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Decellularized lung tissue enhanced mesenchymal stem cells proliferation and differentiation

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Abstract

The combination of mesenchymal stem cells (MSCs) and extracellular matrix (ECM)- derived scaffolds present a promising approach in tissue engineering and regenerative medicine. ECM physically supports stem cells niche and influence stem cell proliferation and differentiation. In this study, we investigated the effects of decellularized lung tissue (DLT) on proliferation and chondrogenic properties of MSCs. MTT assay showed that DLT enhanced the cell viability of MSCs. Alcian blue and immunostaining confirmed that DLT ameliorate the chondrogenesis when compared with tissue culture plate. Decellularization of lung tissue seems to be a good tool to proliferation and differentiation of MSCs.

Keywords: Mesenchymal Stem Cells, Decellularization, Chondrogenesis

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1. Introduction

Tissue engineering is a novel approach which seeks to develop methods to replace or repair damaged organs and tissues. In this method, tissues can be engineered using cells which are grown in a porous scaffold biomaterials [1].

Design of biomaterials that can mimic the structural and biochemical function of natural extracellular matrix (ECM) play an important role in cell adhesion, migration, proliferation, and differentiation [2]. In addition to the natural and synthetic scaffolds in tissue engineering [3], decellularization of tissues is pursued as a new strategy to produce ECM-derived biomaterials [4]. The use of ECM derived from decellularized tissue has been shown to influence cell viability and efficiency in vitro and induce regenerative healing in vivo [5]. It should be understood that many agents including thickness, density, and cellularity of desired tissues affect the outcome of decellularization of tissue [4] (for more details see Carpo et al.). The studies conducted to date have highlighted the importance of mechanical properties of the scaffold which it will be incorporated [6] on the differentiation of MSCs [7]. They explored

that seeding MSCs onto decellularized lung tissue influence the differentiation into epithelial phenotypes. In another study, Young et al. have demonstrated that decellularized lung matrix scaffold is a promising tool to produce in vitro smooth muscle [8]. They concluded the benefits of cell- ECM interaction in the contraction of bronchial smooth muscle. As airway cartilage plays a key role in the action of lung, in this study, we investigated the effects of decellularized lung tissue on the chondrogenic differentiation of MSCs [9].

2. Materials and Methods

2.1. Isolation and culture of MScs

MSCs were isolated from femur and tibia bone marrow of Wistar rats using flashing method and cultured under standard condition (37°C, 5% CO2 and humidified chamber) with DMEM containing FBS 10%. Only MSCs of three to sex passages were used to seed.

2.2. Decellularization of lung tissue

Male Wistar rats were obtained from animal facility of Hamadan University of Medical Sciences and kept in the controlled environment until using.

2.3. MSCs isolation and lung decellularization

We used SDS- triton x method to decellularise the lung. The lungs of anesthetized rats were dissected out and washed in PBS for 20 min. the lung was incubated in SDS 1% (48 h) and triton x- 100 1% (1 h). Next we washed lungs in disterilized water for 48 h followed by PBS for 3 h.

2.4. Hematoxylin and Eosin (H&E) staining

Native and decellularized lungs tissue (DLT) were fixed in paraformaldehyde 4% and after tissue processing were sectioned in 5μM diameter. Sections were stained using H&E.

2.5. Scanning electron microscopy

After primary fixation in glutaraldehyde 2.5 %, the samples were postfixed in secondary fixation (1% osmium tetroxide). Following uranyl acetate staining and embedding in EPOS, the 70 nm sections were obtained and after uranyl acetate and lead citrate staining were observed with a scanning microscope.

2.6. DNA content assay

The total DNA content was assessed using phenol/ chloroform extraction method as a protocol described by Köchl et al. [10].

2.7. Cell seeding and Chondrogenic cell differentiation

DLT were cut into 200 mg hydrated scaffolds and incubated in antibiotics (1% gentamicin, 10% penicillin/ streptomycin) for 24 h. Decontaminated DLT scaffolds were transferred into the 24 wells plates and exposed to the medium. MSCs were seeded at a density of 5 x 10^4 cells onto the DLT. To assess the impact of the DLT scaffolds on the cellular behaviour, we used a control group that MSCs were seeded into tissue culture plates and incubated by medium. After 24, 72 hours and one week, the cell viability was examined using MTT (0.5 mg/ ml) assay test according previous protocol [11].

To induce chondrogenesis, after 48 h the medium was replaced with chondrogenic supplemented (0.01 mM dexamethasone, 50 μg/ ml ascorbic acid, 5 μg/ ml insulin- transferrin- selenium X premix, 100 μg/ ml sodium pyruvate, and 10 ng/ml TGF- beta 3) mediums. The medium was changed every 2 days for 21 day.

2.8. Fixation of specimens and Alcian blue staining

Specimen were harvested and fixed by 4% paraformaldehyde. The tissues were dehydrated, embedded in paraffin, and sectioned at a 5μm thickness. Cells and tissues were incubated in 1 % Alcian blue (pH= 1.0) for 30 minutes at room temperature. In this method sulphated proteoglycan are stained blue.

2.9. Immunostaining

Nonspecific sites of fixed specimens were blocked using 10% goat serums. Then they incubated in primary antibody to Collage II (1:20) for 1 h at 37 °C in a humidified chambers and followed by FITC conjugated secondary antibody (1:200). DAPI used as a counterstain to visualise nuclei.

2.10. Statistical analysis

Data were presented as mean ± S.E.M and analysed using SPSS 16 with Student's t-test and one way ANOVA with Tuckey post hoc comparison test.

3. Results

3.1. Organ decellularization

SDS/triton x decellularization method was established to isolate ECM of lung tissue that successfully produced acellular organ which were nearly transplant (Figure 1). The result of H&E staining confirmed the absence of cells in DLT at the end of decellularization process (Figure 1). The graph of SEM indicated a continues three- dimensional ECM microstructure (Figure 2). DNA content assay showed the efficiency of decellularization method. As shown in figure 2, it was optimal to remove the cellular contents with ≈ 98% reduction. There was just 41.59 ± 3.3 ng of DNA / mg in DLT.

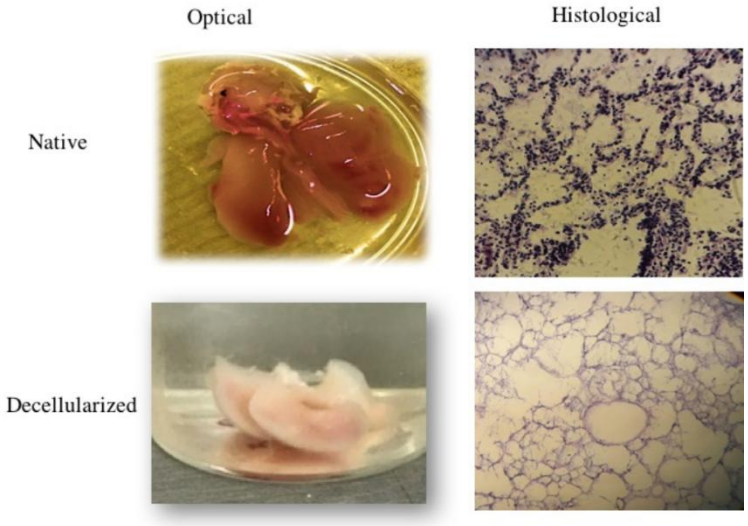

Figure1. H&E staining lung tissue confirmed the absence of cells in decellularized lung tissue at the end of decellularization process.

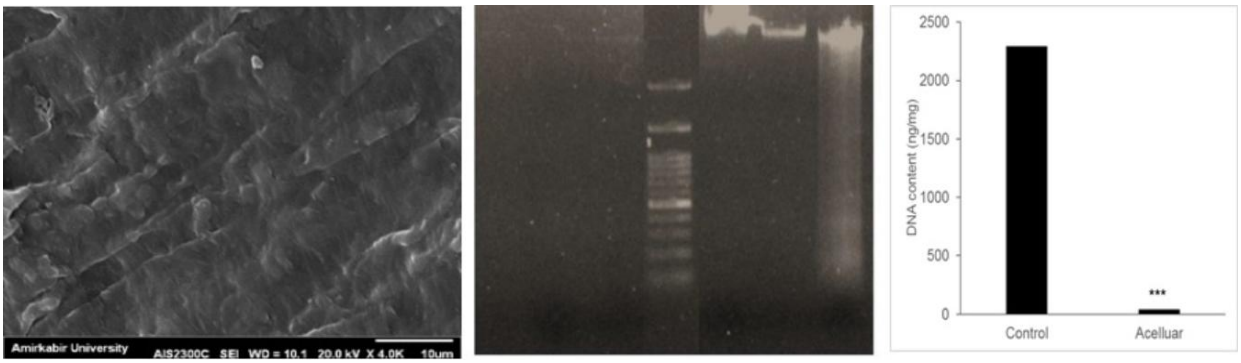

Figure 2. The graph of SEM indicated a continues three- dimensional ECM microstructure and DNA content assay showed the efficiency of decellularization method. 41.59±3.3 ng of DNA / mg in DLT.

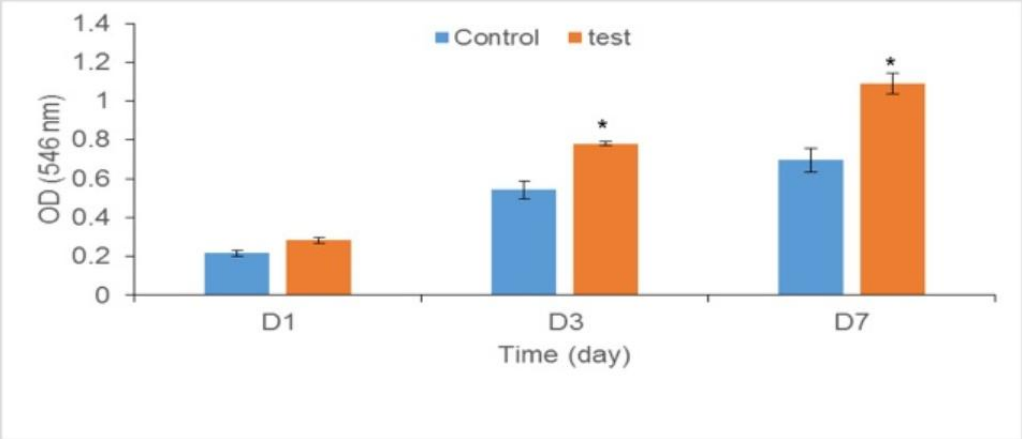

Figure 3. MTT assay on the first, third and seventh days. Our results showed that when MSCs were cultured on DLT, the level of proliferation increased.

3.2. MSCs seeding and proliferation assay

MTT assay measures the activity of cellular dehydrogenase which is an indicator of the number of live and metabolically active cell present. Our results showed that when MSCs were cultured on DLT, the level of proliferation increased (Figure 3). Significant increase in proliferation was time dependent.

3.3. Chondrogenesis differentiation on DLT scaffold

To determine if growth on DLT improve the chondrogenesis, we did differentiation on DLT-coated scaffolds for 21 days. Alcian blue and immunostaining for COL II (as a marker of cartilage) showed the positivity in DLT more than uncoated wells (Figure 4).

4. Discussion

In this study, we used LDT as a model to examine the hypothesis that the decellularization would enhance the differentiation efficiency of MSCs. Seeding of MSCs on LDT revealed high levels of viability and chondrogenesis when compared to tissue culture plates. We used SDS/ triton- x 100 to decellularization because SDS disrupts the interaction between proteins, affect the ECM glycosaminoglycan which reduces the mechanical stability of scaffolds [12]. As well, SDS completely remove the nuclear and cytoplasmic competent [13]. Tarahani Nia et al. reported that decellularization of lung tissue using SDS maintains the proteins of ECM that provide cellular connectivity [14]. Triton- x 100 is a non- ionic detergent that has also been used for decellularization of connective tissues with minimised change in tissue biochemistry [15]. Importance of 3D tissue architecture has been indicated in directing the cellular response [16]. Fibrillar basement network of ECM composed of proteins such as collagen, fibronectin, and laminin which serving as anchoring points for the cells and mediating adhesion to receptors on the cell surface [17]. Our histological studies results showed the preservation of collagen within matrix and absence of cells. We observed porous matrix that plays an important role in the cell infiltration and nutrient movement through the tissue [18]. The main advantage of utilizing the decellularized ECM is that its microenvironment enhances tissue regeneration. The growth factor, cytokines, and chemokines contents of ECM provide bioactive cues for cell proliferation and differentiation [19]. The results of this study showed that chondrogenic differentiation capacity of DLT was more than the tissue culture plates. Consistent to the results of this study, Ji et al. reported that decellularization matrix of adipose- derived MSCs enhanced the cell viability of retinal progenitor cells and promoted the differentiation of progenitor cells toward retinal neurons [20]. They used ammonium hydroxide triton/ NH4OH method to decellularization. In another study, nepherospher cells culture on a decellularized kidney scaffold promoted

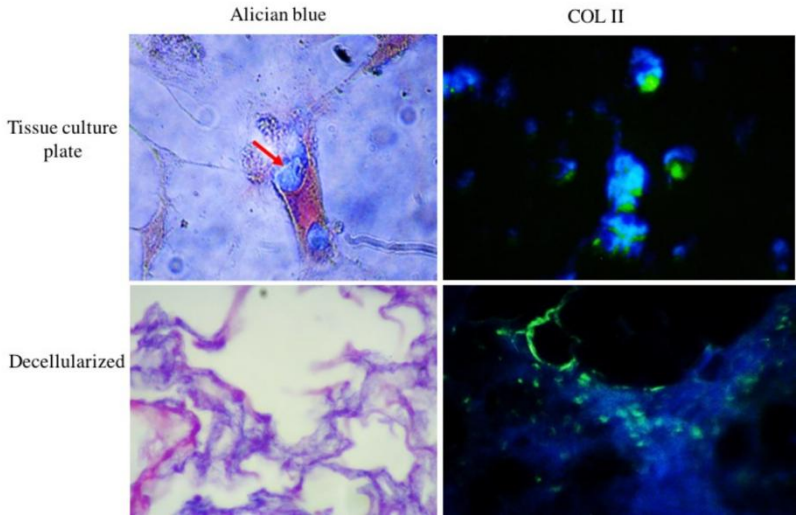

Figure 4. Chondrogenesis differentiation on DLT scaffold by Alcian blue and immunostaining for COL II (as a marker of cartilage) showed the positivity in DLT more than uncoated wells.

the differentiation toward the endothelium and epithelium[21, 23]. They cultured nepherospher cells on the scaffold with basal medium without growth factor and observed that cells were able to generate proximal and distal tubular structure.

5. Conclusion

It has been described that decellularized scaffold preserves transforming growth factors, insulin- like growth factor, and hepatocyte growth factor and subsequently influences differentiation [24]. Taken together, the results of this study showed that Decellularization of lung tissue seems to be a good tool to proliferation and differentiation of MSCs.

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Conflict of interest statement

The authors report no conflicts of interest.

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