Experimental diet-induced diabesity mice model

Mice were randomly assigned for 16 weeks into two groups and were fed, either a High-Fat Diet (HFD) to induce the model of type 2 diabetes or received continuous feeding of a chow diet (NC). The caloric profile of fat, protein and carbohydrate on the HFD diet (Research Diets Inc.) was 60%, 20% and 20% respectively whereas 11%, 36%, and 53% for the normal chow diet (SSNIFF, GMGH). Body weight was monitored weekly and thereafter daily throughout the duration of the study. The diabetic state was confirmed by IPGGT (Intra-peritoneal glucose tolerance test) and IPIST (intra-peritoneal insulin sensitivity test). Briefly, for IPGGT and IPSIT test, NC-and HFD-fed mice were starved for eight hours (1). Then a solution of 20% glucose (1g/kg body weight) or insulin (0,5U/kg body weight) was administered by intra-peritoneal (IP) injection). Blood glucose levels were measured at different time points (0, 15, 30, 45, 90, 120 min). HFD fed mice cleared glucose less effectively after i.p. glucose injection than animals fed chow diet (Fig. S1A). The rate of glucose clearance upon i.p. insulin injection was also lower in HFD fed mice (Fig.S1B), demonstrating that after four months of HFD, the C57BL/6J, 5-LOX KO and 12/15-LOX KO mice developed all the characteristics of type 2 diabetes.

Experimental murine model of wound healing

Experimental non-invasive device

The wound skin chamber is composed of 2 distinct parts created from 3D printing technology in bio-compatible materials (2,3).

The first part is a 500 µl capacity tubular chamber, open at both ends with an outgrowth at the base for attachment on the device to the wounded body part. This flange has the peculiarity to be structured through a parametric mesh filling, a thickness of 200 microns and only 30% of density which provides the best imaginable conformability. With functional specifications and an original design taking into account of the animal morphology; this part fits perfectly on the back of non- or diabetic mice. So, this chamber was made in Nylon 618 and fixed with a bio-compatible Silicon Glue (Kwik-Sil, World Precision Instrument) to obtain a perfectly sealed around the wound.

The second part is a cap in Acrylonitril Butadiene Styrene (ABS) taking place on the top of the tubular chamber to maintain the wound environment sterile. To avoid a damaging maceration on the wound bed, the upper part is provided with a porous membrane allowing gas exchange.

Excisional skin injury and topical treatments

Mice were anesthetized with 2-5% isoflurane in oxygen for induction and 1%-2.5% in oxygen for maintenance. The hair back of each mouse was shaved and subsequently wiped with 70% ethanol. Full-thickness wounds (0.8 cm of diameter) were created on the back of the mice to the *panniculus carnosus* with a biopsy punch from NP Medical. The skin chamber was fixed on the wound edges without contact with the injured tissue using bio-compatible silicon glue (Kwik-Sil, World Precision Instrument). During the wound closure kinetics, daily monitoring is performed. The primary endpoint was to show 100% of wound closure in NC fed mice versus HFD fed mice. The detachment of a chamber is exceptional, if this occurs, the affected mice are excluded from the study.

Depending on the protocols, wound was treated either by: 200µl of Sodium Chloride (NaCl), (BAXTER) used as vehicle solution or 36µg/wound/day of Aspirin (Sigma) or 140 ng of LTA_4m (Cayman Chemical) from the 3rd to the 14th day post-injury. All active ingredients are dissolved into the vehicle solution. The wounds were followed until complete closure or, on different days post-injury, they were washed with PBS to harvest the cells present in the exudate and the healing tissue was recovered.

Evaluation of wound closure

Process of wound healing was observed from days 0 to 21 (total closure) after wounding on each mouse. Between 3–5 high resolution standardized images (same distance and luminosity between camera and anesthetized mice) were taken of the wounds for each animal.

The surface of the wound was evaluated using an imaging software package (Adobe Photoshop CS4). The external surface of the skin chamber was used as a reference to convert the number of pixels to unit area mm^2. Wound surface results were expressed as a percentage of wound closure using the following calculation:

$$\%\ \text{Wound closure} = \frac{\text{Day 0 wound area} - \text{Wound area on particulary day}}{\text{Day 0 wound area}} \times 100$$

Evaluation of wound contraction

We drew four dots, approximately 1 cm square, around the wound. The photos taken daily and their analysis on Image J software, allowed us to calculate the area of the square. These measurements were used to calculate the percentage of wound closure attributed to contraction as follows:

$$\%\ \text{Wound contraction} = \frac{\text{Day 0 square area} - \text{Square area on particulary day}}{\text{Day 0 wound area}} \times 100$$

Isolation of wound macrophages

Macrophages were isolated from the skin of NC or HFD-fed mice as previously described by Lou et al.(4). Briefly, skin was cut into small pieces and incubated with dispase II to allow separation of the dermal and epidermal layers. The separated epidermal and dermal sheets were then incubated with Trypsin-EDTA and RPMI solution containing DNase and type IV collagenase. F4/80+ macrophages were purified using the autoMACSTM Separator (Miltenyi).

References

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