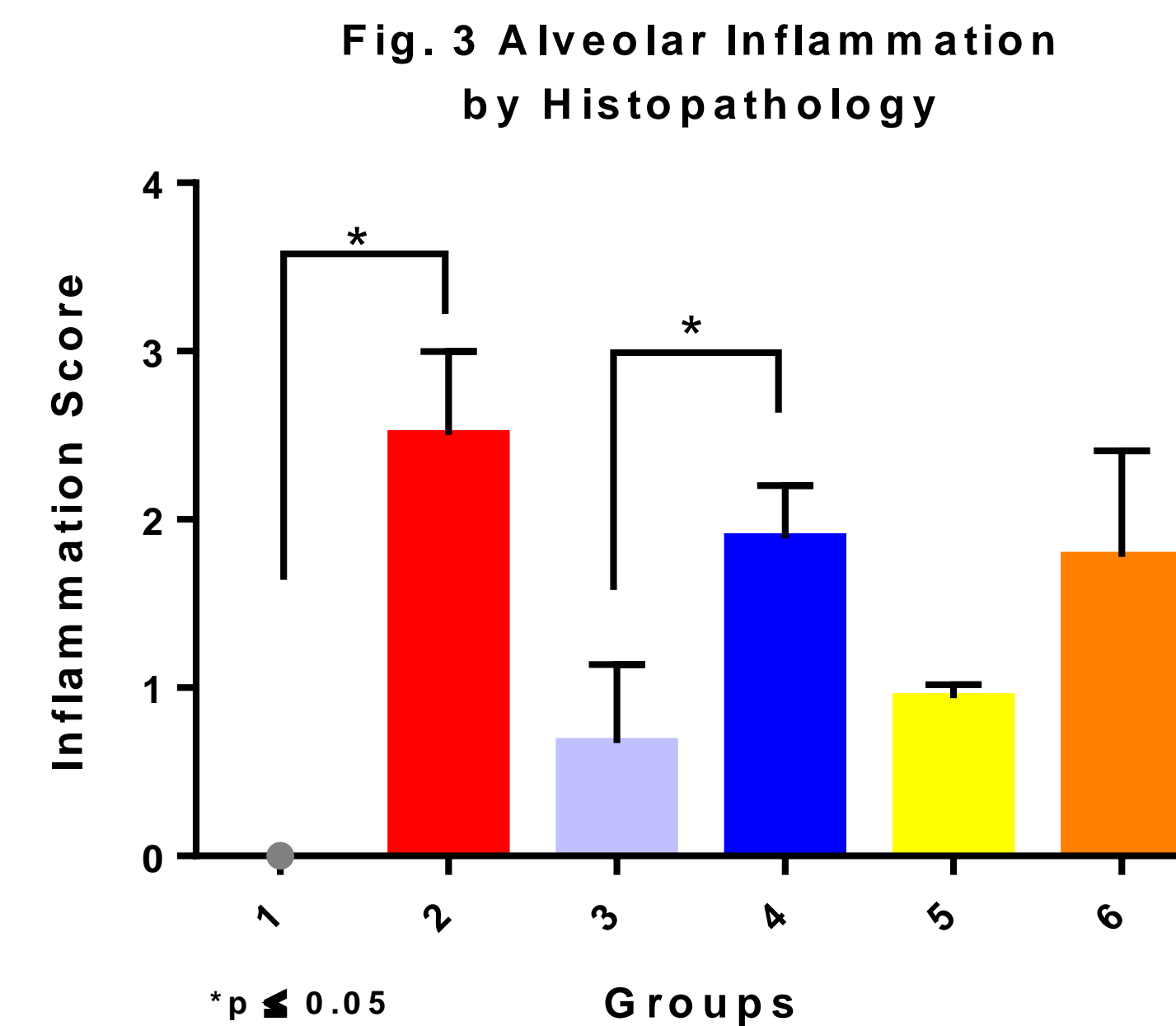
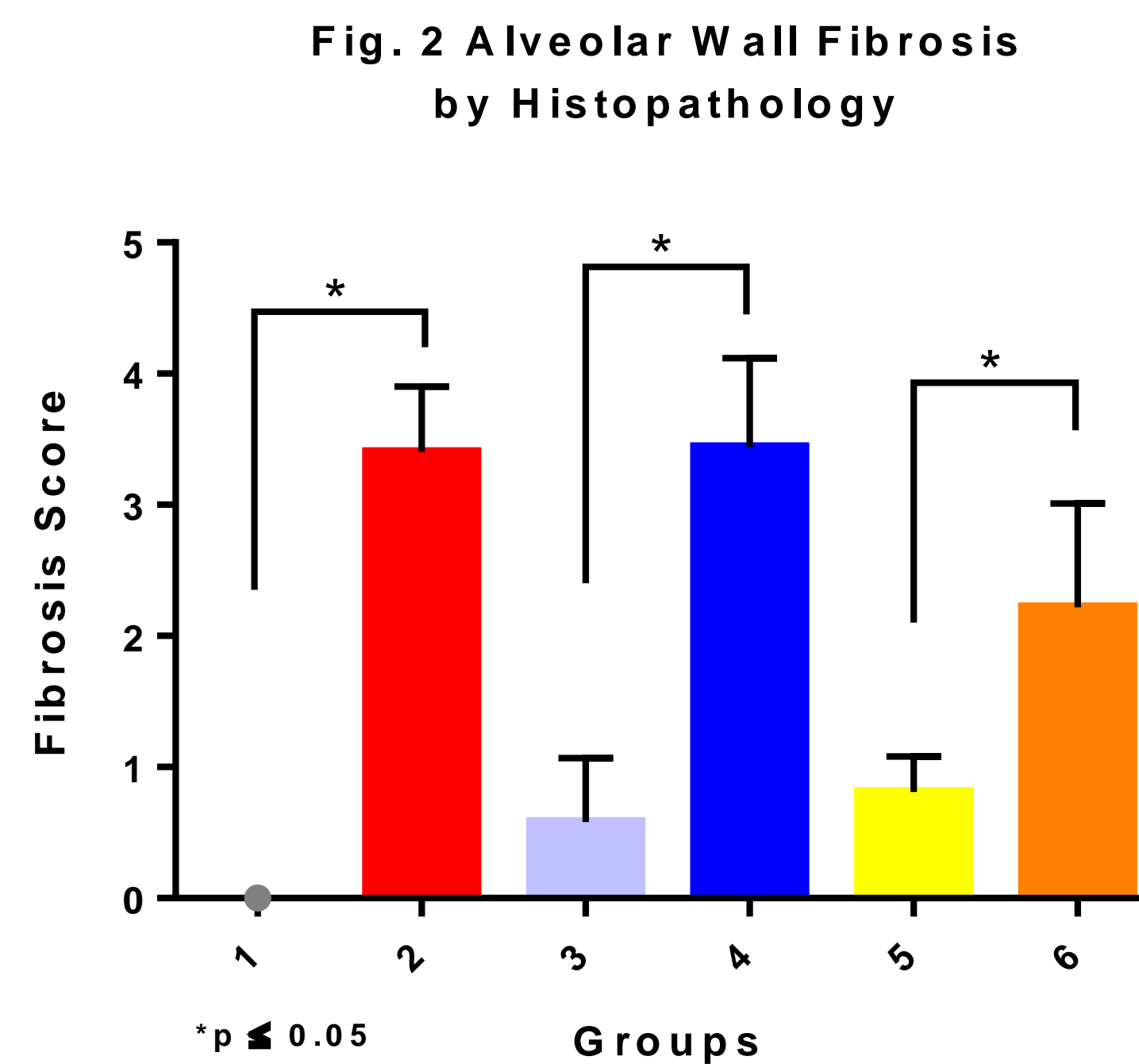
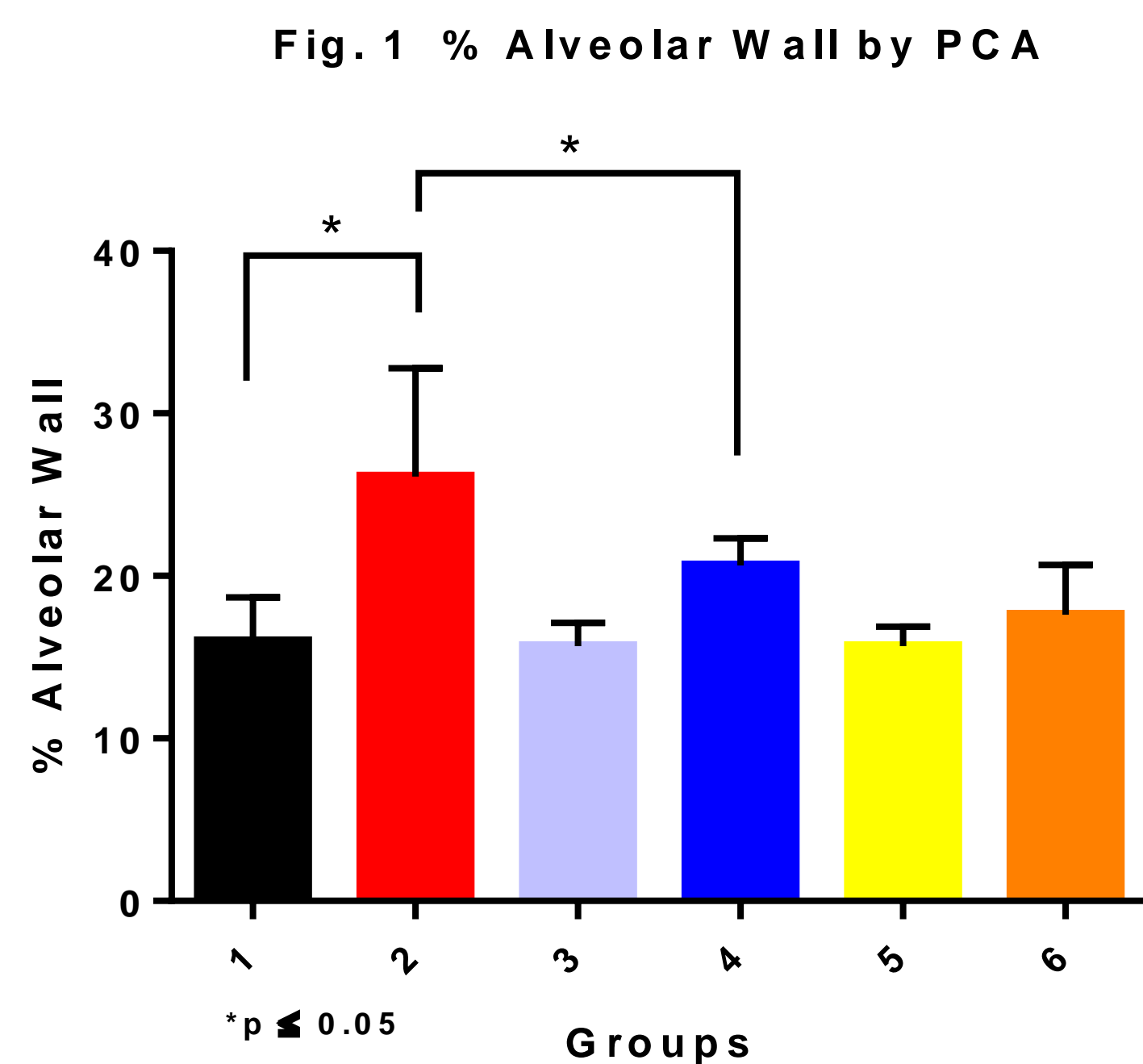
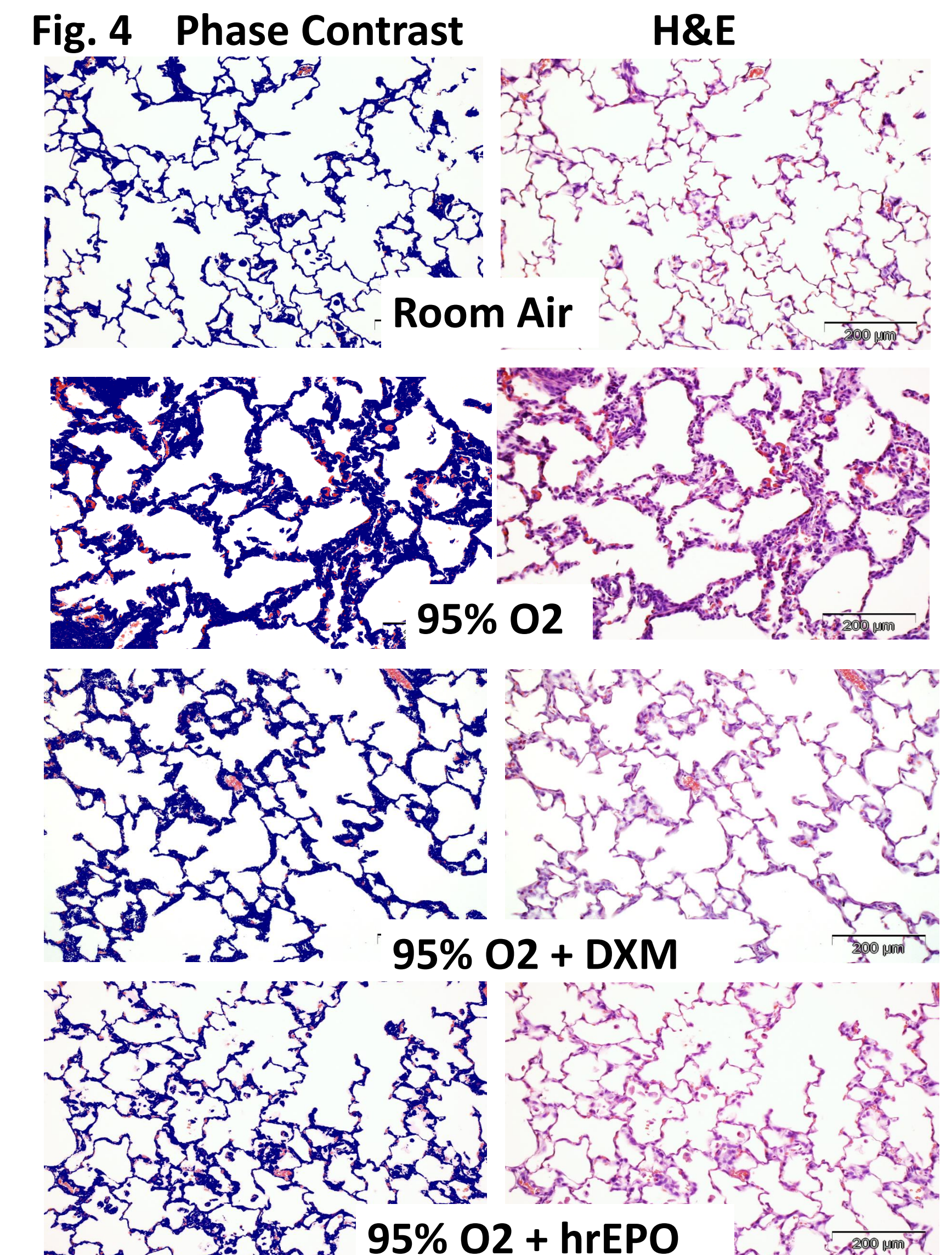


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Summary – Hyperoxia-induced lung injury is well characterized in neonatal rats; hallmarked by alveolar wall fibrosis, alveolar space enlargement and inflammation. Standard endpoints include histopathology and mean linear intercept (MLI), which is a laborious measurement of alveolar lengths. By contrast to MLI, phase contrast analysis (PCA) has the advantage of measuring septal and alveolar volume, both of which more broadly capture the magnitude of changes induced by this model. Treatment with anti-inflammatory therapeutics (dexamethasone (DXM) and erythropoietin (hrEPO) reversed the extent of hyperoxia-induced lung injury as measured by PCA and traditional histopathological assessments. PCA provides a quantitative and sensitive readout for the assessment of pulmonary changes that is simpler and a more efficient method for quantification of pulmonary volume changes following hyperoxia compared to customary approaches like MLI.

Model Induction - Newborn Sprague-Drawley rats were exposed to 95% oxygen for 10 consecutive days from postnatal Day 4-14 and maintained at room air until Day 28. A subset of normoxic pups were maintained entirely in room air as negative controls. Hyperoxic and normoxic pups were randomized to receive either 1) Normal saline on Day 14; 2) once-daily tapering dose regimen of DXM IP for 6 days (0.5mg/Kg on Day 10-11, 0.25mg/Kg on Day 12-13, 0.125mg/Kg on Day 14-15) or 3) once-daily hrEPO IP (76ug/Kg on Day 4 and 7). Consistent with typical research use in rats, animals treated with hrEPO were sacrificed on Day 14 upon removal from hyperoxic chambers whilst all other groups were sacrificed on Day 21.

Phase Contrast Analysis and Histopathology – Lungs were perfused with 10% NBF and fixed for routine H&E staining. For PCA quantification, RGB images from lung slides of 5 regions of interest were first converted to binary images with pixel thresholds to differentiate airspace and adjacent tissue. Quantification of pixel areas representing alveolar tissue are presented as % alveolar wall. Additionally, lung sections were scored for alveolar wall fibrosis and alveolar inflammation by standard histopathology.



Results – Hyperoxygenation increases alveolar wall thickness (% alveolar wall), as well as alveolar volume. A partial recovery from oxygen-induced changes was elicited by DXM, and a full recovery was present with hrEPO (Fig.1). Histopathologically, hyperoxygenation produced uniform and consistent pulmonary lesions characterized by a multifocal to diffuse distribution of lesions throughout the lung (Fig. 4). This was accompanied by fibrotic thickening of the alveolar walls with macrophages within alveolar spaces and adherent to alveolar walls. Treatment with DXM and hrEPO reduced the severity of septal fibrosis and alveolar (Fig. 2 and 3).

Conclusion - PCA is a reproducible and sensitive method for quantification of morphological changes associated with hyperoxia-induced alveolar responses. Measurement of anti-inflammatory interventions on tissue responses demonstrated the utility of PCA as a complementary endpoint to standard histopathology. This simple and robust approach to quantification allows for more efficient screening of anti-inflammatory therapeutics than does MLI in the assessment of pulmonary oxidative injury.