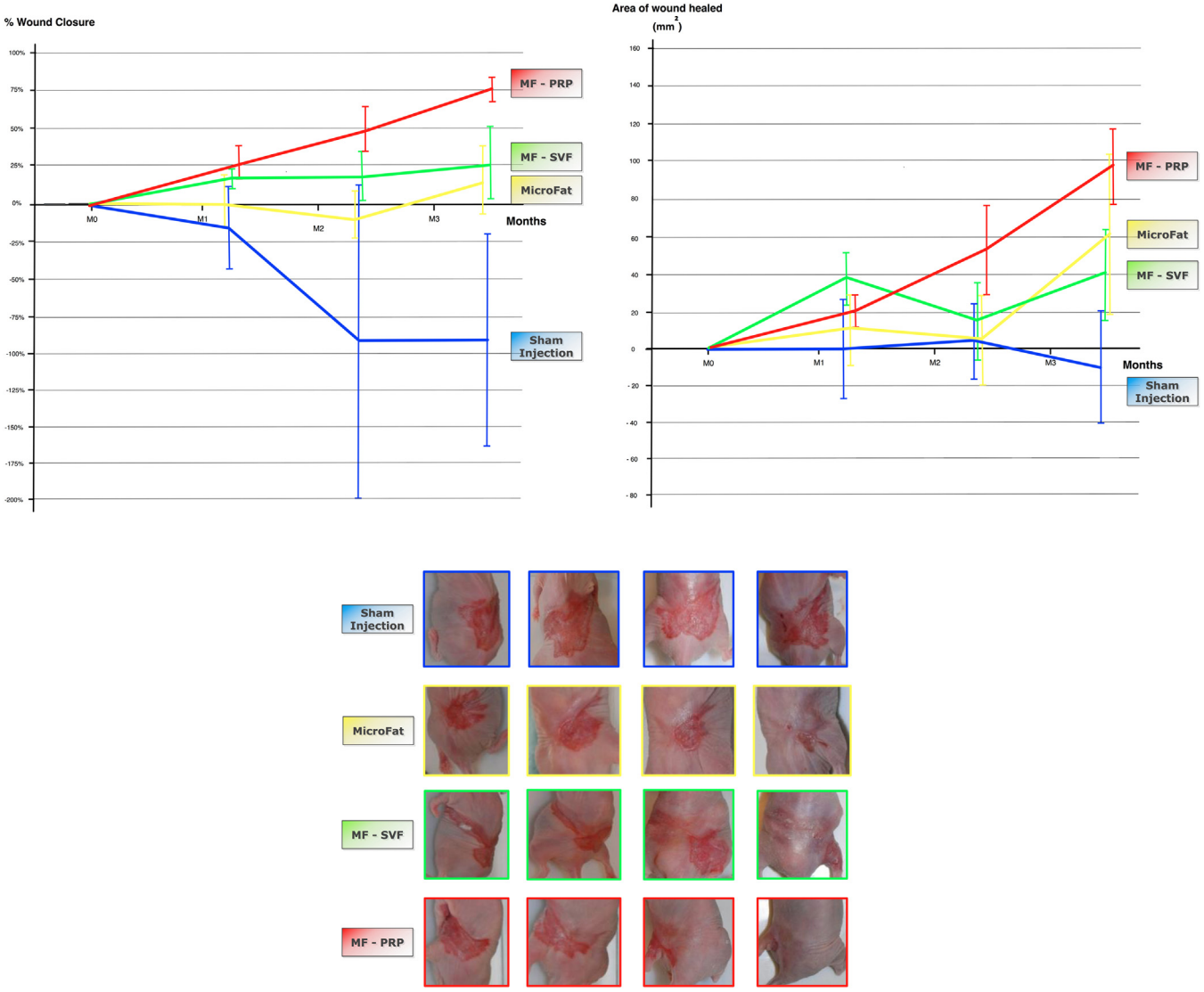

Fig. 3 – Comparison of wound healing from irradiation-induced skin ulcers after treatment with a sham-injection (Ringer's lactate), microfat (MF), MF+platelet-rich plasma (PRP) and MF+the stromal vascular fraction (SVF) during the 3 month follow-up.

Experimental Conditions	Dermal Thickness (µm)	Epidermal Thickness (µm)	Total Skin Thickness (µm)
Sham – Injection	380 ± 121	70 ± 27	450 ± 148
Micro-Fat	191 ± 14	20 ± 0	211 ± 14
SVF + MF	174 ± 18	21 ± 1	196 ± 18
PRP + MF	163 ± 21	24 ± 2	188 ± 24
Control	173 ± 6	22 ± 1	176 ± 6

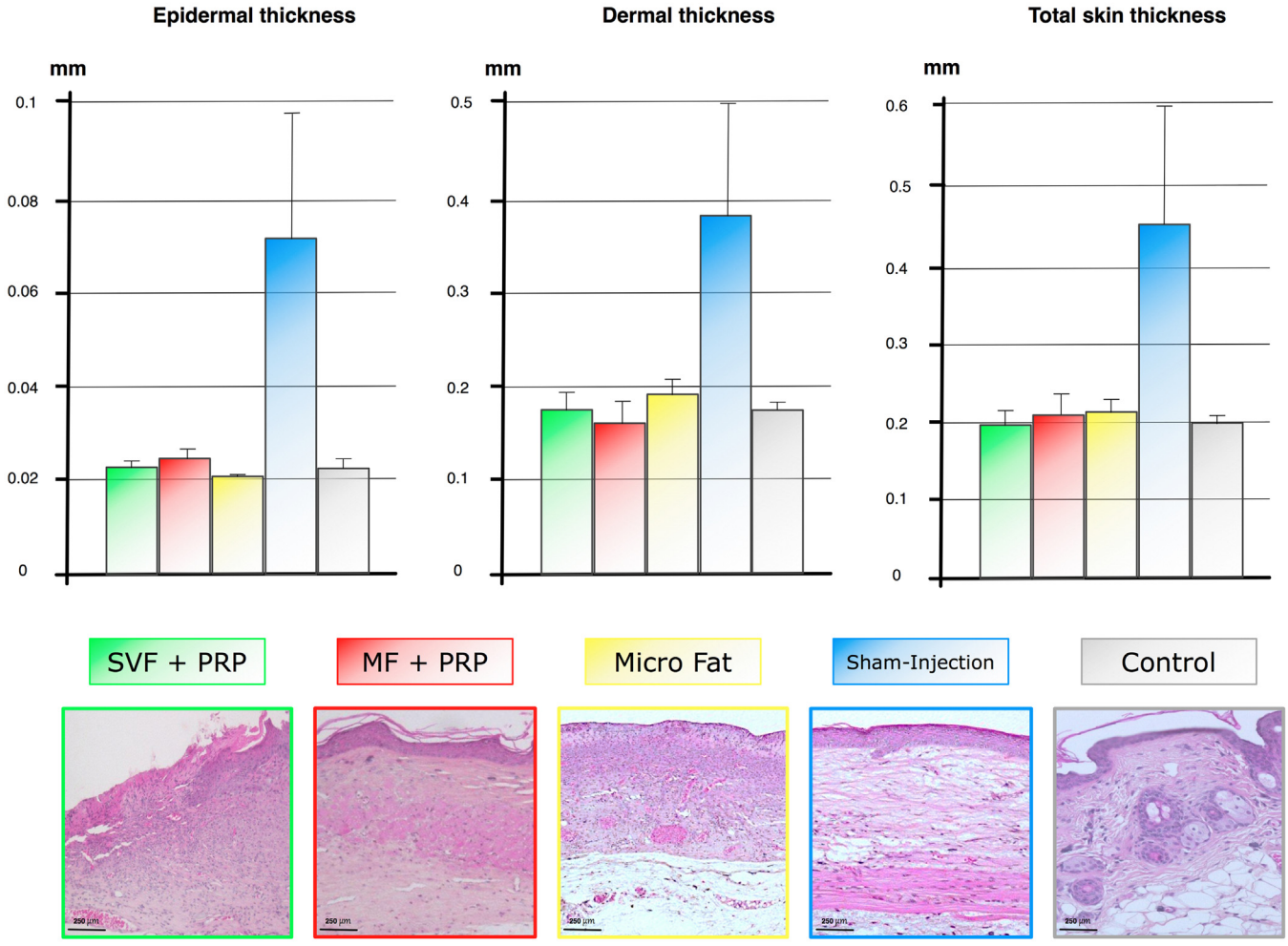

Fig. 4 – Histological findings of the wounds, 3 months after treatment by MF+PRP (A) or sham-injection (Ringer's lactate) (B): the regenerative epidermal tongues are thicker, with more hyperplasia, and the dermis displays a higher cellularity in the treated group (A) compared to sham-injection (B). Small clusters of adipocytes are integrated within the granulation tissue of the wound treated by MF+PRP (yellow arrows).

stated compared to sham injected mice (Fig. 4). A significant reduction in dermal thickness and epidermal thickness secondary to skin sclerosis induced by radiation was observed in the periphery of the wound after treatment with MF, MF +PRP, and MF+SVF compared to the placebo ($P<0.05$). No significant difference was detected among the three groups treated with cell-based therapy products. Data for dermal, epidermal and total skin thicknesses after treatment with the

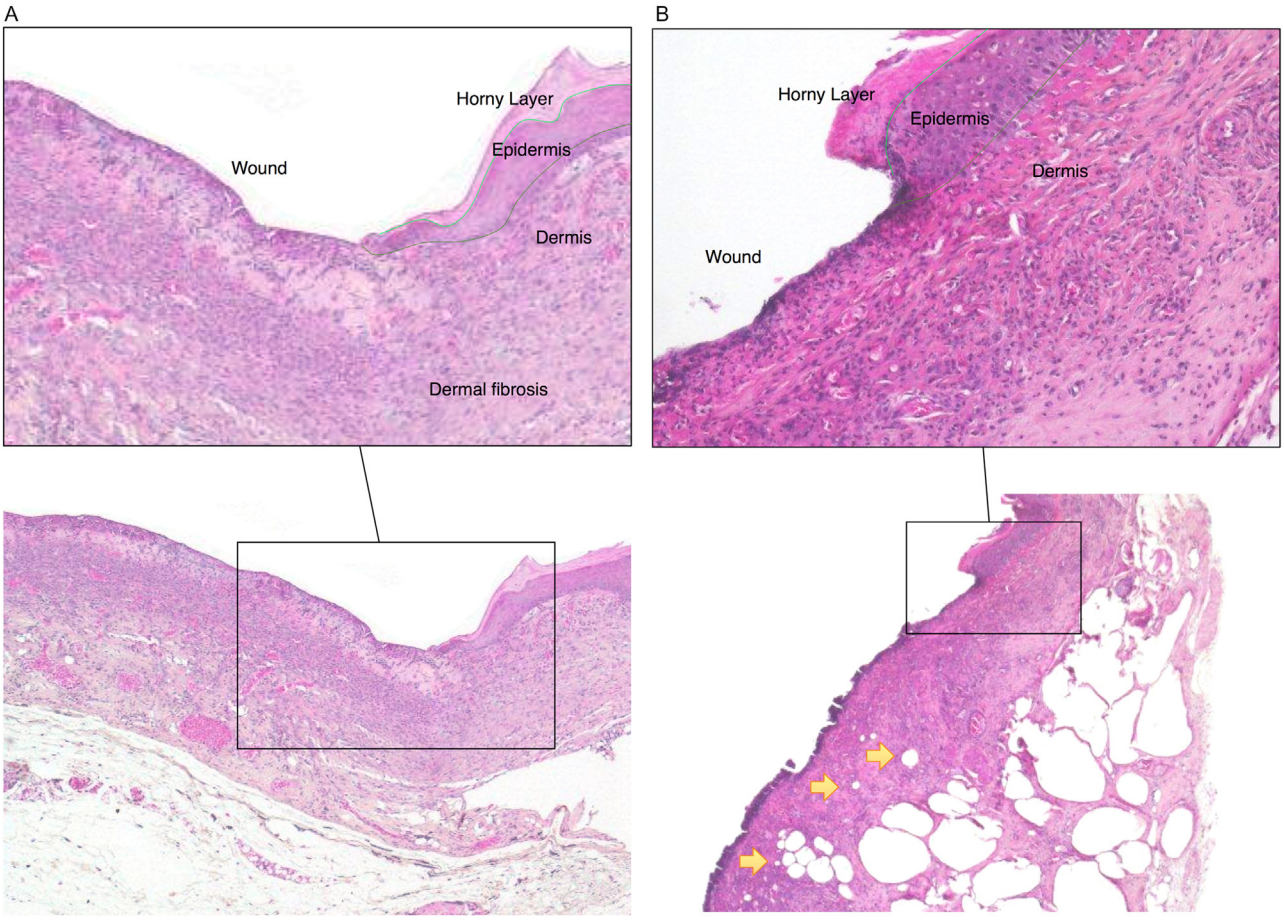

Fig. 5 – Comparison of epidermal, dermal and total skin thicknesses at 3 months after treatment by sham-injection (Ringer's lactate), MF, MF+PRP and MF+the SVF versus the healthy control group.

MF+PRP, MF+SVF, MF and placebo are shown in Fig. 5. No differences in scar thickness were observed between the different treatments and the sham-injection. The appearance of blood vessels and the density in areas treated with MF, MF +PRP and MF+SVF denoted an increase in vessel density, particularly in the MF+SVF group showing the greatest increase in density and diameter of vessels (Fig. 6).

3.6. *Fat graft viability*

The MF injected in the subcutaneous plane was still present at 3 months post-treatment in all three groups. The MF formed homogeneous, adherent and well-vascularised fat nodules under the panniculus carnosus (Fig. 7).

Discussion

Radiodermatitis is characterised by fibrotic transformation of the subcutaneous soft-tissues associated with a decrease in cutaneous vascularisation [26,27]. Immediately after irradiation, acute radiodermatitis is associated with dermal inflammation and epidermal involvement. The chronicity of dermal inflammation is responsible for the fibrotic changes and epidermal thickening, which lead to epidermal hypovascularisation, skin ulcerations and necrosis [28]. Medical or accidental exposure of a large area as well as elevated "hot spot" radiation exposure of the skin lead to severe skin damages named "cutaneous radiation syndrome" (Hopwell). Irradiation severly impairs wound healing, depending on the radiation dose, depth of penetration and quality. Our model is relevant to the human accident situation of large area irradiation exposure. In this situation, the skin ulceration observed is irremediably extensive [29] as in our murine sham-injected group. Treatment of these radiation-induced wounds presents a challenge for clinicians. Actually, autologous fat grafting seems to be the more efficient procedure to attenuate these lesions [10,11,30] in alternative to radical skin excision and use of a cover flap. However, further experimental and clinical studies have shown interesting effects of using PRP and SVF to treat these cutaneous radiation lesions.

Our radiation-induced skin lesion model used was adapted from previous experimental studies. Thanik et al. and Sultan et al. described a murine model of dorsal radiodermatitis carried out after a one-time dose of 50 Greys and 45 Greys of radiation therapy respectively via a linear accelerator [2,9]. In

Experimental Conditions	Dermal Vascularization
Sham - Injection	-
Micro-Fat	+
SVF + MF	++
PRP + MF	+

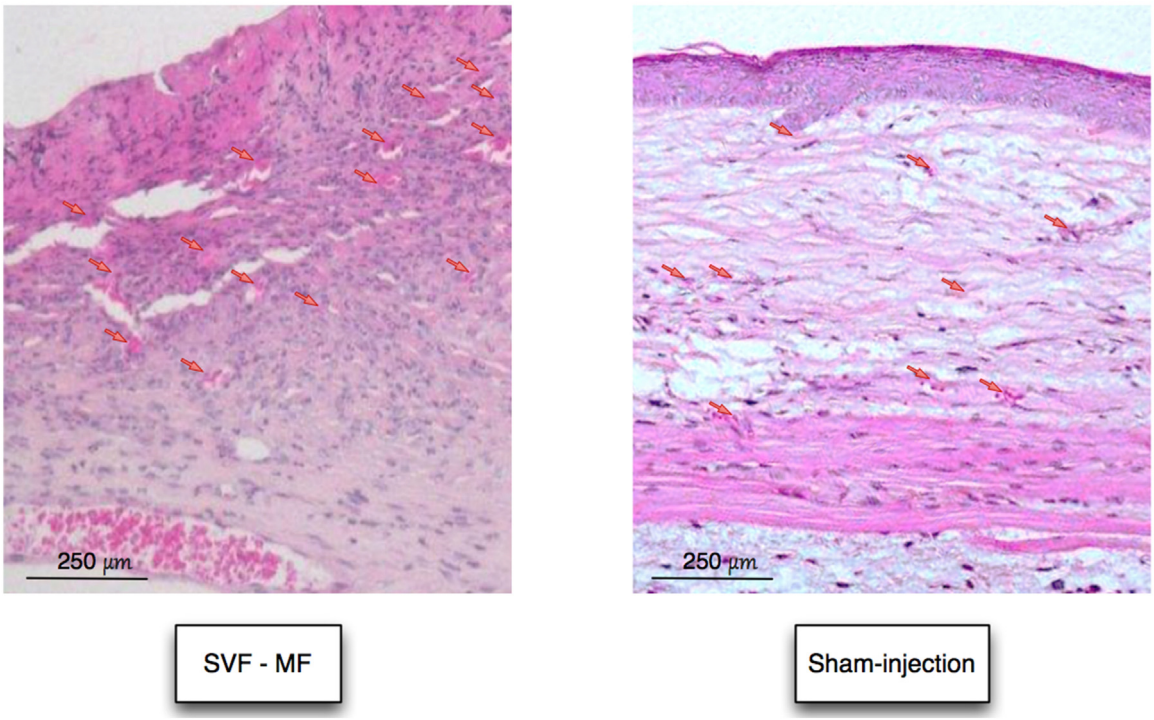

Fig. 6 – Skin vascularisation after treatment by sham-injection (Ringer's lactate), MF, MF + PRP and MF + the SVF. The number and diameter of dermal capillaries increased after treatment with MF + SVF (left) in comparison to the sham injection (right).

our study, a one-time dose of 60 Greys was delivered by linear accelerator to obtain a late dorsal skin necrosis, ulcerations and wounds 12 weeks after radiation with a reduced mortality. However, a limit of this impaired wound healing model is the contraction of the murine panniculus carnosus leading to the wound contraction, absent in physiologic human healing. In fact, in conventional murine models of wound healing, a full-thickness wound is performed with punch biopsy and wound contraction is prevented with donut-shaped silicone splint fixed around the wounds [30]. In our murine wound model, the irregular and unpredictable shape of the radiodermititis wounds did not allow the use of those splints.

Sultan et al. evaluated previously the effect of fat grafting on radiation skin damages in a similar murine model. Four weeks after irradiation, the authors reported overabundant collagen deposits resulting in dermal thickening and a decrease in skin microvascularity. Four weeks after treatment by fat grafting or by sham injection, they reported an epidermal thicknesses of $36.30 \pm 6.1\,\mu m$ in the sham-grafted group versus $8.27 \pm 0.64\,\mu m$ in the fat-grafted group [9]. Comparatively, Thanik et al. evaluated a novel mouse model of cutaneous injury and reported 6 weeks after 45-Grey irradiation an increase of dermal thickness and skin fibrosis [2]. In our study, we reported 6 months after a 60-Grey irradiation thus 3 months after treatment, a thickening of the epidermis (epidermal thickness $= 70 \pm 27\,\mu m$) and the dermis ($380 \pm 121\,\mu m$) in the sham-grafted group compared to the healthy control group (epidermis thickness: $22 \pm 1\,\mu m$; dermis thickness: $173 \pm 6\,\mu m$) and the treated groups (epidermis thickness: 20 to $24 \pm 2\,\mu m$; dermis thickness: 163 to 191 $\pm 21\,\mu m$), revealing a decrease of skin fibrosis in the 3 irradiated and treated groups.

A subcutaneous injection of fat is a simple and validated treatment for irradiation-induced cutaneous lesions. Mojallal et al. showed an increase in extracellular matrix, collagen fibre and neovascularisation around the transplanted fat [31].

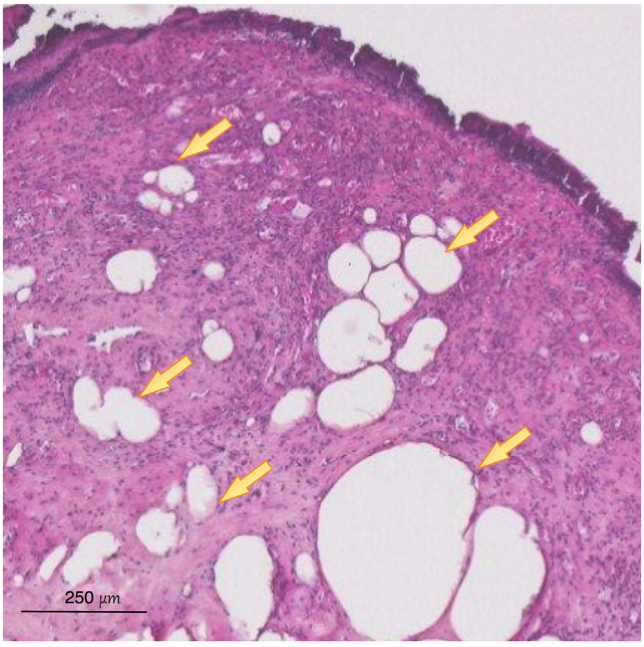

Fig. 7 – Subcutaneous fat viability at 3 months after treatment with MF+PRP.

Phulpin et al. showed improved skin quality after subcutaneously injecting autologous fat in 11 patients with chronic radiodermatitis of the head and neck [11]. They observed an improved vascular network in the treated skin associated with a decrease in the irradiated morphological patterns and no skin ulcerations. In a similar study, Rigotti et al. showed improved healing in 10 of 11 patients with thoracic cutaneous ulcerations secondary to irradiation after a subcutaneous autologous fat transfer [10]. The histological analysis showed improved neovessel formation and dermal hydration. The authors concluded that the adipose-derived stem cells (ASCs) contained in the SVF of the fat were the principal skin-regenerating agents. The action of ASCs in the treatment of radiodermatitis lesions has been studied previously in pigs [20,32]. In these studies, injecting autologous ASCs locally into the skin lesions decreased the subcutaneous defects and muscle fibrosis in comparison to the controls. The authors reported a decrease in disorganised myofibres, associated with a decrease in the size of the fibrotic and necrotic areas, after ASCs were injected compared to the control. Iddins at al. used the SVF from fat, a concentrate of growth factors and non-expanded ASCs, in a clinical case report. The SVF was used in association with 0.2 cm^3 of MF after all reference treatments failed to treat a chronic ulceration of the thumb secondary to accidental irradiation [33]. The mixture was injected in the subcutaneous plane of the pulp. The wound healed completely within 290 days, with a prominent decrease in finger paraesthesia due to a decrease in subcutaneous fibrosis. In our experimental study, the SVF did not show superior efficacy to the other products with respect to ulcer healing. However, the skin treated with MF+SVF showed a greater increase in vascular density than that treated with the other mixtures (Fig. 7).

PRP seems to be effective for healing chronic wounds. Once activated, the PRP secretes more than 30 pro-healing growth factors [34]. Fujita et al. demonstrated the efficacy of PRP injections into skin lesions in a murine model of impaired wound healing after irradiation [35]. They showed a significant decrease in the ulcer at 15 days after the PRP injection associated with a significant increase in the number of neovessels and collagen formation compared to the control. In our study, injecting the MF+PRP mixture was more efficient for increasing wound healing compared to MF alone or MF+SVF (Fig. 4). At 3 months after the injection, the healing was significantly better in the MF+PRP group compared to the sham-injected group ($P<0.001$). However, we observed an important variation of the wound size in the sham-injection group. The randomisation of the mice distribution ensures no selection bias. As reported by Agay et al. in a Minipig model of cutaneous radiation syndrome, the skin ulceration appeared variable and extensive, principally in the control group [29]. Thus, microfat treatment seems to decrease the post irradiation ulcer size variability, promoting wound healing in the 3 treated groups.

The effectiveness of fat, PRP and SVF has been shown in several animal models, and a few clinical case studies, as a treatment for skin lesions induced by radiation. In our study, we assessed MF alone and MF in association with PRP or SVF. PRP and SVF are fluid products that diffuse rapidly. A previous study by our laboratory demonstrated the superiority of these products when used in association with MF [36]. PRP and SVF seem to stay in the subcutaneous plane for a longer period, which could explain the increased effectiveness. Our study suggests that MF+PRP is the most efficient product for wound healing, with a wound healing rate of 81% compared to 16% and 21% for MF and MF+SVF, respectively. However, the initial wound area in the MF+PRP group ($121.3\pm21.5\,mm^2$) had a tendency to be inferior to the MF+SVF group ($176.5\pm27.2\,mm^2$) without significant difference ($P=0.434$, Kruskal–Wallis Test), despite the initial randomisation. This tendancy could be pejorative for the SVF effect compared to the PRP effect. This possible bias could be avoided in a future experimental study with a different design using each mouse as its own control.

All three products decreased skin sclerosis similarly. MF was still present at 3 months after injection in the three treated groups. The clinical results obtained with SVF were disappointing and were not significantly superior to MF alone. However, a superior rate of neoangiogenesis was reported in the group treated with SVF. The low clinical impact of SVF on wound healing could be explained by the non-optimal mixture of 10% SVF and 90% MF. A future study with a 50%/50% proportion of MF and SVF should be performed using the same radiation-induced skin-lesion model.

Conclusion

In this study, a mixture of MF and PRP proved most efficient to increase healing in a chronic impaired wound healing model after skin radiation. MF alone and MF+SVF resulted in increased healing rates compared with the sham-injection, but not significantly. An increase in neoangiogenesis and decrease in subcutaneous sclerosis seemed to be the principal

mechanism of action of these new combined cell-based therapies.

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Declarations

Ethics approval and consent to participate.

This experimental study was approved by the National Animal Care and Ethics Committee (#00506.02).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BB: conception, drafting, final approval, agreement to be accountable for all aspects of the work.

JE: conception, drafting, final approval, agreement to be accountable for all aspects of the work.

MV: conception, revision, final approval, agreement to be accountable for all aspects of the work.

CC: design, revision, final approval, agreement to be accountable for all aspects of the work.

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