Extracellular Matrix Powder Protects Against Bleomycin-Induced Pulmonary Fibrosis

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Introduction
Regardless of etiology, pulmonary fibrosis refers to a group of lung diseases characterized by inflammation, fibroblast proliferation, and excessive collagen deposition. Although the molecular mechanisms underlying pulmonary fibrosis are poorly understood, current evidence suggests epithelial injury contributes to the development of fibrosis. Regenerative medicine approaches using extracellular matrix (ECM) scaffolds derived from porcine urinary bladder matrix (UBM) have been shown to promote site-specific constructive tissue remodeling in part due to release of bioactive degradation products and to modulation of the immune response. These properties of ECM scaffolds led to the hypothesis that ECM powders will promote normal tissue remodeling and attenuate bleomycin-induced pulmonary fibrosis.

Materials and Methods
All animal experiments were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Ten-week-old male C57BL/6 mice (unless otherwise noted) were intratracheally instilled as previously described [1] with 0.07 units of bleomycin sulfate (Hospira, Inc, Lake Forest, IL) with or without 280 µg UBM-ECM powder [2] or digested UBM-ECM [3]. Control mice were treated with 0.9% saline vehicle with and without 280 µg of ECM. Mice were euthanized either 5 or 14 days after exposure. Bronchoalveolar lavage fluid (BALF) was obtained as previously described and processed for total protein measurements, total white cell counts, cell differentials [1], and protein concentration [4] as detailed in the online supplement. Lungs were inflation fixed with 10% buffered formalin and paraffin embedded for histological analysis by a pathologist blinded to the sample groups. All comparisons between groups were compared with one-way ANOVA with Tukey’s post-test or two-way ANOVA with Bonferroni’s post-test using Graphpad Prism 4. A p-value of ≤ 0.05 was considered statistically significant.

Results
Compared to saline or ECM controls, bleomycin-exposed mice had similar increases in inflammation in the bronchoalveolar lavage fluid regardless of ECM treatment. However, 14 days after exposure, lung histology and collagen levels revealed that mice treated with bleomycin and UBM-ECM powder had negligible fibrosis while mice given only bleomycin had marked fibrosis. More impressively, administration of the UBM-ECM powder 24 hours after bleomycin exposure also significantly protected against pulmonary injury. In addition, in vitro epithelial cell migration and wound healing assays reveal that UBM-ECM promotes epithelial cell chemotaxis and migration.

Fig 1. UBM-ECM attenuates bleomycin-induced pulmonary fibrosis. Fibrosis was measured in wild type C57BL/6 mice 14 days after exposure. Average histology score was determined by a blinded pathologist9 and the scoring system was as follows: 0=no fibrosis, 1=0-25% fibrosis, 2=25-50%, 3=50-75%, 4=75-100%. **p<0.001

Discussions and Conclusions
Although there was no change in the amount of inflammation and only a modest decrease in protein in the BALF, UBM-ECM was able to dramatically limit the fibrosis that results from bleomycin injury even with delayed treatment. Overall, our preliminary results strongly suggest that UBM-ECM can minimize bleomycin-induced pulmonary fibrosis. Future investigations into the mechanisms in which UBM-ECM lessens fibrosis are forthcoming.

References

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Disclosures
TWG serves on the Scientific Advisory Board for Acell, Inc., which commercializes UBM.