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Research Article

Antibiotic Resistance of Enterobacteriaceae in Wastewater Samples from the Mindoube Municipal Landfill, Libreville, Gabon

Asada Leelahavanichkul*

Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

***Correspondence:** Asada Leelahavanichkul, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: alelahavanit@gmail.com

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ABSTRACT

Background: Selecting the most suitable Acellularized Dermal Matrix (ADM) for clinical applications requires careful consideration of various factors, including differences in manufacturing processes and potential immune responses. Both in-vitro and in-vivo tests are essential for evaluating the performance of ADMs and predicting their behavior post-implantation.

Methods: In this study, ADMs derived from human and porcine sources were implanted into mouse skin to assess macrophage reactions using RAW264.7 cells as a model system.

Results: The histological analysis revealed that human ADM induced a higher degree of reaction and fibrosis in mouse skin compared to porcine ADM at 2 weeks post-operation. However, these differences became less pronounced and similar between the two groups at 14 weeks post-operation. Furthermore, human ADM elicited a greater upregulation of pro-inflammatory genes in macrophages at 24 hours post-stimulation compared to porcine ADM.

Conclusion: The findings suggest that macrophage responses to ADMs could serve as predictive indicators of in-vivo responses. Understanding these responses may aid in the selection of appropriate ADMs for specific clinical applications.

Keywords: Acellularized Dermal Matrix; Human; Porcine; Fibrosis; Wound.

INTRODUCTION

The healing process of surgical wounds involves a series of complex steps, including hemostasis, inflammation, cell proliferation, and wound remodeling. To aid in this process, various graft materials, such as homologous and xenogenous grafts, are utilized in surgical procedures for both medical and cosmetic purposes.

Among these graft materials, Acellularized Dermal Matrices (ADMs) derived from human or porcine skin are commonly employed due to their ability to facilitate wound healing by providing a scaffold for tissue regeneration. However, variations in the quality of ADMs may arise from different manufacturing processes. Moreover, excessive inflammation during the proliferation phase of wound healing can lead to abnormal fibrosis (scar formation).

Macrophages play a crucial role in the wound healing process and are primarily responsible for orchestrating tissue repair and regeneration. Given the potential differences in ADM quality from various manufacturers, selecting the most appropriate ADM based on macrophage responses could be advantageous in differentiating their qualities [1].

Therefore, both in-vivo and in-vitro tests of ADMs were conducted to evaluate their performance and macrophage reactions, aiming to inform the selection of ADMs with optimal healing properties.

MATERIALS AND METHODS

Animal Model and In-vitro Experiments

Male C57BL/6 mice, 12 weeks old, obtained from Nomura Siam International, Bangkok, Thailand, were used in accordance with the approved protocol (029/2563) of the Faculty of Medicine, Chulalongkorn University. Acellularized Dermal Matrices (ADMs) from human and porcine sources (0.3×0.3 cm^2) were implanted on the left (human ADM) and right sides (porcine ADM) of the mice's backs. Tramadol, 20 mg/kg diluted in 0.5 mL normal saline, was subcutaneously administered post-surgery and at 6 hours post-operation. Mice were euthanized by cardiac puncture under isoflurane anesthesia, and samples were collected at various time points. Paraffin-embedded skins were fixed with 10% neutral buffered formalin and processed with Hematoxylin and Eosin (H&E) and Masson's trichrome staining for cell accumulation and fibrosis area evaluation, respectively. Image analysis was performed using the ImageJ program on 100x magnified images at the borders of ADM grafts from each animal [2].

For in-vitro experiments, ADMs (50 mg) were minced thoroughly in 1 mL Phosphate Buffer Solution (PBS) and sonicated for 10 minutes before centrifugation to separate the soluble fraction. RAW264.7 macrophages (ATCC) at 2×10^6 cells/well were incubated with ADM preparations (0.5 mg ADM/well) or Dulbecco's Modified Eagle Medium (DMEM) for 24 hours at 37°C with 5% carbon dioxide. The supernatant TNF-α levels were evaluated by ELISA, and the cells were used for polymerase chain reaction (PCR). Gene expression in mouse skins or macrophages was evaluated by Quantitative

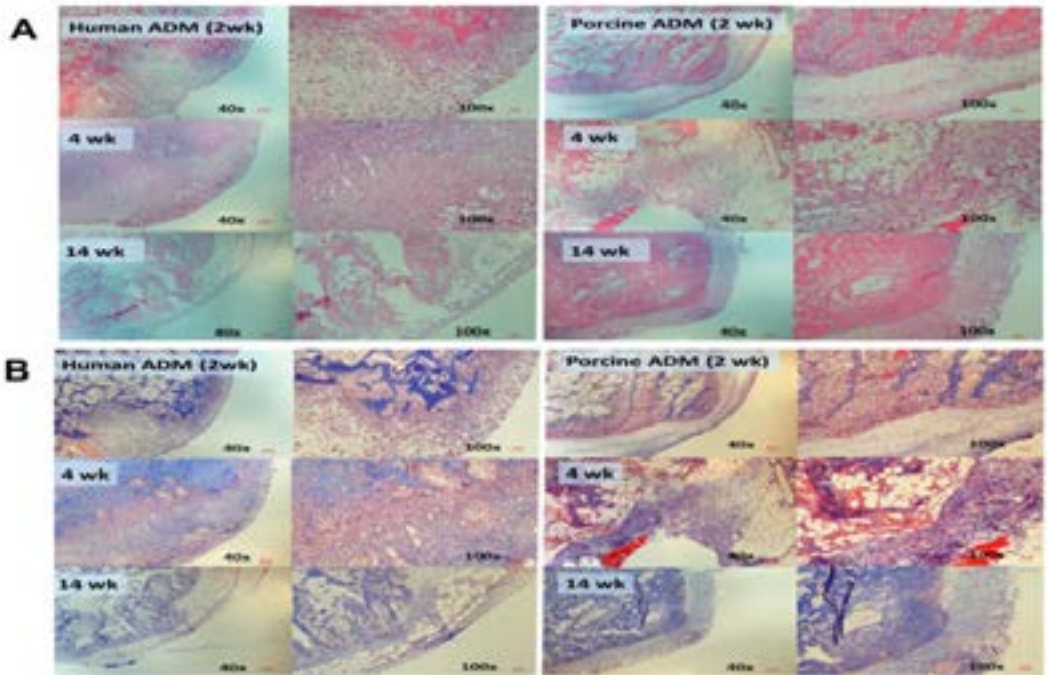

Figure 1: The characteristics of mouse grafts as indicated by pathology, the representative pictures after staining by Hematoxylin & Eosin (H&E) or Masson's trichrome color with cell infiltration and fibrosis area (A-D) and the expression of several genes (E-H) are demonstrated (n = 5-6/ time-point). 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).

real-time polymerase chain reaction (qRT-PCR) using SYBR® Green PCR master mix, normalized by β-actin with the ΔΔCT method. The list of primers for PCR is presented.

Statistical Analysis

Data were presented as mean ± standard error (SE). Differences between groups and time-point data were analyzed by one-way analysis of variance (ANOVA) with Tukey's analysis and repeated measured ANOVA, respectively, using SPSS 11.5 software. A p-value of < 0.05 was considered statistically significant.

RESULTS

Cell Accumulation and Fibrosis Activation by Acellularized Dermal Matrix in Mice

Evaluation of H&E and Masson's trichrome staining revealed that human ADM induced higher cell infiltration and fibrosis area at 4 weeks post-operation compared to porcine ADM (Figure 1). Both human and porcine ADMs exhibited peak inflammatory cell infiltration at 4 weeks post-operation, with fibrosis initiation observed at the same time point and sustained until 14 weeks post-operation. Although human ADM showed greater fibrosis area at 4 weeks post-operation, there was no significant difference in fibrosis area between human and porcine ADMs at 14 weeks post-operation. Notably, prominent inflammation was evident at 1 week post-operation, with human ADM inducing more profound inflammation, as indicated by iNOS and TNF-α expression, compared to porcine ADM. Despite detectable fibrosis at 4- and 14-weeks post-operation, significant upregulation of TGF-β, a fibrosis-associated gene, was observed only at 14 weeks post-operation [3].

Macrophage Response to Acellularized Dermal Matrix

Subsequent evaluation involved incubating macrophages with extracts from ADM to assess their responses. Although ADM did not induce supernatant TNF-α from macrophages, human ADM upregulated pro-inflammatory genes (iNOS, IL-1β, and TNF-α), along with an anti-inflammatory gene (Fizz-1), while porcine ADM induced only TNF-α and Fizz-1.

The significant upregulation of pro-inflammatory genes in macrophages following activation by human ADM compared to porcine ADM may be associated with the more severe inflammation observed in mouse grafts at 4 weeks post-operation. However, neither human nor porcine ADM could activate TGF-β, a pro-fibrotic gene, in macrophages at 24 hours post-incubation, possibly due to the short incubation period insufficient for fibrosis activation.

DISCUSSION

Given the variability in ADM preparation and the multitude of manufacturers involved, selecting the appropriate ADM is crucial for clinicians. Surprisingly, our study revealed a more pronounced inflammatory reaction against human ADM compared to porcine ADM at 4 weeks post-operation, contrary to previous reports [4-7]. While the fibrosis and outcomes at 14 weeks post-operation did not differ between human and porcine ADM, the heightened reaction at 4 weeks may pose inconvenience to patients. Although in-vivo testing can effectively differentiate ADM inflammatory effects, it is a time-consuming and complex procedure. In contrast, the in-vitro test may offer a more practical approach.

To address this, we proposed a protocol for testing macrophage responses by assessing the upregulation of pro-inflammatory cytokines, including iNOS and IL-1β (markers of M1-macrophage polarization), along with TNF-α (a standard inflammatory cytokine). However, as the macrophage reactions were not severe enough to induce significant production of inflammatory cytokines, exploring gene expression may provide a more sensitive measurement in this context. This approach could offer valuable insights into predicting in-vivo ADM responses and aid in the selection of appropriate ADMs for clinical use.

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.

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