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Title: In-vivo microscopy of the effect of surfactant on alveolar morphology

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Body: Changes in alveolar morphology induced by intra-tracheal delivery of CUROSURF (CS, Chiesi) were evaluated after opening a pleural window allowing in-vivo microscopic imaging (x300) of sub-pleural alveoli (fig 1a) revealing in physiological, non surfactant deprived conditions, a remarkable degree of geometrical inhomogeneity. Data were collected in 7 male anesthetized, tracheotomized and mechanically ventilated rabbits (0,75-1 Kg) who received intra-tracheal instillation of 300 μ l, corresponding to 16 mg/Kg, of CS. Images were acquired up to 20 minutes after instillation. Each acquisition (10 images/second) was triggered during the expiratory phase. After defining a ROI (Region Of Interest), alveolar morphology was analyzed through an image processing program (ImageJ). Surfactant instillation, on average, caused an increase in alveolar area (fig 1b, closed circles), peaking at about 10% after 10 minutes and returning towards baseline after 20 minutes. The large standard deviation reflects the variability in caliber following CS instillation as shown for individual alveoli in one representative animal (fig 1c, closed symbols). No changes in alveolar geometry were observed in animals not receiving CS (open circles). Data suggest that CS instillation favors gas diffusion by increasing alveolar surface area, despite a large inhomogeneity in alveolar distension, likely reflecting local differences in surfactant distribution and/or alveolar compliance.