Introduction
Upper airway obstruction is a common cause of poor performance in horses. In general, airway obstructions arise from a reduction in neuromuscular function or from a decrease in the mechanical stiffness of the structures of the upper airway. These reductions in muscular control and stiffness eventually decrease the ability of the airway to resist inspiratory or expiratory pressures, causing laryngeal collapse. We propose to restore airway patency through methods that replace the damaged tissue and improve the stiffness of airway structures. Tissue engineering methods have shown promise for the replacement of a wide variety of damaged, diseased, or missing tissue structures. The objective of the present study was to establish a tissue engineering approach to the creation of viable constructs which approximate the shape and size of equine airway structures.

Methods
Briefly, computed tomography (CT) imaging was performed on intact equine larynges (Figure 1a). CT images were then used to create three-dimensional computer models of the cartilaginous structures of the larynx (Figure 1b). Anatomically shaped injection molds were then created from the three-dimensional models using rapid prototyping (Figure 1c). These molds were then seeded with chondrocytes resuspended within alginate prior to static tissue culture (Figure 1d).

A bovine source was selected for the present, preliminary study due to increased availability of bovine as compared to equine tissues. Cells were harvested from either auricular cartilage (elastic cartilage – epiglottis construct) or from the cartilage of the articulating surfaces of the femoro-patella groove and condylar head (hyaline cartilage – arytenoid construct) using 0.2% collagenase digestion. Isolated cells were then resuspended in 2.2% alginate in PBS at a concentration of 25 million cells/mL. The cell/alginate mixture was then combined with 1% calcium sulfate crosslinking solution prior to injection into the mold. The injected mold was placed into a calcium chloride post-crosslinking solution for 1 hour at room temperature prior to de-molding of the construct. Constructs were then cultured in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum and a 1% antibiotic/antimycotic solution for up to 4 weeks post seeding and then evaluated for biochemical content (DNA, collagen, GAG, elastin), biomechanical properties (compression testing), and histologic architecture (hematoxylin and eosin, Safranin O, Verhoeff’s elastic stain).

Results
Three-dimensional computer reconstructions of individual airway structures were utilized in the creation of molded constructs which were found to approximate the size and shape of equine tissue structures. It was shown that it is possible create constructs consisting of chondrocytes from both elastic and hyaline cartilage sources, and that it is possible to seed such constructs while maintaining 75%+ cell viability. Extracellular matrix content was observed to increase with time in culture and was accompanied by an increase in mechanical stiffness.

Conclusions
We have shown that it is possible to create viable constructs which approximate both the shape and size of cartilaginous structures of the equine airway. Additional investigation is required to determine the optimal culture time prior to implantation as well as to evaluate the ability of constructs created in this manner to integrate with native tissues following implantation. If successful, such an approach would represent a significant improvement upon the currently available treatments for damaged airway cartilage in horses and may provide clinical options for replacement of damaged tissues during treatment of obstructive airway diseases.

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