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An ectopic approach for engineering a vascularized tracheal substitute☆

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ABSTRACT

Tissue engineering can provide alternatives to current methods for tracheal reconstruction. Here we describe an approach for ectopic engineering of vascularized trachea based on the implantation of co-cultured scaffolds surrounded by a muscle flap. Poly(L-lactic-co-glycolic acid) (PLGA) or poly(ε-caprolactone) (PCL) scaffolds were seeded with chondrocytes, bone marrow stem cells and co-cultured both cells respectively (8 groups), wrapped in a pedicled muscle flap, placed as an ectopic culture on the abdominal wall of rabbits ($n = 24$), and harvested after two and four weeks. Analysis of the biochemical and mechanical properties demonstrated that the PCL scaffold with co-culture cells seeding displayed the optimal chondrogenesis with adequate rigidity to maintain the cylindrical shape and luminal patency. Histological analysis confirmed that cartilage formed in the co-culture groups contained a more homogeneous and higher extracellular matrix content. The luminal surfaces appeared to support adequate epithelialization due to the formation of vascularized capsular tissue. A prefabricated neo-trachea was transferred to the defect as a tracheal replacement and yielded satisfactory results. These encouraging results indicate that our co-culture approach may enable the development of a clinically applicable neo-trachea.

1. Introduction

The trachea consists of multiple c-shaped cartilage segments with soft tissue connections that form a round tube. The cartilage component has a defined rigidity and elasticity to maintain the flow of air with proper dynamics [1]. The inner tracheal surface is covered with columnar epithelium. This specialized lining features cilia that remove extraneous particles from the air, while goblet cells secrete mucus to protect the trachea from external stimuli [2,3]. These functions are unique and irreplaceable. Due to the lack of similar autologous tissue, tracheal reconstruction is difficult to achieve. Patients with large segmental tracheal lesions therefore commonly require a permanent tracheostomy after tumor excision [4]. Currently, tracheal reconstruction includes the use of an artificial prosthesis [5,6], a composite graft [7,8] or tissue-engineered constructs to provide airway conduits [9,10]. However, prosthetic alloplastic materials often cause clinical problems, such as airway obstruction, and have a high risk of infection. Adequate epithelialization of the lumen and vascularization of the replacement are also essential for decreasing the rates of infection and dehiscence.

Tissue engineering has the potential to facilitate the development of improved methods of tracheal reconstruction. A fully autologous engineered trachea would overcome the limitations of current graft or synthetic approaches. However, to realize its potential, a clinically viable tissue engineered tracheal replacement must include the following components: 1) a bio-degradable and -compatible scaffold to provide the framework for the cells to produce cartilage and soft tissue of the appropriate cylindrical shape; 2) the ability to induce the formation of a functional

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epithelial lining (cultured or migrated from the native trachea); and 3) an extensive vascular supply to support the volume of tissue required for clinical application. Current approaches to tracheal tissue engineering have not been able to achieve all of these goals.

The use of polyglycolic acid (PGA) fibers in tracheal replacement with cell-polymer constructs was first reported in 1994 [11]. These fibers produced hyaline cartilage after four weeks of implantation in nude mice. Although PGA, poly(lactic acid) (PLA), and poly(L-lactic-co-glycolic acid) (PLGA) fibers have been utilized in engineered tracheal scaffolds, these substances do not provide adequate mechanical strength upon implantation [12–14]. As a consequence, the airway collapses, and the tracheal construct fails. Cell sheet-based reconstruction techniques have been increasingly applied to tracheal tissue engineering; these techniques involve the culture of chondrocytes with extra-cellular matrix (ECM) materials [15,16]. However, this approach is limited in its ability to produce a smooth trachea-like cylinder with suitable rigidity. In recent years, poly(ε-caprolactone) (PCL) has been used as a cartilage scaffold material due to its mechanical characteristics, slow bio-degradation, non-toxic degradation products, and good bio-compatibility.

Chondrocytes [17] or bone marrow stem cells (BMSCs) [18–20] have been employed as the cellular components of engineered tracheal cartilage. Chondrocytes produce cartilage similar to that found in the tracheal rings and can provide suitable mechanical strength. However, the clinical use of chondrocytes is limited by de-differentiation during *in vitro* expansion and a scarcity of autologous donor cartilage. BMSCs, which are inherently pluripotent and expandable, could be applied in neo-trachea formation. When cultured alone, their low induction efficiency requires stimulation with different growth and differentiation factors, which restricts their utility. This problem can potentially be resolved by co-culturing BMSCs and chondrocytes [21,22], which are expected to promote the differentiation of BMSCs into chondrocytes and increase the expression of cartilaginous ECM.

The neo-tracheal lining poses a major challenge. Its construction may rely on epithelial migration from adjacent portions of the host trachea into the lumen of the engineered construct [23] or culture of respiratory epithelium *in vitro* with subsequent grafting into the lumen [24]. Both methods require a stable vascular bed for epithelialization. Conduits from *in vivo* ectopic culture could produce a vascularized lamina to support the survival of epithelial cells. Conduits from ectopic prefabrication may also have the potential to promote epithelial migration on the lumenal surface (lamina equivalent) once a tracheal defect is replaced by a vascular transplant.

Vascularized conduits have the ability to resist infection and diminish tissue necrosis and lumen stenosis, which is critical for clinical applications. The movement of ectopic conduits to the trachea is also an important issue. A vascular transplant is preferred to a graft transplant for tracheal replacement to ensure that the blood supply is maintained. Okumus *et al.* demonstrated the feasibility of circumferential trachea reconstruction using a prefabricated axial bio-synthetic flap based on the lateral thoracic fascia (of a rabbit) as a vascular supply [25].

In this study, an methodology (Fig. 1) that combines these cellular and scaffold approaches was developed to construct a tracheal substitute for clinical application. We fabricated a tracheal scaffold using ring-shaped elements constructed of either PLGA or PCL seeded with chondrocytes and BMSCs. These scaffolds were pre-cultured for seven days *in vitro*, followed by implantation onto the abdominal wall of New Zealand white rabbits. A muscle flap (*cutaneous maximus*) was wrapped around the assembled constructs. The

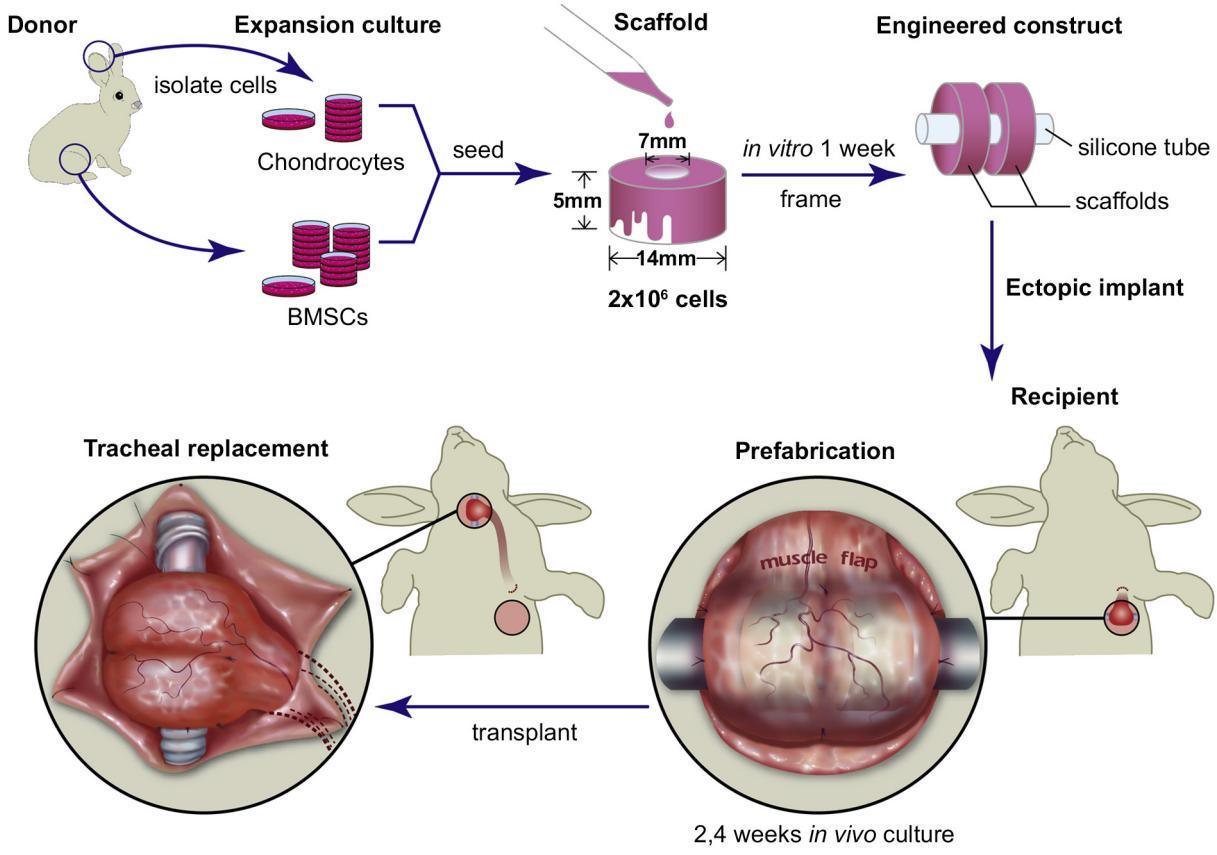

Fig. 1. Diagram of the culture, fabrication, and implantation of the tissue-engineered tracheal constructs.