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The effects of storage and sterilization on de-cellularized and re-cellularized whole lung

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ABSTRACT

Despite growing interest on the potential use of de-cellularized whole lungs as 3-dimensional scaffolds for *ex vivo* lung tissue generation, optimal processing including sterilization and storage conditions, are not well defined. Further, it is unclear whether lungs need to be obtained immediately or may be usable even if harvested several days post-mortem, a situation mimicking potential procurement of human lungs from autopsy. We therefore assessed effects of delayed necropsy, prolonged storage (3 and 6 months), and of two commonly utilized sterilization approaches: irradiation or final rinse with peracetic acid, on architecture and extracellular matrix (ECM) protein characteristics of de-cellularized mouse lungs. These different approaches resulted in significant differences in both histologic appearance and in retention of ECM and intracellular proteins as assessed by immunohistochemistry and mass spectrometry. Despite these differences, binding and proliferation of bone marrow-derived mesenchymal stromal cells (MSCs) over a one month period following intratracheal inoculation was similar between experimental conditions. In contrast, significant differences occurred with C10 mouse lung epithelial cells between the different conditions. Therefore, delayed necropsy, duration of scaffold storage, sterilization approach, and cell type used for re-cellularization may significantly impact the usefulness of this biological scaffold-based model of *ex vivo* lung tissue regeneration.

1. Introduction

Increasing interest in the use of de-cellularized complex whole organ scaffolds for *ex vivo* tissue engineering has provided both opportunity and also unique challenges. Among the unresolved issues which require clarification include defining optimal, organ specific approaches for de-cellularization and for sterilization and storage of de-cellularized organs prior to re-cellularization [1–4]. With respect to trachea and lung, a number of recent publications have comparatively assessed different de-cellularization protocols. Notably, the resulting architecture and extracellular matrix (ECM) protein composition of either trachea or lungs may differ substantially between the different regimens utilized [5–7]. Whether this will subsequently affect re-cellularization of scaffolds and therefore the generation of functional tissue suitable for transplantation, remains unresolved [4,5]. Methods of optimal sterilization and storage have been suggested for trachea [7,8] but not yet clearly delineated for de-cellularized lungs. One further consideration is that of post-mortem time prior to lung harvest and de-cellularization, a practical issue for procurement of human lungs. A number of hours or even days may pass prior to post-mortem

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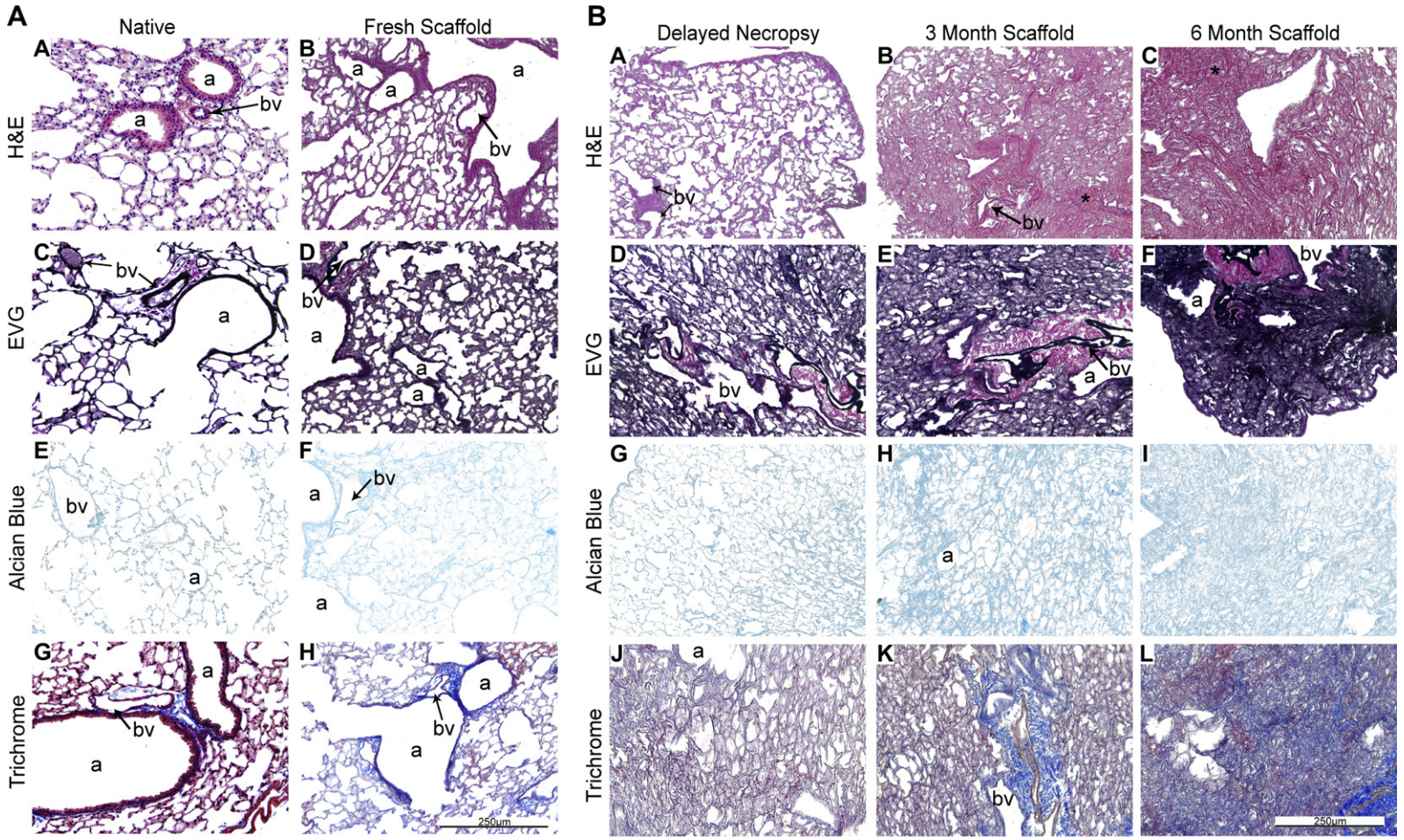

Fig. 1. (A) De-cellularization of freshly obtained mouse lungs maintains the native architecture. Representative photomicrographs comparing native (control) mouse lung and freshly de-cellularized mouse lungs are depicted. a = airways, bv = blood vessels. $N = 6$ for each condition. Original magnification 200×. (B) Delayed necropsy results in preservation of native architecture whereas storage for 3-month and 6-months results in progressive atelectasis and loss of normal architecture. Representative photomicrographs depict the effects of delayed necropsy and of 3 and 6 month storage on freshly de-cellularized mouse lungs. * = atelectatic tissue, a = airways, bv = blood vessels. $N = 6$ for delayed necropsy & 3-month scaffolds, $N = 4$ for 6-month scaffolds. Original magnification 200×. (C) Peracetic acid treatment preserves while irradiation destroys de-cellularized lung tissue architecture. Representative photomicrographs demonstrate the architecture of irradiated and peracetic acid-treated freshly de-cellularized lungs. * = abnormal architecture due to irradiation treatment, a = airways, bv = blood vessels. $N = 6$ for each condition. Original magnification 200×. (D) Inflation of sterilized or stored de-cellularized lungs restores native architecture. Representative photomicrographs demonstrate that atelectasis of stored lungs can be reversed. a = airways, bv = blood vessels. $N = 6$ for each condition. Original magnification 200×.