Mitochondrial transplantation as a new therapy to improve tissue regeneration in surgical wounds: preclinical trials in mice

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Introduction

Surgical skin wounds can result in abnormal healing processes leading to keloids and non-cosmetic scars. These are commonly associated with excessive inflammation, abnormal collagen deposits and fibrotic tissue[1]. In addition, dermal wound healing can be influenced by the composition and density of skin microbiota which may prolong inflammation, delay re-epithelialization and increase the probability of infection[2]. Even though there are treatments available for surgical wound healing, adequate restoration of the dermal tissue cannot be guaranteed.

Accurate determination of the therapeutic potential of novel agents that improve wound repair and promote *in vivo* regeneration, after *in vitro* validation, is critical in translational medicine. Studies that evaluated the regenerative potential of mesenchymal stem cells (MSCs) have revealed that such effects might be due to regenerative factors that promote cell proliferation, migration and immunoregulation. One of those factors is the mitochondria transfer, which could be use to restore the function of damaged cells [3,4].

Hence, the present project aims to evaluate the use of mitochondria as a therapeutic agent to improve cutaneous surgical wound healing in a mouse model. This can be done by analysing wound healing post-mitochondrial treatment by the expression of genes related to proliferation, inflammation and regeneration; histopathology assessment; and identification of pathogenic microorganisms in surgical wounds.

Methodology

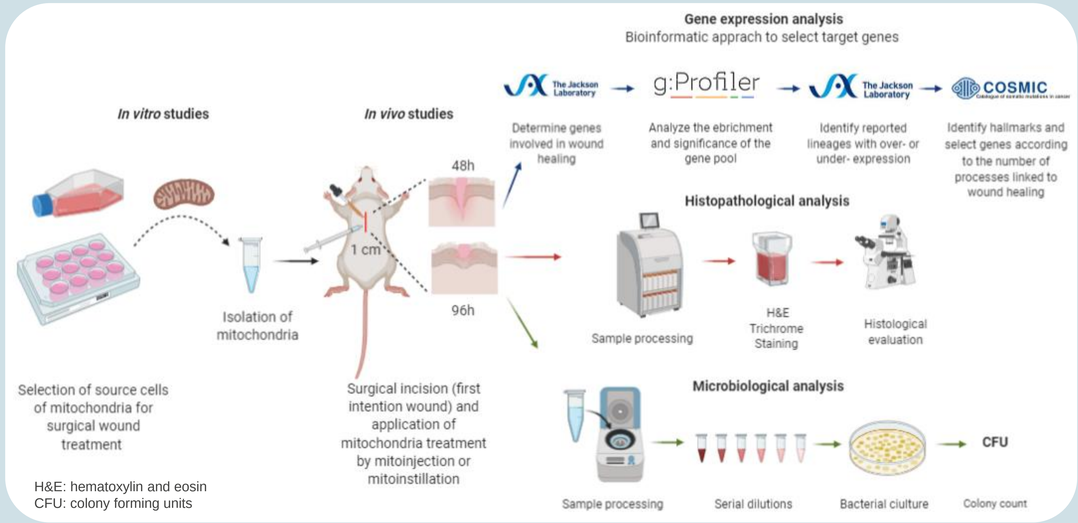

Figure 1. Diagram of the assessment of mitochondria as a therapeutic agent for surgical wound healing by gene expression, histopathology and microbiology.

Results: mitochondria source

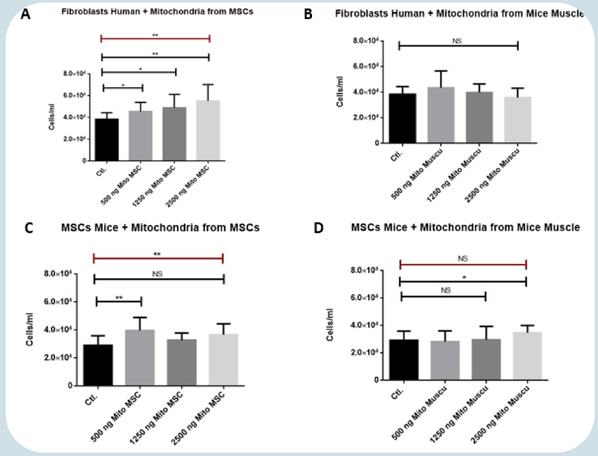

Figure 2. Proliferation effects of mitochondrial transplantation according to the source cell and doses. A) Mitochondria from MSCs transplanted to human fibroblasts. **B)** Mitochondria from mouse muscle transplanted to human fibroblasts. **C)** Mitochondria from MSCs transplanted to mouse MSCs. **D)** Mitochondria from mouse muscle transplanted to mouse MSCs. Kruscal-Wallis test (in red) and t-Student's non-parametric test, with Mann-Whitney comparative ranks (in black). (n=3, 3 repetitions per condition). NS: not statistically significant, *p<0.05, **p<0.01.

Results: gene expression analysis, wound histology and bacterial colony identification

Table 1. Hallmark genes and its associated number of biological processes involved in wound healing.

Gene	*MTOR*	*HIF1A*	*PTEN*	*EZH2*	*KDR*	*HRAS*	*EGFR*
Biological Process	44	23	25	12	11	10	17

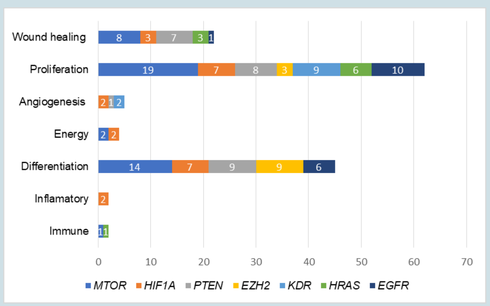

Figure 3. Hallmark genes and their associated biological processes involved in wound healing.

Seven genes were identified as hallmarks genes in wound healing according to the number of biological processes linked to tissue healing, proliferation inflammation, among others.

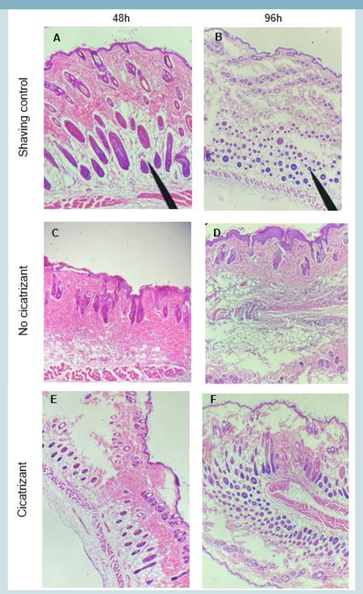

Figure 4. Representative H&E sections of mouse skin biopsies at 48h and 96h after first intention wounding with or without cicatrizant. **A)** Conserved tissue architecture. Dermis shows collagen of variable density without inflammatory changes. **B)** Dermis shows edematous lax collagen and no inflammatory changes. **C)** Uneven healing, persistence of fibrin and polymorphous inflammatory infiltration. **D)** Complete healing with polymorphous inflammatory infiltration. **E)** Fibrous proliferation zone of laminated morphology with vertical projection. **F)** A small spot of fibrosis in reticular dermis.

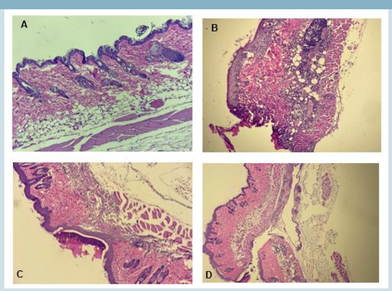

Figure 5. Representative H&E sections of mouse skin biopsies with or without mitochondrial treatment (50 ng/µl). **A)** Shaving control. Thin epidermis, numerous hair follicles, papillary dermis with thin collagen bands distributed randomly. Thicker, more regular and homogenized collagen fibers are identified in the reticular dermis. **B)** Vehicle control. Incomplete repair. Polymorphonuclear inflammatory infiltrate accompanied by fibrinoid material. Inflammation reaches the adipose tissue and muscle fibers. **C)** First intention wound mitoinjected. There is retraction and persistence of inflammatory infiltration but in lesser quantity. Areas with different phases of healing, image shows an intermediate state. **D)** First intention wound mitoinstilled. Bottom-up repair process with increased vascularity at the base of the lesion. Edema and polymorphic inflammatory infiltration of predominantly perivascular distribution below the muscle fibers.

Table 2. Colony count from mouse skin samples with or without mitochondrial treatment.

Sample	Dilution/Culture media	Colony number	CFU/ml
Shaving control	10^{-2} NA	TNTC	TNTC
	10^{-3} NA	TNTC	TNTC
Vehicle control	10^{-2} NA	TNTC	TNTC
	10^{-3} NA	0	0
First intention wound mitoinjected (25 ng/µl)	10^{-2} NA	0	0
	10^{-3} NA	2	2000
First intention wound mitoinstilled (25 ng/µl)	10^{-2} NA	70	7000
	10^{-3} NA	0	0

NA: nutrient agar
TNTC: too numerous to count

It was observed that skin biopsy is more appropriate for sampling than brushing. The table shows the results of the colony count from Nutrient Agar media. Control samples (shaving and vehicle control) presented a very high bacterial load, compared to samples with mitochondrial treatment (injected and instilled). This allows us to presume that the mitochondrial treatment has some effect on the normal load of microorganisms on skin wounds.

Future assays include: gene expression analysis of the hallmark genes identified in the present work, histopathological analysis of skin wounds treated with different doses of mitochondria and identification of pathogenic microorganism in skin wound samples.

Conclusions

- MSCs are better sources of mitochondria than muscle cells for proliferative effects in both allogenic (mouse MSCs) and xenogeneic (human fibroblasts) treatment, showing a potential effect to promote surgical wound healing.
- *MTOR, HIF1A, PTEN, EZH2, KDR, HRAS* and *EGFR* were identified as the hallmark genes in wound healing.
- The use of cicatrizant allowed the resolution of the wound with adequate collagen architecture, apparently aesthetic. Results to be correlated with subsequent assays.
- Mitochondrial treatment allowed partial wound resolution with some inflammatory infiltration, may be due to the presence of ectoparasites.
- Mitochondrial treatment for skin wounds has a possible effect on the load of microorganisms.

References

1. Recent Understandings of Biology, Prophylaxis and Treatment Strategies for Hypertrophic Scars and Keloids. **DOI: 10.3390/ijms19030711**
2. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. **DOI:10.1111/j.1524-475X.2009.00489.x**
3. MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. **DOI:10.1038/srep09073**
4. Primary allogeneic mitochondrial mix (PAMM) transfer/transplant by MitoCeption to address damage in PBMCs caused by ultraviolet radiation. **DOI: 10.1186/s12896-019-0534-6**