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Research Paper

Mechanical properties of acellular mouse lungs after sterilization by gamma irradiation ☆

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ABSTRACT

Lung bioengineering using decellularized organ scaffolds is a potential alternative for lung transplantation. Clinical application will require donor scaffold sterilization. As gamma-irradiation is a conventional method for sterilizing tissue preparations for clinical application, the aim of this study was to evaluate the effects of lung scaffold sterilization by gamma irradiation on the mechanical properties of the acellular lung when subjected to the artificial ventilation maneuvers typical within bioreactors. Twenty-six mouse lungs were decellularized by a sodium dodecyl sulfate detergent protocol. Eight lungs were used as controls and 18 of them were submitted to a 31 kGy gamma irradiation sterilization process (9 kept frozen in dry ice and 9 at room temperature). Mechanical properties of acellular lungs were measured before and after irradiation. Lung resistance (R_L) and elastance (E_L) were computed by linear regression fitting of recorded signals during mechanical ventilation (tracheal pressure, flow and volume). Static (E_{st}) and dynamic (E_{dyn}) elastances were obtained by the end-inspiratory occlusion method. After irradiation lungs presented higher values of resistance and elastance than before irradiation: R_L increased by 41.1% (room temperature irradiation) and 32.8% (frozen irradiation) and E_L increased by 41.8% (room temperature irradiation) and 31.8% (frozen irradiation). Similar increases were induced by irradiation in E_{st} and E_{dyn}. Scanning electron microscopy showed slight structural changes after irradiation, particularly those kept frozen. Sterilization by gamma irradiation at a conventional dose to ensure sterilization modifies acellular lung mechanics, with potential implications for lung bioengineering.

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1. Introduction

Organ bioengineering has emerged as a potential therapeutic alternative for severe and irreversible diseases. Considerable research efforts are currently devoted to biofabricate organs from acellular scaffolds. Decellularization aims to remove the cellular content leaving the anatomical 3D structure of the organ intact and freed from genetic material from the donor (Gilbert et al., 2006; He and Callanan, 2013). The acellular organ is then used as a non-immunogenic scaffold to rebuild the organ by seeding it with stem/progenitor cells able to adequately repopulate the different niches within the organ (Ross et al., 2009; Uygun et al., 2010).

Lung bioengineering is a field of application particularly active in recent years because of the lack of viable lungs for transplantation and the reduced long term patient survival after the intervention. The investigation is focused on finding the best protocols for lung decellularization (Jensen et al., 2012; Melo et al., 2014a; Wallis et al., 2012), determining whether donor lungs from patients with aged or diseased lungs are suitable and selecting the best types of stem/progenitor cells for lung repopulation (Cortiella et al., 2010; Melo et al., 2014a; Ott et al., 2010; Petersen et al., 2010; Wagner et al., 2014). As knowledge in these issues is advancing and thus the idea of lung biofabrication is progressively seen as a potential alternative for future clinical routines, practical aspects that will be crucial in terms of real life feasibility are progressively being considered. Therefore, it is of great importance to study the best useful procedures – e.g., freezing/thawing (Nonaka et al., 2014a) and storage duration (Bonenfant et al., 2013) – to provide on-the-shelf acellular lungs ready for recellularization.

An important issue to be determined in handling acellular lungs before they are subjected to recellularization is sterilization to suppress any risk of transmission of viruses and bacteria from the donor to the receiver of the transplanted tissue/organ. Indeed, potential transmission of bacterial and viral infections such as HIV and hepatitis C has been reported in applications of tissue engineering (Eastlund, 2006; Kajbafzadeh et al., 2013). Therefore, the effects of different sterilization methods on different types of tissue have been studied to establish the optimal procedures. To this end, it is very important to take into account that aggressive sterilization methods that ensure full elimination of pathogens can also deteriorate structural components in the tissue, specifically its mechanical performance (Gouk et al., 2008; McGilvray et al., 2011).

Sterilization by gamma irradiation is a common procedure in materials and medical devices for clinical application, such as surgical grafts, due to the high penetrability of photons in the irradiated matter, attributed to its homogeneous dose distribution (IAEA, 2008) (Kaminski et al., 2012). However, gamma irradiation on allograft bone and soft tissues may cause structural damage, altering the biomechanical integrity of the tissue, particularly at high radiation doses (Dziedzic-Goclawska et al., 2005; Nguyen et al., 2007).

The need for testing the mechanical consequences of sterilizing lung scaffolds is particularly relevant since the lung is an organ with high structural and mechanical complexity that is physiologically subjected to continuous deformation cycling during breathing. Therefore, the mechanical properties of the organ scaffold should be preserved as much as possible after sterilization to ensure optimal organ regeneration. However, there are no data on the mechanical effects induced in lungs, whether native or decellularized, when subjected to a conventional irradiation dose ensuring sterilization of health application material (25–30 kGy). In the only data available to date on the effects of gamma radiation on acellular lungs (Bonenfant et al., 2013) the dose was so low (5 Gy/min for 12 min) that it cannot be taken as an accurate reference for safe sterilization procedures (IAEA, 2008). Regardless of the fact that the radiation dose was low, the authors observed that irradiated acellular lungs presented diffuse heterogeneous thickness, fused septa and spaced alveolar aspect, and laminin and collagen I with a more intense appearance due to agglomeration and tissue thickness (Bonenfant et al., 2013). However, as the authors did not measure any mechanical index, it is unknown whether this low dose (60 Gy) affected tissue mechanics. Therefore, the aim of this study was to evaluate the effects of gamma-irradiation sterilization with a conventional dose (Balsly et al., 2008; Loty et al., 1990) on the mechanical properties of acellular lungs subjected to the physiological conditions of cyclic mechanical stretch typical of ventilation. Since it is not clear whether gamma irradiation at low temperatures could be advantageous (due to reduction of the mobility and reactivity of free radicals) or detrimental for tissues (Gouk et al., 2008), we carried out the irradiation study in acellular lungs kept frozen or at room temperature.

2. Methods

2.1. *Lung decellularization*

This study was approved by the Ethical Committee for Animal Research of the University of Barcelona. Lungs were obtained from 26 male C57BL/6 mice (7–8 weeks old) intraperitoneally anesthetized with urethane (1.0 g/kg) and sacrificed by exsanguination. The lungs and trachea were excised and stored at −80 °C until start of the decellularization protocol. The lungs were decellularized following a variant of previously described procedures in mice (Melo et al., in press; Nonaka et al., 2014a,b). As explained below, decellularizing media were infused by the trachea only, with no perfusion through the pulmonary artery since a previous study showed that this procedure resulted in minor differences in rat lungs (Melo et al., 2014a). Lungs were first subjected to a four-times repeating cycle that consisted in thawing the lungs in a water bath at 37 °C and freezing them again at −80 °C. Subsequently, all lung samples were cleaned to remove any attached esophageal, lymphatic and connective tissues. The lungs were then submitted to 6–8 washes by tracheal instillation of 2 mL of PBS containing streptomycin (90 mg/mL), penicillin (50 U/mL) and amphotericin B (25 mg/mL) until the liquid extracted from the lungs had a transparent appearance. This step was repeated with 2.5 mL of de-ionized water several times, then treated with tracheal instillation of 2.5 mL of 1% sodium dodecyl sulfate (SDS)