Conclusion/Summary

By varying the HAD type and composition with copolymerizing fibrillar collagens our approach seems feasible to create customized artificial extracellular matrices. These matrices can be used to establish a defined environment at the implant surface selectively attracting and storing biological mediators relevant for bone healing and remodeling. Coating titanium implants with these matrices seems promising to improve healing of bone prosthesis and therefore to enhance stability of the regenerated tissue.

Acknowledgement

We gratefully acknowledge financial support by the DFG (PAK 105) and the BMBF (01EZ0748).

The authors like to thank Dipl.-Ing. Sascha Heinemann for support in AFM investigations.

References

[1] Bierbaum S, Douglas T, Hanke T, Scharnweber D, Tippelt S, Monsees TK, Funk RH, Worch H. Journal of Biomedical Material Research 2006, 77, 551.
[2] Douglas T, Hempel U, Mietrach C, Heinemann S, Scharnweber D, Worch H. Biomol Eng. 2007, 24, 455.
[3] Wolf-Brandstetter C, Lode A, Hanke T, Scharnweber D, Worch H. J Biomed Mater Res A. 2006, 79, 882.
[4] Stadlinger B, Pilling E, Huhle M, Mai R, Bierbaum S, Scharnweber D, Kuhlisch E, Loukota R, Eckelt U. Int J Oral Maxillofac Surg. 2008, 37, 54.
[5] Bessho K, Carnes DL, Cavin R, Chen HY, Ong JL. Clin Oral Implants Res 1999, 10, 212.
[6] Boyne PJ. J Bone Joint Surg Am 2001, 83-A Suppl 1(Pt 2): S146-150.
[7] Raschke M, Wildemann B, Inden P, Bail H, Flyvbjerg A, Hoffmann J, Haas NP, Schmidmaier G. Bone 2002 30, 144.
[8] Szczesny G. Pol J Pathol 2002, 53, 145.
[9] Douglas T, Heinemann S, Mietrach C, Hempel U, Bierbaum S, Scharnweber D, Worch H. Biomacromolecules 2007, 8, 1085.

THE ROLE OF DECELLULARIZATION IN BIOMATERIALS MANUFACTURING FROM XENOGENEIC TISSUES

Artur Turek[1]*, Beata Cwalina[2]

[1] Medical University of Silesia,
Department of Biopharmacy,
Sosnowiec, Poland
[2] Silesian University of Technology,
Environmental Biotechnology Department,
Gliwice, Poland
* e-mail: aturek@vip.interia.pl

Abstract

Biological heart valves have represented an important area of the tissue-derived biomaterials. Decellularization processes are considered to be useful for manufacturing of biodegradable scaffolds which make it possible to create living and functioning tissues. These processes result in elimination of most disadvantages of GA-stabilized tissues. Acellular tissues may be obtained using various chemical, enzymatic and mechanical methods. Decellularization processes give the possibility of creating biomaterials for cell seeding which are not immunogenic, cytotoxic and calcifying.

[Engineering of Biomaterials, 77-80, (2008), 19-20]

Introduction

Biological heart valves manufacturing has represented an important area of the tissue-derived biomaterials use over the past 40 years. During this time period, various allogeneic and xenogeneic tissues have been investigated and applied for this purpose [1]. Although many allograft features such as relative resistance to infection and also hemodynamic properties are very important for biomaterials fabrication [2-4], these tissues have limited supply.

On the other hand, fabrication of xenogafts is not limited. The immunological barrier has been broken due to various stabilization processes [5].

Glutaraldehyde (GA) is a chemical cross-linking reagent routinely used in stabilization processes. Apart from stabilization of extracellular matrix, GA-treatment leads to reduction of immunological response [5]. However, GA in tissue-derived biomaterials is responsible for their premature calcification as well as thrombosis [6] and cytotoxicity [7]. Although GA-modified biomaterials are biologically inert, cellular debris is not completely removed from them.

Decellularization processes are considered to be useful for manufacturing of biodegradable scaffolds which make it possible to create living and functioning tissues [8]. It is widely accepted that decellularization processes result in elimination of most disadvantages of GA-stabilized tissues. However, till now little is known about the safety of decellularized xenogeneic tissues.

Xenogeneic tissues

Both porcine heart valves and bovine fibrous pericardium are often used in cardiosurgery. The main component of their tissue structures is collagen type I. Xenogeneic collagen antigenicity is known to be low because of inter-species similarity of amino-acid sequences [9]. It is very important from preparative point of view that the pericardium structure is more homogenous than heart valve structure. Pericardium is composed of two layers of fibrous tissue whereas the heart valve cusp has three distinct layers: fibrous, spongy and ventricular. Moreover, porcine aortic valve anatomical structure is more complicated than the pericardium one. Thus, both the cell debris extraction and prosthetic valve manufacturing from pericardium tissues are simpler than the processing of whole heart valves.

Some authors point out that application of biomaterials made of porcine and bovine tissues may increase the risk of some cross-species disease transmission [10-12]. Even decellularized porcine valves may be responsible for transmission of porcine endogenous retroviruses [11]. However, Leyh and co-workers [12] have shown (using sheep model) that decellularized porcine matrix did not cause such health hazard. On the other hand, the bone grafts are considered to be a possible source of dangerous prion proteins [10].

Tissues decellularization

The tissue stabilization method depends on the tissue type. Decellularization is one of important steps belonging to stabilization processes, consisting in extraction of immunogenic agents [1]. Low molecular components are removed from extracellular matrix during this process, which does not result in the tissue preservation.

Acellular tissues may be obtained using various chemical, enzymatic and mechanical methods [8,13]. One of chemical reagents used for this purpose is sodium dodecyl sulfate (SDS) which is considered to play an important role in the tissues decellularization as well as prevention of their calcification. SDS influences cells, proteoglycans and phospholipids extraction. For decellularization procedures, SDS is dissolved in 0.09% NaCl solution to obtain SDS-concentrations of 0.03-0.5%.

Histological observations performed after such treatment showed extracellular matrix without cells. Besides, no degradations of collagen, elastin and glycosaminoglycans were observed [13].

The tissues may also be decellularized using solutions containing trypsin and ethylenediaminetetraacetic acid [8]. The trypsin 0.05%-0.25% solutions in EDTA allow to obtain acellular matrix scaffolds to cells seeding [14]. Synergistic effect was observed in the experiment performed with the usage of SDS and trypsin combination to the xenogeneic tissues treatment [15]. It is worth denoting that decellularization processes do not always result in removing all cells and cell debris.

Conclusions

Decellularized porcine valve and bovine pericardium may be employed as scaffolds in the tissue engineering. Decellularization processes give the possibility of creating biomaterials for cell seeding which are not immunogenic, cytotoxic and calcifying.

References

[1] Schmidt CE, Baier JM. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. Biomaterials 2000, 21: 2215-31.
[2] Yacoub M, Rasmi NR, Sundt TM, Lund O, Boyland E, Radley-Smith R, Khaghani A, Mitchell A. Fourteen-year experience with homovital homografts for aortic valve replacement. J Thorac Cardiovasc Surg 1995, 110: 186-93.
[3] Doty JR, Salazar JD, Liddicoat JR, Flores JH, Doty DB. Aortic valve replacement with cryopreserved aortic allograft: ten-year experience. J Thorac Cardiovasc Surg 1998, 115: 371-9.
[4] Yankah AC, Klose H, Petzina R, Musci M, Siniawski H, Hetzer R. Surgical management of acute aortic root endocarditis with viable homograft: 13-year experience. Eur J Cardiothorac Surg 2002, 21: 260-7.
[5] Khor E. Methods for the treatment of collagenous tissue for bioprotheses. Biomaterials 1997, 18: 95-105.
[6] Thoma RJ, Phillips RE. The role of material surface chemistry in implant device calcification: a hypothesis. J Heart Valve Dis 1995, 4: 214-21.
[7] Gendler E, Gendler S, Nimni ME. Toxic reactions evoked by glutaraldehyde – fixed pericardium and cardiac valve tissue bioprosthesis. J Biomed Mater Res 1984, 18: 727-36.
[8] Bader A, Schilling T, Teebken O, Brandes G, Steinhoff G, Haverich A. Tissue engineering of heart valves - human endothelial cell seeding of detergent acellularized porcine valves. Eur J Cardiothorac Surg 1998,14: 129–284.
[9] Timpl R. Immunology of the collagens. In: Piez KA, Reddi AH (Eds.) Extracellular matrix biochemistry. Elsevier, New York, 1984.
[10] Wenz B, Oesch B, Host M. Analysis of the risk of transmitting bovine spongiform encephalopathy through bone grafts derived from bovine bone. Biomaterials 2001, 22: 1599-1606.
[11] Moza AK, Mertsching H, Herden T, Bader A, Haverich A. Heart valves from pig and the porcine endogenous retrovirus: experimental and clinical data to assess the probability of porcine endogenous retrovirus infection in human subjects. J Thorac Cardiovasc Surg 2001, 121: 697-701.
[12] Leyh RG, Wilhelmi M, Walles T, Kallenbach K, Rebe P, Oberbeck A, Herden T, Haverich A, Mertsching H, Acellularized porcine heart valve scaffolds for heart valve tissue engineering and the risk of cross-species transmission of porcine endogenous retrovirus. J Thorac Cardiovasc Surg 2003, 126: 1000-1004.
[13] Booth C, Korossis SA, Wilcox HE, Watterson KG, Kearney JN, Fisher J, Ingham E. Tissue engineering of cardiac valve prostheses. I: development and histological characterization of an acellular porcine scaffold. J Heart Valve Dis 2002, 11: 457-462.
[14] Steinhoff G, Stock U, Karim N, Mertsching H, Timke A, Meliss RR, Pethig K, Haverich A, Bader A. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: in vivo restoration of valve tissue. Circulation 2000, 102: III50-III55.
[15] Korossis SA, Wilcox HE, Watterson KG, Kearney JN, Ingham E, Fisher J. In-vitro assessment of the functional performance of the decellularized intact porcine aortic root. J Heart Valve Dis 2005, 14: 408-422.