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Review

The development of artificial organs using decellularization technology

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Abstract

As a result of advances in medical technology and legal revisions in relation to organ transplantation, the number of patients who receive organ transplants is increasing annually. However, the number of patients who need an organ transplant still far exceeds the number of donors, and many patients are forced to wait for their transplant. The development of artificial organs is rapidly advancing together with research into stem cells such as induced pluripotent stem (iPS) cells, and is yielding results. However, there is little prospect of the development of artificial organs that fully imitate complex spatial structures and functions. Decellularized structures, which are fabricated by removing the cellular components that cause rejection from the biological tissue, function as biological scaffolds that have minute structures and mechanical properties derived from living organisms. Decellularized grafts treated using recipient serum have been found to suppress post-transplant immunorejection in comparison with grafts treated with surfactants. With regard to calcification, which is a problem in the case of long-term transplantation, clues to a solution have been obtained through improvements in the decellularization efficiency by means of dessication stress and the tissue preservation effects of trehalose. Furthermore, dessication stress has also been found to be effective if used together with surfactant treatment methods.

Key words: biological scaffold, decellularization, drying process, trehalose, recipient's serum

Introduction

In the human body, if certain organs have fallen into a state of severe dysfunction, then, in many cases, receiving an organ transplant is the only radical cure. As a result of advances in medical technology and legal revisions regarding organ transplantation, the number of organ transplant cases is increasing every year. However, according to statistics collected by the Japan Organ Transplant Network (JOT), the number of patients requiring an organ transplant still far exceeds the number of donors. It is thought that this current situation will be improved by the development of implantable artificial organs that have superior biological compatibility and will function stably for long periods of time.

In this article, we discuss the current situation regarding decellularization technology, which is receiving attention as one approach to artificial

organ development, and the results of the authors' research to date.

Organ transplantation and artificial organs

Same-species transplantation from humans to humans is the general rule in organ transplantation. In the 1900s, transplants using heterologous mammals were also performed, but no promising results were obtained (Miyagawa, 2007). Untreated heterologous organs have never achieved long-term engrafting within the human body. This is because the immune system in the recipient tries to eliminate the heterologous organ (heterologous cells), resulting in rejection. Transplantation of some non-human tissues into humans is possible by deactivating the antigenicity of the heterologous tissues through treating them with drugs such as glutaraldehyde. For example, bioprosthetic valves comprising porcine heart valves that have been treated with the above-mentioned drugs are being used clinically as alternative tissues for human heart valves. However, the bioprosthetic valves that are currently being used cannot be said to be complete alternative tissues, as reoperation is unavoidable after several years or decades owing to post-transplantation calcification and dysfunction. Furthermore, for organs that have complex functions, it is difficult to maintain the functions and deactivate their antigenicity.

While the development of artificial organs has been actively pursued for many years as an alternative means of organ transplantation, no artificial organs exist that fully mimic the functions of biological organs. For example, artificial hearts are in most cases limited to use as an auxiliary measure until heart transplantation. Implantable artificial kidneys (dialyzers) have not been put practical use, and patients must receive artificial dialysis treatment if their renal failure progresses. In all cases, patients must accept many restrictions on their everyday lives in order to receive the benefits of artificial organs, and their quality of life (QOL) is significantly impaired.

There is much anticipation regarding research into stem cells such as induced pluripotent stem (iPS) cells as one possibility for overcoming the shortage of organs for transplantation. However, as it is difficult to construct three-dimensional organs that have complex functions, there is little prospect of practical applications. In the regeneration of organs using tissue engineering methods, "cells," "scaffolds," and "signals" are regarded as the three elements of design (Makris et al., 2015). In order to construct a three-dimensional organ, it is essential to provide a scaffold for the cells to engraft and function adequately.

Decellularization of tissues and organs

Decellularization research has been receiving attention as one strategy for developing artificial organs that fully imitate the complex spatial structures and functions of biological organs. Decellularized tissues can be fabricated by removing the donor-derived cellular components from human or other mammalian tissues, using the various methods discussed below. If the immunogenic cellular components are removed, post-transplantation rejection can be avoided. In the long term, the decellularized tissues will form scaffolds, and self-assembly can be anticipated as a result of infiltration and engrafting by the recipient cells. There have been various studies on decellularized tissues to date, and some of them are being used clinically. For example, Cryo Valves® (CryoLife, Inc., Georgia, United States of America) are widely known decellularized human tissues that are currently available commercially. It was reported (Bechtel et al., 2008) that 52 months after transplanting CryoValves® into the pulmonary artery valve position in 23 adult patients who were to undergo the Ross procedure, no clinical differences were observed compared with conventional homograft transplantation cases. In Japan, the first decellularized bioprosthetic valve transplant operation was carried out in October 2014, on a patient who was due to undergo PVR (pulmonary valve replacement). In 2016, good short-term postoperative progress was reported (Ueno et al., 2016). The pulmonary artery valve used was a bioprosthetic valve developed by the German Society for Tissue Transplantation (Hannover, Germany), and decellularized by CorLife (Hannover, Germany). Besides these, tissues with a relatively simple structure such as the dermis and bronchi, have reached the stage of clinical application (Crapo et al., 2011).

Several research groups have reported that decellularization technology can be applied to many different organs. Ott et al. (2008) received attention after reporting that as a result of seeding a decellularized rat's heart with myocardium cells, the heartbeat restarted. The above-mentioned study by Ott et al. (2010), together with that of Petersen et al. (2010), applied decellularization technology to artificial lung development. They recellularized a decellularized rat lung using various kinds of cells, and then performed a single-

lung transplant into the same species and same position. The transplanted recellularized lung recommenced gas exchange *in vivo*. However, similar reports of the development of organs with active functions remain limited to extremely short-term follow-ups using small animals, and the functions after recellularization were not able to sustain active life. The fact that organ transplantation is procedurally difficult may be one reason for this, but it is thought that, for organs that have complex spatial structures and functions, changes in mechanical properties and the minute structure of the extracellular matrix (ECM) accompanying decellularization have a stronger effect on post-recellularization functions.

Decellularization treatment methods

The advantage of decellularized tissues lies in providing a scaffold for recipient cells that has a complex spatial structure derived from a living organism by removing the cellular components, while preserving the ECM structure. In much of the research, surfactants such as sodium dodecyl sulfate (SDS) and TritonX-100 (octylphenol ethoxylate) are used in combination with various enzymes in order to remove the cellular components. However, surfactants have a strong effect on ECM structures, and there are concerns that they may destroy the spatial structures that are the precise advantage of using materials derived from living organisms. In our studies, we found that after using a thrombogenic assessment method based on platelet aggregation (Ishino et al., 2015 b) to assess porcine carotid arteries treated with SDS, the platelet aggregation capability significantly decreased in comparison with untreated blood vessels. This is thought to have been caused by denaturing the collagen of the intravascular luminal surface together with the removal of endothelial cells as a result of SDS treatment. While the non-ionic surfactant Triton X-100 is known to cause less damage to tissues compared with SDS, it is, however, inferior with regard to decellularization efficiency (Hrebikova et al., 2013). With surfactant treatment methods, it is difficult to reconcile preserving the ECM structure and decellularization, and it is necessary to select a suitable treatment agent for the target tissue or organ, and optimize the conditions pertaining to it, such as the concentration and treatment duration. Furthermore, the residual surfactant after treatment exhibits cytotoxicity, and it has been reported that it obstructs *in vivo* remodeling after transplantation (Liem et al., 2013). Therefore, in the case of the above-mentioned CryoValves® for example, decellularization is carried out using a hypotonic solution and enzymes, without using any surfactant. Freezing-thawing methods (Neubauer et al., 2007) and ultra-high hydrostatic pressure methods (Fujisato et al., 2005) are both known treatment methods that do not use surfactants, and their effectiveness has been reported.

Our approach

Decellularization treatment using recipient serum

The use of non-self components such as surfactants and enzymes, in the fabrication of decellularized tissues, may lead to side effects and unknown infections after transplantation in the recipient, being a matter of marked concern. Therefore, we attempted decellularizing tissues using self-derived serum collected from the recipient. With this method, it was possible to decellularize tissues while preserving microstructures derived from the living organism, owing to the action of the active ingredient in the serum (we believe that complement activation is the primary mechanism involved in decellularization). The immune rejection response upon the subcutaneous transplantation of a porcine carotid graft (5×5 mm) treated by this method into rats could be reduced when compared with the immune rejection response using an SDS-treated graft. (Ishino et al., 2015 a)

We believe that the results of this research can be applied to the development of artificial organs that may self-assemble in the early stages after transplantation and develop fully into an organ as the recipient grows **(Figure 1)**. However, we observed advanced calcification in some grafts 30 days after subcutaneous transplantation.

Improvement of calcification suppression and decellularization efficiency

The mechanism leading to the calcification of grafts has gradually been clarified. For example, it is known that calcification is induced by the denaturing of elastic fibers (Dao et al., 2005) and by cellular residues resulting from insufficient decellularization treatment (Schmidt et al., 2000). In our studies involving rat subcutaneous transplantation experiments, we noted two different outcomes : cases showing calcification of the graft along the longitudinal axis of the elastic fibers, and cases of diffuseness. It is considered that the main reason for the former was probably denaturing of the elastic fibers, and that for the latter was

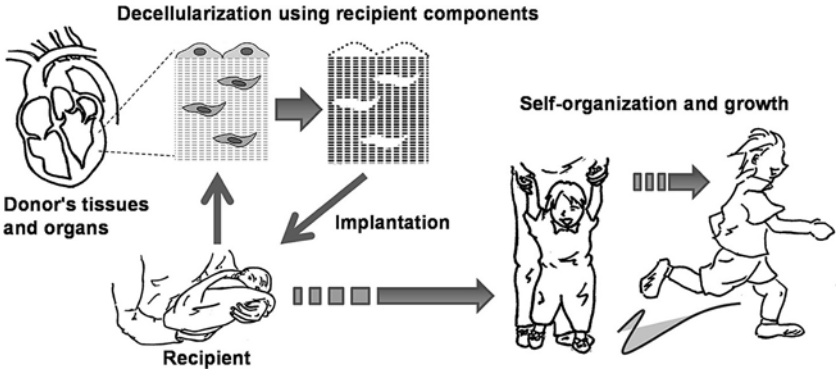

Figure 1 Expected results of decellularization technology using recipient components

probably calcification of the cellular residues.

When decellularizing tissues using serum, freezing-thawing treatment is necessary before the serum treatment, as it has a lower decellularization efficiency compared with surfactant treatment. Trehalose has the effect of suppressing ice formation during freezing, and Pulver et al. (2014) used trehalose to reduce the destruction of the ECM structure due to freezing-thawing treatment. Trehalose has not been found to be toxic to humans and is suitable as a treatment solution for graft fabrication. In addition, Sakurai et al. (2008) clarified that trehalose is an essential substance for the cryptobiosis of *Polypedilum vanderplanki,* which is found in Africa, and that it protects living organisms from extremely dry conditions. Therefore, in order to preserve the fiber structure while frozen, we carried out freezing-thawing and serum treatment after the penetration of trehalose into the ECM. In parallel with our study of the freezing-thawing method, we also examined the decellularization of tissue that had undergone desiccation treatment. No transporters for trehalose exist in the cell membranes of humans or other mammals. This means that if tissue is desiccated after penetration by trehalose, although the cells that need to be removed are exposed to dessication stress, the ECM will be protected. As for stress resulting from freezing, if decellularization is promoted through desiccation stress, the material will still be suitable for fabricating decellularized tissues.

As the results, trehalose significantly preserved the structural and mechanical properties of tissues from stress resulting from either freezing or dessication (Ueda et al., 2016). Furthermore, for tissue that had been exposed to dessication stress, the efficiency of decellularization by subsequent serum treatment improved, and the tissue was successfully decellularized without any freezing treatment (Ozaki et al., 2016). The findings where by desiccation treatment using trehalose reduces the denaturing of elastic fibers and leads to improvements in the decellularization efficiency provided clues to solving the two calcification issues mentioned above.

Including a desiccation process in the decellularization treatment will be of marked benefit for the use of biotissues. In addition to being able to preserve precious biomaterials for long periods of time, it will also be possible to guarantee their safety as grafts by performing ethylene oxide gas (EOG) sterilization (Iwasaki et al., 2013). As the glass transition temperature of trehalose is above 60° C (Sakurai et al., 2008), it is thought that even if EOG sterilization is performed, the tissue preservation effects will not be lost. Furthermore, Iwasaki et al. (2013) are developing a technique for the long-term desiccation preservation of decellularized tissue using trehalose (application number : PCT/JP2013/059842). However, there have been no other reports of techniques for improving the decellularization efficiency by applying dessication stress.

As a result of further study, we were able to understand that the decellularization efficiency is also improved by applying dessication stress before surfactant treatment (Ishino et al., 2017, unpublished data). If these findings are applied, then it may be possible to decellularize tissues and organs by means of surfactant treatments at lower concentrations and with shorter durations than those used conventionally. Treatment using a low-concentration surfactant will reduce post-transplantation cytotoxicity, and treatment with a shorter duration will promote preservation of the minute structure of organs **(Figure 2)**.

Conclusion

In recent years, research into stem cells such as iPS cells has been actively pursued, and applica-

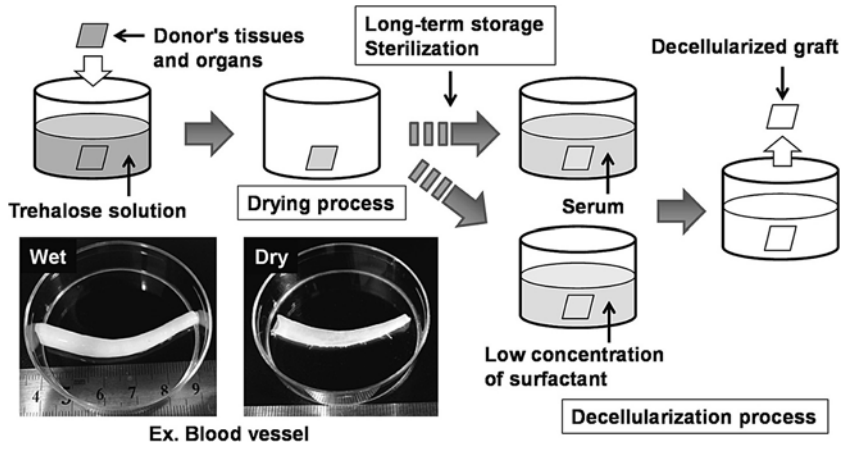

Figure 2 Decellularization treatment including drying process

tions to organ regeneration are anticipated. However, constructing organs that have complex spatial structures and functions is difficult, and it is thought that for the time being, the achievement of practical applications lies some way off.

The results of our research to date in relation to decellularization technology have yet to reach the stage of providing a scaffold for organ construction. However, we anticipate that the various results that we have introduced through this article will contribute to the development of artificial organs in the future, and our studies are ongoing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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