Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Histologic analyses of different concentrations of TritonX-100 and Sodium dodecyl sulfate detergent in lung decellularization

H. Tebyanian[1], A. Karami[1], E. Motavallian[2], J. Aslani[3], A. Samadikuchaksaraei[4], B. Arjmand[5], M. R. Nourani[3*]

[1] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Department of General Surgery, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3] Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences; Tehran, Iran
[4] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran; Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
[5] Cell Therapy and Regenerative Medicine Research Center, Endicrinology and Metabolism Cellular-Molecular Sciences Institute, Tehran Univertsity of Medical Sciences, Tehran, Iran

Correspondence to: r.nourani@yahoo.com

Received April 1, 2017; **Accepted** May 18, 2017; **Published** August 15, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.7.8

Abstract: Pulmonary diseases cusecs a large portion of mortality in the world. There is no more cure for pulmonary diseases and many approaches are needed for finding ways to cure. Nowadays, implantation and drugs are only ways for curing those people who are facing with these diseases. Tissue engineering and regenerative medicine have been appeared as multidisciplinary field and also, they presents new therapeutic approaches for pulmonary diseases. One of these therapeutic approaches is decellularization which removes cellular but leaves intact important extracellular matrix (ECM) proteins and three-dimensional (3D) architecture and also, this approach has been studied for in-vitro and ex-vivo. In this study, we aimed to investigate a comparison of different concentrations of Triton X-100 and Sodium dodecyl sulfate detergents in lung decellularization in order to evaluate the effects of different concentrations and times of mentioned detergents on three dimensional and ECM proteins lung. Two detergents (Triton-X100 and Sodium dodecyl sulfat) were used with different concentrations for decellularizing rat lungs for maintaining of three-dimensional lung architecture and ECM protein compositions which have significant roles in differentiation and migration of stem cells. Results showed that SDS 0.05%, 0.1% and Triton-X100 0.1% could maintain 3D, elastin and collagen better than other concentrations in 24 and 48 h- decellularization. We concluded that these approaches can help to achieve three-dimensional architecture and extracellular matrix of lung with minimum destruction for next step such as recellularization and in-vivo study.

Key words: Rat lung; Decellularization; SDS, Triton X-100.

Introduction

Pulmonary disease is one of significant which it decreases the life quality and increase the premature death. Nowadays, transplantation is only way for survival and also, there is a shortage of lungs for transplantation (1-3). Additionally, chronic obstructive pulmonary diseases (COPD) such as emphysema and chronic obstructive bronchitis, have caused more than 120,000 deaths in adults in the US in one year (4). Then, on one hand there is a substantial demand for implanting healthy lung tissues in patients of all ages. On the other hand, unfortunately, many patients on the waiting list for lung transplant pass out before suitable donor organ get found because of the critical shortage of transplantable donor lungs. Moreover, immunosuppression is another lifelong drawback for transplant recipients (1). In this regard, tissue engineering and regenerative medicine have presented as multidisciplinary field and techniques for engineered lung tissue for transplantation (1-3). There are different pediatric and adult pulmonary diseases which they have not effective treatment. But, there is a new approach to use stem cells in natural or synthetic scaffolds which may help to cure pulmonary disease. In addition, some studies have investigated to mimic the three dimensional of lung with using a diversity of biomaterial and natural scaffold as collagen and Gelfoam. But, engineered scaffolds did not have three dimensional matrix due to the complex 3D architecture of the lung. Decellularization is a new technique for removing all cellular materials and produce an intact whole lung, which is an important characteristic of a three dimensional matrix for cellular differentiation (5, 6).

There are major technical elements and challenges before medical application. ECM bioscaffold and decellularization method are significant parameters which are intrinsic to achieve successful recellularization and regenerative process and also, the quality of the ECM directly depends on decellularization method. There are various parameters which should be consider such as type of decellularization agent, concentration, decellularization time, perfusion and… for maintaining 3D and ECM protein composition of lung (7, 8). Some researchers have been investigated on tissue and organs decellularization (9-11). Decellularization approaches can maintain some of the mechanical and biology features. Lung includes all of ECM properties which essential

for biochemical and biophysical functions and ECM is important for attachment, proliferation and differentiation lung cells (12). Many researchers tried to use biomaterial to design matrices that mimic lung ECM but it would be better to use natural lung extracellular matrices which has all of properties such as biocompatibility, elasticity and so on. These features support all of requirements for breathing and oxygenation (13). ECM scaffolds are complex bio-structures constructed of a variety of structural and functional compositions that are specially organized and suitable for the intended tissue (14). Molecules such as collagen, laminin, elastin, and fibronectin are among these compositions (1).

As an instance, using a bipolar detergent such as CHAPS for decellularization of rat lungs retains collagen and some elastin leaving airway and vascular structures intact but glycosaminoglycans (GAGs) are removed. On the other hand, using SDS preserves alveolar and vascular structures and allows recellularization and oxygen exchange, whereas, decellularization of mouse lungs with Triton X-100 plus sodium deoxycholate with DNase remains collagen, elastin, and GAGs (1). In this study, we aimed to investigate a comparison of different concentrations of Triton X-100 and Sodium dodecyl sulfate detergent in lung decellularization in order to evaluate the effects of different concentrations and times of mentioned detergents on three dimensional and ECM proteins lung. Evaluation of structural characteristics of the ECM proteins and 3D was determined by histology tests.

Materials and Methods

Animals and lung extraction

8 male healthy rats (250–300 gr) were purchased from animal researcher center of Baqiyatallah University of Medical Sciences and housed in this center. Rats were controlled in standard environment (humidity and temperature) on a 12/12 hours light/dark cycle with standard food and water, following experimental procedures approved by the Ethical Committee for Animal Research of Baqiyatallah University of Medical Sciences. The rats were anesthetized with intraperitoneal with Ketamine and Xylazine (100 mg/kg and 10 mg/kg). A midline incision was made along the throat, and the trachea was exposed. Right ventricle perfusion were perfused with 250cc phosphate buffered saline (PBS) containing Heparin 5000u/ml and 1% penicillin and streptomycin (P/S) (Figure 1). Lung was sliced to use in decellularization processes.

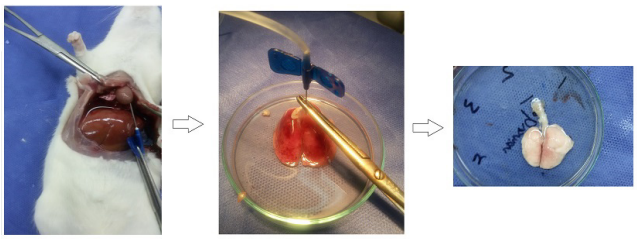

Figure 1. Perfusion of PBS, Heparin and P/S.

Decellularization processes

Sliced lungs were similarly rinsed and treated by SDS and Triton-X100 with different concentrations in three times (24, 48 and 72 h) which are shown in Table 1. Lastly, sliced lungs were rinsed and treated with 1M sodium chloride for 1 h at room temperature. In this study, mixed detergent of SDS and Triton-X100 were used in three times (48, 96 and 144 h) which are shown in Table 2. Sliced lungs were flushed with PBS before they were used for experiments.

Lung histology

H&E staining protocol was utilized to tissue assay. Sliced lungs tissue (before and after decellularization) were fixed in paraformaldehyde 10%, after fixation transferred to tissue processor device to dehydration and clearing. Finally, they were embedded in paraffin and sectioned in 5 micron thickness. Sections on glass lam were stained with H&E, Trichrome- Masson staining and Elastin staining and then, they were observed in a light microscope (15-19). H&E staining was utilized to visually quantify the nuclei loss and three-dimensional lung architecture by comparing nuclei counts in paraffin sections of normal and decellularized sliced lungs. Nuclei were counted from three random images at 100 × magnification using a counting tool. Trichrome- Masson staining and Elastin staining were done for evaluating the level of collagen and elastin, respectively.

Statistical analysis

All studies were conducted at least in biological triplicate. Data were statistically analyzed by t-test. P values less than 0.05 were considered significant.

Table 1. SDS and Triton X-100 detergent for rat lung decellularization.

Time	Methods	SDS approaches	Methods	Triton X-100 approaches
24 h	1	SDS 0.05% 23h, NaCl 1 M 1h	13	Triton X-100 0.05% 23h, NaCl 1 M 1h
	2	SDS 0.1% 23h, NaCl 1 M 1h	14	Triton X-100 0.1% 23h, NaCl 1 M 1h
	3	SDS 0.15% 23h, NaCl 1 M 1h	15	Triton X-100 0.15% 23h, NaCl 1 M 1h
	4	SDS 0.2% 23h, NaCl 1 M 1h	16	Triton X-100 0.2% 23h, NaCl 1 M 1h
48 h	5	SDS 0.05% 47h, NaCl 1 M 1h	17	Triton X-100 0.05% 47h, NaCl 1 M 1h
	6	SDS 0.1% 47h, NaCl 1 M 1h	18	Triton X-100 0.1% 47h, NaCl 1 M 1h
	7	SDS 0.15% 47h, NaCl 1 M 1h	19	Triton X-100 0.15% 47h, NaCl 1 M 1h
	8	SDS 0.2% 47h, NaCl 1 M 1h	20	Triton X-100 0.2% 47h, NaCl 1 M 1h
72 h	9	SDS 0.05% 71h, NaCl 1 M 1h	21	Triton X-100 0.05% 71h, NaCl 1 M 1h
	10	SDS 0.1% 71h, NaCl 1 M 1h	22	Triton X-100 0.1% 71h, NaCl 1 M 1h
	11	SDS 0.15% 71h, NaCl 1 M 1h	23	Triton X-100 0.15% 71h, NaCl 1 M 1h
	12	SDS 0.2% 71h, NaCl 1 M 1h	24	Triton X-100 0.2% 71h, NaCl 1 M 1h

Table 2. Mixed detergents for rat lung decellularization.

Detergents	Time	Methods	Decellularization Methods
Triton X-100+ SDS	48 h	25	Triton X-100 0.05% 24h, SDS 0.05% 24h
		26	Triton X-100 0.1% 24h, SDS 0.1% 24h
		27	Triton X-100 0.15% 24h, SDS 0.15% 24h
		28	Triton X-100 0.2% 24h, SDS 0.2% 24h
		29	Triton X-100 0.1% 24h, SDS 0.05% 24h
		30	Triton X-100 0.15% 24h, SDS 0.1% 24h
	96 h	31	Triton X-100 0.05% 48h, SDS 0.05% 48h
		32	Triton X-100 0.1% 48h, SDS 0.1% 48h
		33	Triton X-100 0.15%48h, SDS 0.15% 48h
		34	Triton X-100 0.2% 48h, SDS 0.2% 48h
		35	Triton X-100 0.1% 48h, SDS 0.05% 48h
		36	Triton X-100 0.15% 48h, SDS 0.1% 48h
	144 h	37	Triton X-100 0.05% 72h, SDS 0.05% 72h
		38	Triton X-100 0.1% 72h, SDS 0.1% 72h
		39	Triton X-100 0.15% 72h, SDS 0.15% 72h
		40	Triton X-100 0.2% 72h, SDS 0.2% 72h
		41	Triton X-100 0.1% 72h, SDS 0.05% 72h
		42	Triton X-100 0.15% 72h, SDS 0.1% 72h

Results

Hematoxylin and Eosin Staining of decellularized lungs

Results showed that methods 4-12 could completely remove cells from tissue but methods 1, 2 and 5 could maintain the three-dimensional (3D) lung architecture more than others in SDS methods. 3D structures of lung were completely destroyed by methods 9-12 in SDS methods. It can be understood that the 3D lungs were destroyed by increasing the concentration of SDS to 0.2% (Figure 2). Removing of nuclei was not completely observed by increasing the concentration of Triton X-100 (0.05% to 0.2%) in 24 h-decellularization. Method 13 could relatively maintain the 3D of decellularized lung but it could not completely remove nuclei from tissue compare to other methods in Triton X-100 approaches. Methods 18 and 22 were observed as best methods decellularization in maintaining of structures and removing of nuclei in Triton X-100 approaches in 48 and 72 h-decellularization. Removing of nuclei and maintaining of structures were not detected by increasing the concentration of SDS + Triton X-100 in 48 h-decellularization but methods 29 could relatively remove nuclei and keep structures better than other approaches in SDS + Triton X-100 methods in 48 h-decellularization. And also, method 35 was detected as the least damage to structures and relatively remove nuclei with comparing to other approaches in SDS + Triton X-100 methods in 96 h-decellularization. All of SDS + Triton X-100 methods destroyed structures of decellularized lungs in 144 h-decellularization (Figure 2).

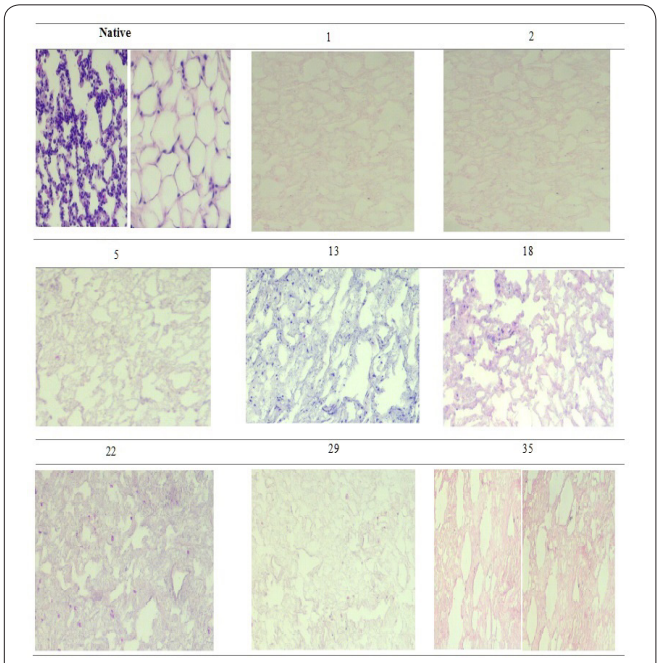

Figure 2. Hematoxylin and Eosin Staining showed structures and nuclei present in native and decellularized lungs. Representative images for all conditions are shown (100×magnification).

Trichrome- Masson and Elastin staining of decellularized lungs

Collagen and elastin were relatively detected more in 24 h in SDS detergent approaches but methods 1, 2 and 5 could retain collagen and elastin more than other approaches in 24, 48 and 72 h-decellularization. Methods 13, 18 and 22 could preserve collagen and elastin better than other approaches in Triton X-100 methods. Mixed approaches of SDS and Triton-X100 were done and methods 29 and 35 could relatively maintain collagen and elastin comparing to other methods in SDS + Triton-X100 approaches in 48, 96 and 144 h-decellularization. According to Tables 1 and 2, collagen and elastin were significantly decreased by increasing the concentration and times (Figure 3 and 4).

Discussion

There are many limitations in lung tissue engineering and regenerative medicine due to complex structure of lung. Researchers have tried to produce this complex three dimensional architecture as a decellularized scaffold (20). Many studies have been done for producing a complex structure of lung from biomaterial but it has a lot of limitations. Recently, decellularization have been presented as decellularized biomimetic scaf-

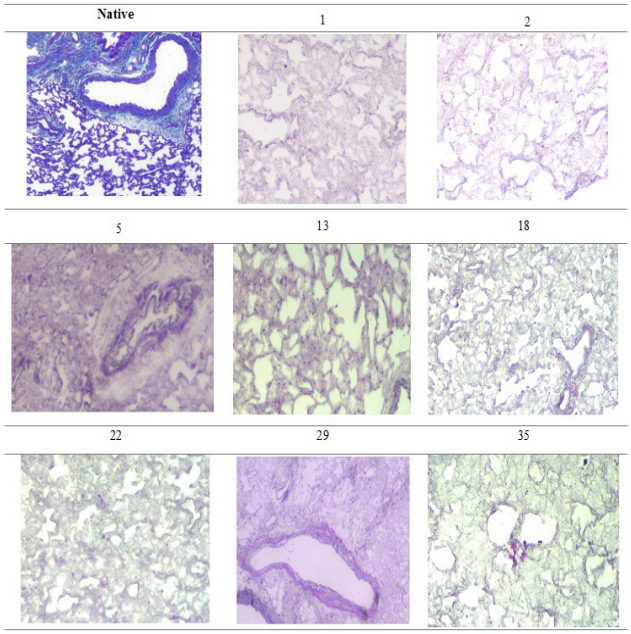

Figure 3. Trichrome-Masson staining showed collagen levels in native and decellularized lungs (Blue color). Representative images for all conditions are shown (100×magnification).

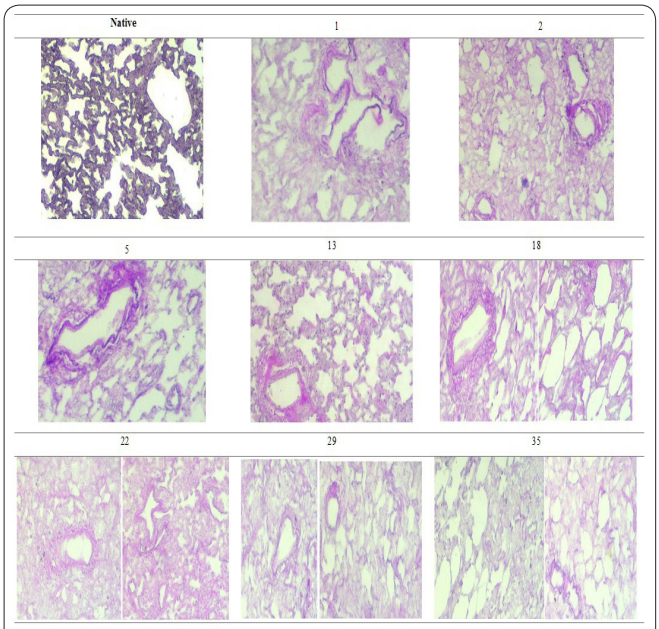

Figure 4. Elastin staining showed elastin levels in native and decellularized lungs (Blue color to black color). Representative images for all conditions are shown (100×magnification).

fold to support differentiated stem cells which it can be an important treatment in pulmonary diseases. The fast making of scaffolds would increase the efficiency of the clinical delivery and availability (5, 15). Native ECM has the optimal substrate for cell attachment, spreading, growth and differentiation. The aim of the decellularization method is to remove cellular and nuclear material while maintaining 3D and ECM protein compositions and also, diminishing any damage to lung ECM. There are various types of decellularization approaches such as physical, chemical and enzymatic methods (21, 22). Physical approaches can cause destruction of the delicate lung ECM which this method includes freezing, pressure, sonication and agitation, while enzymatic approaches cannot be cost-effective due to large volumes of liquid would be needed for decellularization of a whole organ such as proteases, nucleases, and calcium chelators (10). Trypsin has been used in enzymatic method which it can remove fibronectin, laminin, and elastin. Because of the importance of these compositions, enzymatic approaches have not been mostly used in decellularization. But, chemical approaches are cost-effective and have several of techniques such as alkaline or acidic solutions, detergents (SDS, SDC, CHAPS and …) hypotonic or hypertonic solutions, and chelators (10, 21, 22). In this study, we described 42 methods for decellularization of rat lungs and compare dthe results with each others to find the best approaches for decellularization. Significantly, decellularized lung should have the capacity to support suitable growth and differentiation of inoculated cells. Many studies have demonstrated methods of decellularization which they used physical, chemical approaches such as freeze-drawing, CHAPS, SDC, SDS and so on. However, the optimal means of decellularizing lungs while maintaining architecture is not yet clear. In addition, there isn't an optimization of lung decellularization for preserving three dimensional structure and ECM proteins (5, 13, 15, 23). Furthermore, there are various methods for decellularization but there is no concurrence on the best method yet (24). Jensen et al showed evaluation of 24 h-decellularization process (Triton-X100 0.1% and sodium deoxycholate 2%) which they demonstrated that lung scaffolds with preservation of key ECM proteins collagen I, collagen IV, fibronectin, and laminin, but with loss of nuclear proteins (5). Cortiella et al demonstrated that the first attempt to produce and use whole decellularized lung and they compared the influence of decellularized lung by SDS 0.1%, Gelfoam, Matrigel, and a collagen I hydrogel matrix on the mESC attachment, differentiation and subsequent formation of complex tissue and they found that decellularized lung allowed for better retention of cells with more differentiation of mESCs into epithelial and endothelial lineages (13). Neill et al used CHAPS 8 mM and SDS 1.8 mM for human and porcine decellularization and they found that porcine lungs can be decellularized using CHAPS to produce lung ECM scaffolds with properties resembling those of human lungs, for pulmonary tissue engineering and they proposed that porcine lung ECM can be an excellent screening platform for the envisioned human tissue engineering applications of decellularized lungs (25). In this study, two detergents (SDS and Triton X-100) were used for decellularization and also, mixed approaches were designed. Results showed that destroying of ECM and removing of nuclei were increased by increasing the concentration of each detergents and times. In SDS approaches, the best methods were 1, 2 and 5 which could relatively retain the 3D structures and remove nuclei from lung tissue in 24 and 48 h-decellularization. But, method 5 showed better result in comparison with methods 1 and 2 in SDS approaches. In Triton X-100 approaches, methods 13 could maintain the 3D but it was weak in removing of nuclei from lung tissue in 24 h-decellularization. Methods 18 and 22 were the best decellularization approaches in preserving 3D and ECM collagen and elastin in 48 and 72 h-decellularization, respectively. Destroying of collagen and elastin were increased by increasing of the concentration of detergents and times. Mixed approaches of SDS and Triton-X100 showed

that methods 29 and 35 could relatively remove nuclei and relatively preserve collagen and elastin comparing to other methods in SDS + Triton-X100 approaches in 48, 96 and 144 h-decellularization. Wallis et al evaluated the histologic appearance extracellular matrix (ECM) with three different detergent based decellularization methods utilizing either Triton-X 100 (0.1%)/ sodium deoxycholate (0.1%) (Triton/SDC), sodium dodecyl sulfate (SDS) (0.1%), or 3-[(3 cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) (8 mM) and they demonstrated that different detergent based decellularization protocols result in significant differences in histologic appearance (26). Maghsoudlou et al decellularized (sodium deoxycholate 4%) the rat lungs via the intratracheal route removed most of the nuclear material when compared to the other entry points and they showed a presence of collagen, elastin, GAG and laminin with Staining and quantification for ECM components (20). Finally, we concluded that methods 1, 2, 5, 29 and 35 could relatively remove nuclei and also, could maintain 3D, collagen and elastin better than other approaches. These approaches can help to achieve three dimensional architecture and extracellular matrix proteins of lung with minimum destruction for next step such as recellularization and in-vivo study. And also, these results may be appropriate to other decellularized whole organ/species, and may lead the way to further optimization and standardization in the improvement of regenerated organ replacements for transplantation.

Acknowledgements

This study is extracted from Ph.D thesis which has been approved by Applied Biotechnology Research Center, Baqiyatallah University of medical sciences, Tehran, Iran.

Interest conflict

This research was financially supported by Baqiyatallah University of medical sciences, Tehran, Iran.

References

1. Prakash YS, Tschumperlin DJ, and Stenmark KR. Coming to terms with tissue engineering and regenerative medicine in the lung. American Journal of Physiology - Lung Cellular and Molecular Physiology 2015; 309 (7): L625-L638.
2. Garcia O, Carraro G, Navarro S, Bertoncello I, McQualter J, Driscoll B, Jesudason E, and Warburton D. Cell-based therapies for lung disease. Br Med Bull 2012; 101 147-61.
3. Morrisey EE and Hogan BLM. Preparing for the First Breath: Genetic and Cellular Mechanisms in Lung Development. Developmental Cell 2010; 18 (1): 8-23.
4. Miniño AM, Murphy SL, Xu J, and Kochanek KD. Deaths: final data for 2008. National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System 2011; 59 (10): 1-126.
5. Jensen T, Roszell B, Zang F, Girard E, Matson A, Thrall R, Jaworski DM, Hatton C, Weiss DJ, and Finck C. A Rapid Lung Decellularization Protocol Supports Embryonic Stem Cell Differentiation In Vitro and Following Implantation. Tissue Engineering. Part C, Methods 2012; 18 (8): 632-646.
6. Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E, and Niklason LE. Tissue-Engineered Lungs for in Vivo Implantation. Science (New York, N.Y.) 2010; 329 (5991): 538-541.
7. He M, Callanan A, Lagaras K, Steele JAM, and Stevens MM. Optimization of SDS exposure on preservation of ECM characteristics in whole organ decellularization of rat kidneys. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2016.
8. He M and Callanan A. Comparison of methods for whole-organ decellularization in tissue engineering of bioartificial organs. Tissue Engineering Part B: Reviews 2012; 19 (3): 194-208.
9. Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007; 28 (25): 3587-3593.
10. Gilbert TW, Sellaro TL, and Badylak SF. Decellularization of tissues and organs. Biomaterials 2006; 27 (19): 3675-3683.
11. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, and Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 2008; 14 (2): 213-221.
12. Lwebuga-Mukasa JS, Ingbar DH, and Madri JA. Repopulation of a human alveolar matrix by adult rat type II pneumocytes in vitro: A novel system for type II pneumocyte culture. Experimental Cell Research 1986; 162 (2): 423-435.
13. Cortiella J, Niles J, Cantu A, Brettler A, Pham A, Vargas G, Winston S, Wang J, Walls S, and Nichols JE. Influence of Acellular Natural Lung Matrix on Murine Embryonic Stem Cell Differentiation and Tissue Formation. Tissue Engineering Part A 2010; 16 (8): 2565-2580.
14. Badylak SF, Taylor D, and Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng 2011; 13 27-53.
15. Daly AB, Wallis JM, Borg ZD, Bonvillain RW, Deng B, Ballif BA, Jaworski DM, Allen GB, and Weiss DJ. Initial binding and re-cellularization of de-cellularized mouse lung scaffolds with bone marrow-derived mesenchymal stromal cells. Tissue Eng. A 2011; 18 (1-2): 1-16.
16. Babavalian H, Latifi AM, Shokrgozar MA, Bonakdar S, Shakeri F, and Tebyanian H. Healing Effects of Synthetic and Commercial Alginate Hydrogel Dressings on Wounds: A Comparative Study. Trauma Mon; Inpress (Inpress).
17. Babavalian H, latifi AM, Shokrgozar MA, Bonakdar S, Tebyanian H, and Shakeri F. Cloning and expression of recombinant human platelet-derived growth factor-BB in Pichia Pink. Cellular and Molecular Biology 2016; 62 (8): 45-51.
18. Karami A, Tebyanian H, Goodarzi V, and Shiri S. Planarians: an In Vivo Model for Regenerative Medicine. International journal of stem cells 2015; 8 (2): 128.
19. Shakeri F, Tebyanian H, Karami A, Babavalian H, and Tahmasbi MH. Effect of Topical Phenytoin on Wound Healing. Trauma Mon; Inpress (Inpress).
20. Maghsoudlou P, Georgiades F, Tyraskis A, Totonelli G, Loukogeorgakis SP, Orlando G, Shangaris P, Lange P, Delalande J-M, Burns AJ, Cenedese A, Sebire NJ, Turmaine M, Guest BN, Alcorn JF, Atala A, Birchall MA, Elliott MJ, Eaton S, Pierro A, Gilbert TW, and De Coppi P. Preservation of micro-architecture and angiogenic potential in a pulmonary acellular matrix obtained using intermittent intra-tracheal flow of detergent enzymatic treatment. Biomaterials 2013; 34 (28): 6638-6648.
21. Petersen TH, Calle EA, Colehour MB, and Niklason LE. Matrix Composition and Mechanics of Decellularized Lung Scaffolds. Cells, Tissues, Organs 2012; 195 (3): 222-231.
22. Petersen TH. In vitro development of engineered lung tissue. 2009.
23. Song JJ, Kim SS, Liu Z, Madsen JC, Mathisen DJ, Vacanti JP, and Ott HC. Enhanced In Vivo Function of Bioartificial Lungs in Rats. The Annals of Thoracic Surgery; 92 (3): 998-1006.
24. Wagner DE, Bonvillain RW, Jensen T, Girard ED, Bunnell BA,

Finck CM, Hoffman AM, and Weiss DJ. Can stem cells be used to generate new lungs? Ex vivo lung bioengineering with decellularized whole lung scaffolds. Respirology 2013; 18 (6): 895-911.
25. O'Neill JD, Anfang R, Anandappa A, Costa J, Javidfar JJ, Wobma HM, Singh G, Freytes DO, Bacchetta MD, Sonett JR, and Vunjak-Novakovic G. Decellularization of Human and Porcine Lung Tissues for Pulmonary Tissue Engineering. The Annals of thoracic surgery 2013; 96 (3): 1046-1056.
26. Wallis JM, Borg ZD, Daly AB, Deng B, Ballif BA, Allen GB, Jaworski DM, and Weiss DJ. Comparative Assessment of Detergent-Based Protocols for Mouse Lung De-Cellularization and Re-Cellularization. Tissue Engineering. Part C, Methods 2012; 18 (6): 420-432.