

Research Article

Macrophage Responses Against Acellularized Dermal Matrix from Human Versus Porcine Predicts the *In-vivo* Fibrosis

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Abstract

Background: Due to the possible difference in the manufacturer's processes, *in-vitro* and *in-vivo* tests might be beneficial for the selection of an Acellularized Dermal Matrix (ADM).

Methods: ADMs from human and porcine were replaced the mouse skins and tested macrophage reactions using RAW264.7 cells.

Results: In comparison with porcine ADM, human ADM induced higher reaction and fibrosis on mouse histology at 2 wk (but became similar between groups at 14 wk post-operation) and induced higher upregulation of pro-inflammatory genes at 24 h post-macrophage stimulation.

Conclusion: Macrophage responses were proposed to use for the prediction of *in-vivo* ADM responses which might be beneficial for the ADM selection.

Keywords: Acellularized Dermal Matrix; Humans; Porcine; Fibrosis; Wound

Introduction

The healing processes of the surgical wounds are initiated by hemostasis and inflammation, followed by cell proliferation (epithelial regeneration, extracellular matrix with collagen formation, wound contraction and neovascularization) and finally wound remodeling (extracellular matrix degradation and collagen arrangement) [1]. Homologous grafts and Xenogenous grafts have been currently used in several surgical procedures for both medical and cosmetic indications, including abdominal operation, facial plastic surgery, dentistry and rhinoplasty [2]. Acellularized Dermal Matrices (ADM) prepared from human skin (human ADM) or porcine (porcine ADM) are commonly used and the collagen in grafts facilitates wound healing [3]. However, with the possible different qualities from several manufacturers.

Because i) overwhelming in the proliferation phase of the wound healing induced chronic inflammation and abnormal fibrosis (scar), ii) macrophages are the major immune cells responsible for the wound healing process and iii) the possible differences in qualities of ADM in different manufacturer despite some possible benefits of human ADM, the selection of ADM based on macrophage responses might be beneficial to differentiate the ADM qualities [3,4]. Hence, the *in-vivo* and *in-vitro* tests of ADM were performed.

Material and Methods

Animal Model and In-vitro Experiments

Male C57BL/6 12-week-old mice, purchased from Nomura Siam International (Pathum Wan, Bangkok, Thailand), were used following the approved protocol (029/2563) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Acellularized Dermal Matrices (ADM) from human and porcine (0.3x0.3 cm²) were put on the back of the mice on the left (human ADM) and right sides (porcine ADM). Tramadol, 20 mg/kg diluted in 0.5 mL normal saline were subcutaneously administered after surgery and at 6 h post-operation. Mice were sacrificed by cardiac puncture under isoflurane anesthesia with sample collections at several time-point. Paraffined-embedded skins were fixed with 10% neutral buffered formalin before the processes with Hematoxylin and Eosin (H&E) and Masson's trichrome colors for the evaluation of cell accumulation and fibrosis area, respectively, using the ImageJ program (NIH, Bethesda, MD, U.S.A) on the 100x magnified pictures at the borders of ADM grafts from each animal [5].

The ADM 50 mg were thoroughly minced in 1 mL Phosphate Buffer Solution (PBS) and sonicated for 10 mins before centrifugation to separate the soluble fraction. Macrophages (RAW264.7) (TIB-71TM, ATCC, Manassas, VA, USA) at 2x10⁶ cells/well were mixed with ADM preparations (the solution from 0.5 mg ADM/ well) or Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) before 24 h incubation at 37°C with 5% carbon dioxide before the cell collection. The supernatant TNF- α were evaluated by ELISA (Biolegend, San Diego, CA, USA) and the cells were used for polymerase chain reaction. Gene expression in mouse skins or macrophages were evaluated by Quantitative real-time polymerase chain reaction (qRT-PCR) following the previous protocol using SYBR® Green PCR master mix normalized by β -actin with the $\Delta\Delta$ CT method. The list of primers for PCR is presented in Table 1 [6,7].

Statistical Analysis

Data presentation was performed with mean $\pm$ standard error (SE). The differences between groups and the time-point data were examined by one-way analysis of variance (ANOVA) with Tukey's analysis and repeat measured ANOVA, respectively, using SPSS 11.5 software (SPSS, IL, USA). A p-value of < 0.05 was considered for statistical significance.

Results

Cell Accumulation and Fibrosis Activation by Acellularized Dermal Matrix in Mice

Human ADM induced a higher cell infiltration and area of fibrosis at 4 wk post-operation as evaluated by H&E and Masson's trichrome staining, respectively (Fig. 1). With human or porcine ADM, the inflammatory cells were highest at 4 wk and fibrosis began at 4 wk and remained non-significantly different to the 14 wk post-operation (Fig. 1). Although the area of fibrosis from human ADM at 4 wk higher than porcine ADM, fibrosis at 14 wk post-operation was not different between groups (Fig. 1). The prominent inflammation was demonstrated at 1 wk post-operation as indicated by iNOS (in human ADM) and TNF- α (in porcine ADM) and human ADM induced more profound inflammation (iNOS, IL-1 β and TNF- α), at least in some time-points (Fig. 1). Despite a detectable fibrosis at 4 and 14 wk post-operation (Fig. 1), TGF- β (a fibrosis-associated genes) was significantly upregulated only at 14 wk post-operation (Fig. 1).

Macrophage by Acellularized Dermal Matrix

Then, the extract from ADM were incubated with macrophages to evaluate the responses. Although ADM did not induce supernatant TNF- α from macrophages (data not shown), the human ADM upregulated pro-inflammatory genes (iNOS, IL-1 β and TNF- α) with an anti-inflammatory gene (Fizz-1) and the porcine ADM induced only TNF- α and Fizz-1 (Fig. 1).

The prominent pro-inflammatory gene upregulation in macrophages after the activation by human ADM over the porcine ADM (Fig. 1) might be associated with the more severe inflammation in mouse grafts at 4 wk post-operation (Fig. 1). However, both ADM could not activate TGF- β (a pro-fibrotic gene) in macrophages at 24 h post-incubation, possibly due to the too short incubation for the fibrosis activation.

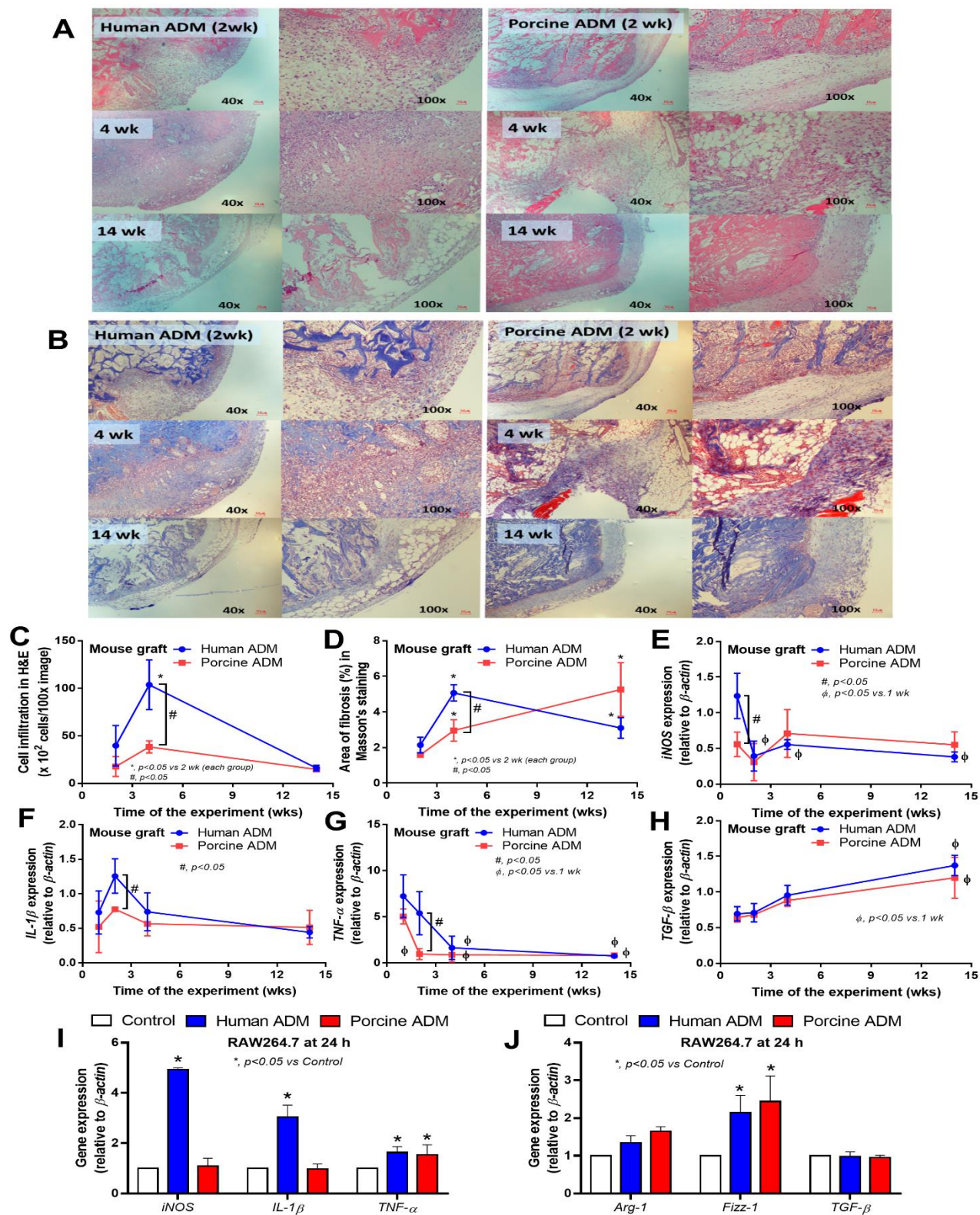


Figure 1: The characteristics of mouse grafts as indicated by pathology, the representative pictures after staining by Hematoxylin & Eosin (H&E) or Masson's trichrome color with cell infiltration and fibrosis area (A-D) and the expression of several genes (E-H) are demonstrated (n = 5-6/ time-point). The gene expression in macrophages (RAW246.7) after activation by media control or ADM (human or porcine) is also demonstrated (triplicate independent experiments were performed).

Discussion

Due to the variation of ADM preparation and a variety of ADM manufacturer, the process to select ADM might be helpful for the clinician. Here, we surprisingly demonstrated the more severe inflammatory reaction against human ADM over porcine ADM at 4 wk post-operation, different from several reports [4]. Although the fibrosis and the outcomes at 14 wk post-operation was not different between human versus porcine ADM, the higher reaction at 4 wk might lead to some inconvenient to the patients. Although the *in-vivo* test might be very useful for the differentiation of ADM inflammatory effect, it is a time-consuming complex procedure and the *in-vitro* test might be more practical to use. Hence, we introduced a protocol to test the macrophage responses using the up-regulation of pro-inflammatory cytokines, including iNOS and IL-1 β (the M1-macrophage polarization pro-inflammatory cytokines) with TNF- α (a regular inflammatory cytokines). Because the macrophage reactions were not severe enough to induce the significant production of inflammatory cytokine, the exploration in the gene expression might be more sensitive in this measurement.

Conclusion

In conclusion, we proposed to test macrophage responses against the ADM extract to predict the ADM reaction and partly for the selection criteria to use ADM from different manufacturers.

Conflict of Interest

The authors have no conflict of interest to declare.

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References

1. Dang CP, Weawseetong S, Charoensappakit A, Sae-Khow K, Thong-Aram D, Leelahavanichkul A. Non-thermal atmospheric pressure argon-sourced plasma flux promotes wound healing of burn wounds and burn wounds with infection in mice through the anti-inflammatory macrophages. *Applied Sci.* 2021;11(12):5343.
2. Jang YJ, Alfanta EM. Rhinoplasty in the Asian nose. *Facial Plast Surg Clin North Am.* 2014;22(3):357-77.
3. Ge L, Zheng S, Wei H. Comparison of histological structure and biocompatibility between human Acellular Dermal Matrix (ADM) and porcine ADM. *Burns.* 2009;35(1):46-50.
4. Ngo MD, Aberman HM, Hawes ML, Choi B, Gertzman AA. Evaluation of human acellular dermis versus porcine acellular dermis in an *in-vivo* model for incisional hernia repair. *Cell Tissue Bank.* 2011;12(2):135-45.
5. Visitchanakun P, Panpetch W, Saisorn W, Chatthanathon P, Wannigama DL, Thim-Uam A, et al. Increased susceptibility to dextran sulfate-induced mucositis of iron-overload β -thalassemia mice, another endogenous cause of septicemia in thalassemia. *Clin Sci (Lond).* 2021;135(12):1467-86.
6. Tungsanga S, Panpetch W, Bhunyakarnjanarat T, Udompornpitak K, Katavetin P, Chanchaoenthana W, et al. Uremia-induced gut barrier defect in 5/6 nephrectomized mice is worsened by candida administration through a synergy of uremic toxin, lipopolysaccharide and (1 $\rightarrow$ 3)- β -d-glucan, but is attenuated by lacticaseibacillus rhamnosus L34. *Int J Mol Sci.* 2022;23(5).
7. Sae-Khow K, Tachaboon S, Wright HL, Edwards SW, Srisawat N, Leelahavanichkul A, et al. Defective neutrophil function in patients with sepsis is mostly restored by *ex-vivo* ascorbate incubation. *J Inflamm Res.* 2020;13:263-74.

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