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European Journal of Cardio-thoracic Surgery 30 (2006) 782—786

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

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Review

Tissue-engineered trachea: History, problems and the future☆

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Received 15 June 2006; received in revised form 21 August 2006; accepted 24 August 2006

Summary

This review tries to summarize the efforts over the past 20 years to construct a tissue-engineered trachea. After illustrating the main technical bottlenecks faced nowadays, we discuss what might be the solutions to these bottlenecks. You may find out why the focus in this research field shifts dramatically from the construction of a tubular cartilage tissue to reepithelialization and revascularization of the prosthesis. In the end we propose a novel concept of 'in vivo bioreactor', defined as the design of a perfusion system inside the scaffold, and explain its potential application in the construction of a tissue-engineered trachea.

Keywords: Tissue engineering; Tracheal surgery; Revascularization; Reepithelialization

1. Introduction

Tissue engineering has emerged as a thriving new field in medical science. It is an interdisciplinary field in which the principles of engineering and life science meet each other to generate biological substitutes for creation, preservation or restoration of lost organ functions [1]. Unlike organ transplantation, tissue engineering offers off-the-shelf tissue and organ substitutes by seeding a patient's own cells on a biodegradable scaffold, representing a promising future of medical science.

The experimental and clinical tracheal repair dates back to as early as the late 19th century, yet till today no clinically convincing tracheal replacement method has been established [2—6]. Belsey [7] summarized the requirements for tracheal replacement to consist of, first, a laterally rigid but longitudinally flexible tube; second, a surface covered with ciliated respiratory epithelium. At the very beginning, many scientists were led to believe in the illusionary simplicity in tissue-engineered trachea reconstruction and took it as no more than developing a tubular cartilage tissue. Later, animal examinations demonstrated the importance of an intact epithelial line since it prevents the in-growth of granulomatous tissue, which leads to fatal airway obstruction. The cilia also help to expel the mucosal fluids and the adhering microparticles. Therefore, the focus in the tissue-engineered trachea researches today has been shifted towards the substitute reepithelialization [8—12]. In this review we will discuss what has been done, what is going on, where the obstacles are, and what might be the practical solutions in the reconstruction of a tissue-engineered trachea.

2. What has been achieved: formation of a tubular cartilage tissue

Although the techniques of tubular cartilage tissue construction are available, there are still some problems involved in each step.

2.1. Sources of chondrocytes

Reconstruction of cartilage tissues has become one of the most popular research topics today in tissue engineering, mainly due to the fact that chondrocytes rely on the permeation of tissue fluid for nutrition supply, and thus exempting cartilage tissues from revascularization. Thanks to their low antigenicity, allogeneic chondrocytes can be used to repair cartilage tissue defects [13,14]. In many countries, scientists are exploring the possibility of establishing cell banks to guarantee a stable supply of chondrocytes [15,16]. Regarding autologous sources, besides mature chondrocytes isolated from nose or rib hyaline cartilage tissues, adult stem cells (ASCs), with their high rate of

☆ Oral presentation at the 8th Annual Tissue Engineering Society International TESI Congress on 10/23/2005.

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1010-7940/$ — see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ejcts.2006.08.023

proliferation and ease of isolation, have proven to be a promising alternative. While the ideal donor site for the isolation of ASCs remains controversial, bone marrow aspirates and adipose-derived stem cells so far stand out as appropriate sources [17]. The antigen markers can be used for ASCs isolation including CD166, CD105, CD55, CD54, CD44 and CD13, among which CD105, the transforming growth factor-ß (TGF-ß) receptor, is identified as chondrogenic potential related cell marker [18]. Meanwhile recent researches demonstrated that CD14+ mononuclear cells obtained from peripheral blood can also differentiate into a mesenchymal progenitor phenotype [19]. Many research teams around the world have successfully induced ASCs into chondrocytes in vitro. Theoretically, ASCs can be obtained without limitation but the induction rate is still on the low side these days [20—22].

2.2. Scaffolds

Tissue engineers favor porous biodegradable scaffolds for they can facilitate the penetration of seeded cells, nutrients, and the clearance of biological waste products. But a high porous rate will inevitably sacrifice the initial stiffness of the scaffold, which is of utmost importance in the case of the tissue-engineered trachea. Researchers have tried many materials, both synthetic and natural, including polyglycolic acid (PGA), polylactide-co-glycolide acid (PLGA), DegraPol, Pluronic F-127, acellular cartilage tissue matrices, as well as some combination of them all [23—32]. Unfortunately, none of these materials has worked wonder. For example, the biodegraded molecules from PGA often leads to a low pH environment, which is detrimental for the survival of seeded cells; the porous rate of acellular tracheal matrices is hard to be controlled; and nearly all these scaffolds show inadequate mechanical strength for a circumferential trachea replacement and usually need an external support device such as a silicon tube.

2.3. Bioreactors

Bioreactors play a key role in almost every step in the reconstruction of tissue-engineered tissues: cell proliferation on a large scale, cell seeding process, 3D cell-scaffold constructs culture [33]. There are various designs ranging from the simplest stirred vessels to much more complex double chamber bioreactors for composite tissue formation. Regarding their application in cell culture, microcarriers are frequently used to increase the culture surface with the advantage of less enzymatic subcultivation steps, which turn out to be the main cause of cell dedifferentiation [34]. Recent studies have demonstrated that perfusion of a cell suspension directly through the pores of 3D scaffolds resulted in higher efficiencies and more uniform cell distributions inside the scaffold [35]. In spite of all these improvements, in most cases the cartilage tissues formed in these bioreactors are thinner and softer than native ones. This is mainly due to the insufficient infiltration of nutrition and oxygen into the center part of newly-formed tissues, as well as the obstruction of biological waste elimination [36,37]. In addition, contamination remains another critical issue especially when a long-term in vitro culture is required. The essential purpose of bioreactor designs, effectively simulating the in vivo regeneration condition in vitro for the off-the-shelf tissues formation, has proven to be extremely difficult to achieve. One possible makeshift is to use nude mice as living bioreactors [38,39]. The results are inspiring, however this approach has a serious limitation in terms of the tissue size: since a tracheal resection less than 6 cm in length can be handled easily by direct anastomosis, tissue-engineered cartilage tissues of small size are therefore clinically irrelevant in tracheal replacement.

3. Problems faced: reepithelialization and revascularization

Now that a tubular bioengineered cartilage tissue is available, is it really a perfect tracheal prosthesis? Earlier animal experiments underwent circumferential replacement of trachea with tubular cartilage tissues only to show frustrating results: most recipients died within 1 week from airway obstruction caused by either the in-growth of granulation tissues or the sputum retention [40]. These results were not surprising at all, since the entire history of trachea replacement has highlighted the importance of a complete reepithelialization process in building a functional tracheal substitute. The experience gained from tissue-engineered skin demonstrated that epithelial cells have better survival chances on a well vascularized wound surface, a clear proof that revascularization is the essence of reepithelialization [41—44].

Oncologists have also done lots of researches on the angiogenesis and vasculogenesis mechanisms, however, with an ironically opposite purpose: they go all out to prevent the in-growth of capillary networks into tumor tissues. On the other hand, tissue engineers work hard to facilitate and accelerate these processes based on the same biological mechanisms [45—48]. Two approaches are frequently used to accelerate the revascularization process these days. In one approach, various growth factors (GFs), such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), and platelet-derived growth factor (PDGF) are administrated into the ischemia area to induce the in-growth of vessels from surrounding normal tissues [49—53]. In the other approach, endothelial cells or their progenitors are applied either systemically or topically in the hope that these cells may contribute to the reconstruction of the capillary network inside the ischemia tissues or organs [54—58]. Many studies showed that both hypotheses work well but some disadvantages still exist.

Regarding the first approach, there are two main challenges, namely, how to maintain the working concentration of these GFs after implantation, and how to terminate their function in time to avoid side effects caused by the GFs over expression? Biochemical engineers suggest integrating GFs into biodegradable scaffolds in the hope that these embedded GFs will be slowly released during the scaffold biodegradation process after implantation. This technique, often known as 'in situ tissue engineering', however, is not only technically demanding but has size limitation in neo-tissue formation without pre-seeded cells inside the scaffold [59—63]. Meanwhile, many promising experiments suggest

the possibility of GFs gene delivery. Unfortunately in these experiments, both viral method (transduction) and nonviral method (transfection) showed technical limitations: the former often goes with likelihood of mutagenesis, carcinogenesis and immune response to viral infection or viral proteins while the latter still suffers from low efficiency of gene delivery into the target cell population [64—68]. The tragic death of a patient due to massive cytokine release gives rise to heated ethical debate in trial gene therapy recently.

Regarding the endothelial cell delivery approach, the dilemma is whether it is really worth to make time-consuming efforts to add the endothelial cells that hardly culture and usually die soon after implantations due to insufficient nutrient supply inside the scaffold.

In addition to revascularization, we still face many other problems in reepithelialization, such as the harvest and proliferation of human respiratory epithelial cells, which are mainly terminal cells unsuitable to be further passaged [69]. Even if we could identify the epithelial stem cell, there are still no widely accepted seeding methods, which can facilitate these cells to rapidly spread and completely cover the inner surface of tubular scaffolds. In early experiments, epithelial cells were simply injected into the lumen of a tubular scaffold placed subcutaneously on the back of a nude mouse. The results, with a maximal surface cover rate of 80%, were disappointing and unacceptable for clinic applications. Recent studies suggested directly suturing cell sheets onto the inner surface of the tissue-engineered trachea substitutes [70], but how to maintain the survival of these epithelial cells during the initial ischemia period remains a challenge.

All these being said, what next?

4. Future perspectives

Let's get down to fundamentals and take a look at normal histological structure of trachea. Between the epithelial layer and the hyaline cartilage tissues we can find a submucosal layer of capillary networks. Normal cartilage tissue preventing the in-growth of capillary vessels is separated from other connective tissues (i.e., submucosa) by a fibrous membrane [71,72]. Unlike parenchymal organs, the trachea is supplied with a network of small vessels inaccessible to direct revascularization through vessel anastomosis. Even immediate autologous orthotropic trachea replacement is bound to fail, despite that such a fresh autograft substitute is far more physiological than even the most optimal tissue-engineered trachea. The capillary net can only penetrate into the implant across the two anastomoses for no more than 2 cm (4 cm bilaterally) and this revascularization process easily takes months [2]. In an implant longer than 3—4 cm, the epithelial cell in the middle will die and thus failing to maintain an intact basement membrane. As a result, granulation tissue hyperplasia into the lumen causes tracheal stenosis.

Such being the case we advanced a novel concept of 'in vivo bioreactor' defined as the design of a perfusion system inside the scaffold for tissue-engineered trachea reconstruction. [Fig. 1] In our opinion, a tissue-engineered trachea scaffold, ideally, should follow the physiological structure mentioned above and leave a porous middle layer facilitating blood vessels' in-growth between the cartilage and epithelial layers. It may also be of advantage if we would add 3 or 4 porous feeding tubes inside this middle layer with lower density. These feeding tubes will be connected to an extracorporeal pump system after implantation in order to form a perfusion system. Through this perfusion system we continuously administrate medium into the scaffold. The medium infiltrates throughout the scaffold and thus maintains the survival of both epithelial cells seeded on the inner surface and the chondrocytes seeded on the outer layer, which has a relatively high density to prevent the tissue-engineered trachea from a collapse.

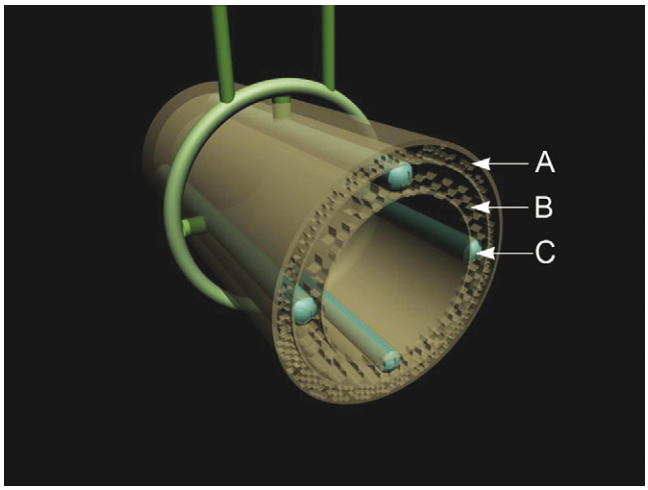

Fig. 1. Sketch of novel tissue-engineered tracheal scaffold with a perfusion system inside. This 'in vivo bioreactor' contains two layers with different density. The inner low-density layer (B) is embedded with porous catheters (C) and allows for in-growth of capillary net, while the outer high-density layer (A) provides the scaffold with desirable mechanical strength.

Contrary to traditional bioreactors where the medium immerses cell-scaffold composites, in our design the medium actually flows inside the scaffold to mimic the way blood stream flows inside the normal tissue. Since we are not yet able to imitate in vivo regeneration environment successfully in vitro, this in vivo bioreactor seeded with cells needs to be implanted as soon as possible to leverage on the recipient's regenerative capabilities. Thus, the recipient serves as her own bioreactor for the maturation of tissue-engineered organ and that is how we are inspired to name our design 'in vivo bioreactor'. To avoid the coagulation-related problems, the perfusion system will be connected to two extracorporeal pumps instead of being directly anastomosed to the recipient's circulation system. One pump will deliver medium while the other will drain the waste. Like a heart—lung machine widely used in cardiac operations, this in vivo bioreactor is supposed to work as an 'artificial heart' to the tissue-engineered trachea until normal revascularization is established. Obviously GFs and epithelial cells can be added into the perfusate to facilitate revascularization process; artificial oxygen carriers, in addition, such as perfluorocarbon (PFC) emulsion, can be added to increase the oxygen incidence inside the medium. The expression level of the GFs can be readily adjusted by changing their medium concentrations. By combining the in vivo and the in vitro parts of tissue engineering researches, which were traditionally

separated, this concept of 'in vivo bioreactor' would hopefully be applied as a more physiological and more clinically practical way in organ regeneration.

Regarding the epithelial cell source for reepithelialization, our hypothesis is as follows: the key issue for a clinically successful reepithelialization of the tissue-engineered trachea may not be the original type of the epithelial cells but rather the environmental signals and an intact basement membrane layer. Allogenic trachea transplantation, in addition to preclinical and clinical research work using autologous skin or aorta, clearly showed that no matter what kind of epithelial cell type was initially chosen they would be replaced by normal tracheal epithelial as long as there was a sufficient blood supply and an intact basement membrane underneath [73—76]. Therefore for the epithelial cell source, we suggest using autologous skin keratinocytes instead of the ciliated tracheal epithelial cells. Fortunately, in the field of tissue engineering, tissue-engineered skin is by far the most successful branch.

To summarize, the tissue-engineered tracheal substitute is still far away from wide clinical application. The source of epithelial cells, and the retarded revascularization and reepithelialization process of the tissue-engineered tracheal substitute are currently the main obstacles. With the idea of 'in vivo bioreactor' we organically combine the traditionally separated in vivo and in vitro parts of tissue engineering research. In our opinion, the inner structure of the scaffolds might be the key to solve some of these pertinent problems. Being a more physiological way in tissue reconstruction, this novel approach may also find itself in clinic applications in many other areas of tissue engineering research, such as tissue-engineered bone.

References

[1] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920—6.
[2] Grillo HC. The history of tracheal surgery. Chest Surg Clin N Am 2003;13:175—89.
[3] Macchiarini P. Primary tracheal tumours. Lancet Oncol 2006;7:83—91.
[4] Okumus A, Cizmeci O, Kabakas F, Kuvat SV, Bilir A, Aydin A. Circumferential trachea reconstruction with a prefabricated axial bio-synthetic flap: experimental study. Int J Pediatr Otorhinolaryngol 2005;69:335—44.
[5] Cibantos Filho JS, de Mello Filho FV, Campos AD, Ellinguer F. Viability of a 12-ring complete tracheal segment transferred in the form of a compound flap: an experimental study in dogs. Laryngoscope 2004;114:1949—52.
[6] Yves J, Pilloud R, Lang FJ, Monnier P. Prefabrication of composite grafts for long-segment tracheal reconstruction. Arch Otolaryngol Head Neck Surg 2004;130:1185—90.
[7] Belsey R. Resection and reconstruction of the intrathoracic trachea. Br J Surg 1950;38:200—5.
[8] Ruszymah BH, Chua K, Latif MA, Hussein FN, Saim AB. Formation of in vivo tissue engineered human hyaline cartilage in the shape of a trachea with internal support. Int J Pediatr Otorhinolaryngol 2005;69:1489—95.
[9] Grimmer JF, Gunnlaugsson CB, Alsberg E, Murphy HS, Kong HJ, Mooney DJ, Weatherly RA. Tracheal reconstruction using tissue-engineered cartilage. Arch Otolaryngol Head Neck Surg 2004;130:1191—6.
[10] Kojima K. Tissue engineered trachea using sheep nasal septum. Med J Malaysia 2004;59:32—3.
[11] Kamil SH, Eavey RD, Vacanti MP, Vacanti CA, Hartnick CJ. Tissue-engineered cartilage as a graft source for laryngotracheal reconstruction: a pig model. Arch Otolaryngol Head Neck Surg 2004;130:1048—51.
[12] Kim J, Suh SW, Shin JY, Kim JH, Choi YS, Kim H. Replacement of a tracheal defect with a tissue-engineered prosthesis: early results from animal experiments. J Thorac Cardiovasc Surg 2004;128:124—9.
[13] Weinand C, Peretti GM, Adams Jr SB, Randolph MA, Savvidis E, Gill TJ. Healing potential of transplanted allogeneic chondrocytes of three sources in lesions of the avascular zone of the meniscus: a pilot study. Arch Orthop Trauma Surg 2006;13:1—7.
[14] Lu Y, Adkisson HD, Bogdanske J, Kalscheur V, Maloney W, Cheung R, Grodzinsky AJ, Hruska KA, Markel MD. In vivo transplantation of neonatal ovine neocartilage allografts : determining the effectiveness of tissue transgluatminase. J Knee Surg 2005;18:31—42.
[15] Csonge L, Bravo D, Newman-Gage H, Rigley T, Conrad EU, Bakay A, Strong DM, Pellet S. Banking of osteochondral allografts: part I. Viability assays adapted for osteochondrol and cartilage studies. Cell Tissue Bank 2002;3:151—9.
[16] Csonge L, Bravo D, Newman-Gage H, Rigley T, Conrad EU, Bakay A, Strong DM, Pellet S. Banking of osteochondral allografts: part II. Preservation of chondrocyte viability during long-term storge. Cell Tissue Bank 2002;3:161—8.
[17] Raghunath J, Salacinski HJ, Sales KM, Butler PE, Seifalian AM. Advancing cartilage tissue engineering: the application of stem cell technology. Curr Opin Biotechnol 2005;16:503—9.
[18] Majumdar MK, Banks V, Peluso DP, Morris EA. Isolation, characterization, and chondrogenic potential of human bone marrow-derived multipotential stromal cells. J Cell Physiol 2000;185:98—106.
[19] Kuwana M, Okazaki Y, Kodama H, Izumi K, Yasuoka H, Ogawa Y, Kawakami Y, Ikeda Y. Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. J Leukoc Biol 2003;74:833—45.
[20] Huang JI, Kazmi N, Durbhakula MM, Hering TM, Yoo JU, Johnstone B. Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient-matched comparison. J Orthop Res 2005;23:1383—9.
[21] Dudics V, Kunstar A, Geher P, Gomor B, Hangody L, Uher F. Mesenchymal stem cells as potential source cartilage repair. Orv Hetil 2005;146:1201—8.
[22] Ichinose S, Yamagata K, Sekiya I, Muneta T, Tagami M. Detailed examination of cartilage formation and endochondral ossification using human mesenchymal stem cells. Clin Exp Pharmacol Physiol 2005;32:561—70.
[23] Grimmer JF, Gunnlarugsson CB, Alsberg E, Murphy HS, Kong HJ, Mooney DJ, Weatherly RA. Tracheal reconstruction using tissue-engineered cartilage. Arch Otolaryngol Head Neck Surg 2004;130:1191—6.
[24] Lee CJ, Moon KD, Choi H, Woo JI, Min BH, Lee KB. Tissue engineered tracheal prosthesis with acceleratedly cultured homologous chondrocytes as an alternative of tracheal reconstruction. J Cardiovasc Surg (Torino) 2002;43:275—9.
[25] Yang L, Korom S, Welti M, Hoerstrup SP, Zund G, Jung FJ, Neuenschwander P, Weder W. Tissue engineered cartilage generated from human trachea using DegraPol scaffold. Eur J Cardiothorac Surg 2003;24:201—7.
[26] Conconi MT, De Coppi P, Di Liddo R, Vigolo S, Zanon GF, Parnigotto PP, Nussdorfer GG. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and traheal epithelial cells. Transpl Int 2005;18:727—34.
[27] Doolin EJ, Strande LF, Sheng X, Hewitt CW. Engineering a composite neotrachea with surgical adhesives. J Pediatr Surg 2002;37:1034—7.
[28] Li Z, Zhang M. Chitosan-alginate as scaffolding material for cartilage tissue engineering. J Biomed Mater Res A 2005;75:485—93.
[29] Galois L, Hutasse S, Cortial D, Rousseau CF, Grossin L, Ronziere MC, Herbage D, Freyria AM. Bovine chondrocyte behaviour in three-dimensional type I collagen gel in terms of gel contraction, proliferation and gene expression. Biomaterial 2006;27:79—90.
[30] Fan H, Hu Y, Li X, Lu R, Bai J, Wang J. Experimental study on gelatin-chondroitin sulfate-sodium hyaluronate tri-copolymer as novel scaffolds for cartilage tissue engineering. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2005;19:473—7.
[31] Masuoka K, Asazuma T, Ishihara M, Sato M, Hattori H, Ishihara M, Yashihara Y, Matsui T, Takase B, Kikuchi M, Nemoto K. Tissue engineering of articular cartilage using an allograft of cultured chondrocytes in a membrane-sealed atelocollagen honeycomb-shaped scaffold [ACHMS scaffold]. J Biomed Mater Res B Appl Biomater 2005;75:177—84.
[32] Chen GO, Wu Q. The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials 2005;26:6565—78.
[33] Portner R, Nagel-Heyer S, Goepfert C, Adamientz P, Meenen NM. Bioreactor design for tissue engineering. J Biosci Bioeng 2005;100:235—45.
[34] Shikani AH, Fink DJ, Sohrabi A, Phan P, Polotsky A, Hungerford DS, Frondoza CG. Propagation of human nasal chondrocytes in microcarrier spinner culture. Am J Rhinol 2004;18:105—12.
[35] Wendt D, Jakob M, Martin I. Bioreactor-based engineering of osteochondral grafts: from model systems to tissue manufacturing. J Biosci Bioeng 2005;100:489—94.

[36] Vunjak-Novakovic G, Meine L, Altman G, Kaplan D. Bioreactor cultivation of osteochondral grafts. Orthod Craniofac Res 2005;8:209—18.
[37] Mahmoudifar N, Doran PM. Tissue engineering of human cartilage and osteochondral composites using recirculation bioreactors. Biomaterials 2005;26:7012—24.
[38] Hisch A, Groger A, Gebert C, Leder K, Ebmeyer J, Sudhoff H, Jovanovic S, Sedlmaier B, Sittinger M. Creating artificial perichondrium by polymer complex membrane macroencapsulation: immune protection and stabilization of subcutaneously transplanted tissue-engineered cartilage. Eur Arch Otorhinolaryngol 2005;262:338—44.
[39] Kojima K, Bonassar LJ, Ignotz RA, Syed K, Cortiella J, Vacanti CA. Comparison of tracheal and nasan chondrocytes for tissue engineering of the trachea. Ann Thorac Surg 2003;76:1884—8.
[40] Kojima K, Bonassar LJ, Roy AK, Vacanti CA, Cortiella J. Autologous tissue-engineered trachea with sheep nasal chondrocytes. J Thorac Cardiovasc Surg 2002;123:1177—84.
[41] Sahota PS, Burn JL, Brown NJ, MacNeil S. Approaches to improve angiogenesis in tissue-engineered skin. Wound Repair Regen 2004;12: 635—42.
[42] Supp DM, Boyce ST. Engineered skin substitutes: practices and potentials. Clin Dermatol 2005;23:403—12.
[43] Trent JT, Falabella A, Eaglstein WH, Kirsner RS. Venous ulcer: pathophysiology and treatment options. Ostomy Wound Manage 2005;51:38—54.
[44] Mason C. Tissue engineering skin: a paradigm shift in wound care. Med Device Technol 2005;16:32—3.
[45] Sato Y. Update on endogenous inhibitors of angiogenesis. Endothelium 2006;13:147—55.
[46] Nyberg P, Xie L, Kalluri R. Endogenous inhibitors of angiogenesis. Cancer Res 2005;65:3967—79.
[47] Brewer GJ. Copper lowering therapy with tetrathiomolybdate as an antiangiogenic strategy in cancer. Curr Cancer Drug Targets 2005;5: 195—202.
[48] Handsley MM, Edwards DR. Metalloproteinases and their inhibitors in tumor angiogenesis. Int J Cancer 2005;115:849—60.
[49] Annex BH, Simons M. Growth factor-induced therapeutic angiogenesis in the heart: protein therapy. Cardiovasc Res 2005;65:649—55.
[50] Kelm JM, Diaz Sanchez-Bustamante C, Ehler E, Hoerstrup SP, Djonov V, Ittner L, Fussenegger M. VEGF profiling and angiogenesis in human microtissues. J Biotechnol 2005;118:213—29.
[51] Li X, Tjwa M, Moons L, Fons P, Noel A, Ny A, Zhou JM, Lennartsson J, Li H, Luttun A, Ponten A, Devy L, Bouche A, Oh H, Manderveld A, Blacher S, Communi D, Savi P, Bono F, Dewerchin M, Foidart JM, Autiero M, Herbert JM, Collen D, Heldin CH, Eriksson U, Carmeliet P. Revascularization of ischemic tissues by PDGF-CC via effects on endothelial cells and their progenitors. J Clin Invest 2005;115:118—27.
[52] Bauer SM, Bauer RJ, Liu ZJ, Chen H, Goldstein L, Velazquaz OC. Vascular endothelial growth factor-C promotes vasculogenesis, angiogenesis, and collagen constriction in three-dimensional collagen gels. J Vasc Surg 2005;41:699—707.
[53] Nakajima H, Sakakibara Y, Tambara K, Iwakura A, Doi K, Marui A, Ueyama K, Ikeda T, Tabata Y, Komeda M. Therapeutic angiogenesis by the controlled release of basic fibroblast growth factor for ischemic limb and heart injury: toward safety and minimal invasiveness. J Artif Organs 2004;7:58—61.
[54] Cao Y, Sun Z, Liao L, Meng Y, han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. Biochem Biophys Res Commun 2005;332:370—9.
[55] Prasad Chennazhy K, Krishnan LK. Effect of passage number and matrix characteristics on differentiation of endothelial cells cultured for tissue engineering. Biomaterials 2005;26:5658—67.
[56] Wu X, Rabkin-Aikawa E, Guleserian KJ, Perry TE, Masuda Y, Sutherland FW, Schoen FJ, Mayer Jr JE, Bischoff J. Tissue-engineered microvessles on three-dimensional biodegradable scaffolds using human endothelial progenitor cells. Am J Physiol Heart Circ Physiol 2004;287:480—7.
[57] Caplice NM, Gersh BJ, Alegria JR. Cell therapy for cardiovascular disease: what cells, what diseases and for whom? Nat Clin Pract Cardiovasc Med 2005;2:37—43.
[58] Nishiyama K, Takaji K, Kataoka K, Kurihara Y, Yoshimura M, Kato A, Ogawa H, Kurihara H. Id1 gene transfers confers angiogenic property on fully differentiated endothelial cells and contributes to therapeutic angiogenesis. Circulation 2005;112:2840—50.
[59] Jung RE, Schmoekel HG, Zwahlen R, Kokovic V, Hammerle CH, Weber FE. Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morphogenetic protein-2. Clin Oral Implants Res 2005;16: 676—82.
[60] Pratt AB, Weber FE, Schmoekel HG, Muller R, Hubbell JA. Synthetic extracellular matrices for in situ tissue engineering. Biotechnol Bioeng 2004;86:27—36.
[61] Yao C, Prevel P, Koch S, Schenck P, Noah EM, Paullua N, Steffens G. Modification of collagen matrices for enhancing angiogenesis. Cells Tissues Organs 2004;178:189—96.
[62] Steffens GC, Yao C, Prevel P, Markowicz M, Schenck P, Noah EM, Pallua N. Modulation of angiogenic potential of collagen matrices by covalent incorporation of heparin and loading with vascular endothelial growth factor. Tissue Eng 2004;10:1502—9.
[63] Kanematsu A, Yamamoto S, Ozeki M, Noguchi T, Kanatani I, Ogawa O, Tabata Y. Collagenous matrices as release carriers of exogenous growth factors. Biomaterials 2004;25:4513—20.
[64] Peak HJ, Campaner AB, Kim JL, Aaron RK, Ciombor DM, Morgan JR, Lysaght MJ. In vitro characterization of TGF-beta1 release from genetically modified fibroblasts in Ca(2+)-alginate microcapsules. ASAIO J 2005; 51:379—84.
[65] Jang JH, Rives CB, Shea LD. Plasmid delivery in vivo from porous tissue engineering scaffolds: transgene expression and cellular transfection. Mol Ther 2005;12:475—83.
[66] Premaraj S, Mundy B, Parker-Barnes J, Winnard PL, Moursi AM. Collagen gel delivery of Tgf-beta3 non-viral plasmid DNA in rat osteoblast and calvarial culture. Orthod Craniofac Res 2005;8:320—2.
[67] Distler JH, Jungel A, Kurowska-Stolarska M, Michel BA, Gay RE, Gay S, Distler O. Nucleofection: a new, highly efficient transfection method for primary human keratinocytes. Exp Dermatol 2005;14:315—20.
[68] Partridge KA, Oreffo RO. Gene delivery in bone tissue engineering: progress and prospects using viral and nonviral strategies. Tissue Eng 2004;10:295—307.
[69] Endres M, Leinhase I, Kaps C, Wentges M, Unger M, Olze H, Ringe J, Sittinger M, Rotter N. Changes in the gene expression pattern of cytokeratins in human respiratory epithelial cells during culture. Eur Arch Otorhinolaryngol 2005;262:390—6.
[70] Kanzaki M, Yamato M, Hatakeyama H, Kohno C, Yang J, Umemoto T, Kikuchi A, Okano T, Onuki T. Tissue engineered epithelial cell sheets for the creation of a bioartificial trachea. Tissue Eng 2006;12:1275—83.
[71] Walsh DA. Angiogenesis in osteoarthritis and spondylosis: successful repair with undesirable outcomes. Curr Opin Rheumatol 2004;16:609—15.
[72] Staudenmaier R, Hoang TN, Kleinsasser N, Schurr C, Frolich K, Wenzel MM, Aigner J. Flap prefabrication and prelamination with tissue-engineered cartilage. J Reconstr Microsurg 2004;20:555—64.
[73] Spaggiari L, Calabrese LS, D'Aiuto M, Veronesi G, Galetta D, Venturino M, Chiesa F. Successful subtotal tracheal replacement (using a skin/omental graft) for dehiscence after a resection for thyroid cancer. J Thorac Cardiovasc Surg 2005;129:1455—6.
[74] Olias J, Millan G, da Costa D. Circumferential tracheal reconstruction for the functional treatment of airway compromise. Laryngoscope 2005;115: 159—61.
[75] Azorin JF, Bertin F, Martinod E, Laskar M. Tracheal replacement with an aortic autograft. Eur J Cardiothorac Surg 2006;29:261—3.
[76] Ito Y, Suzuki H, Hattori Y, Muhammad BA, Takahashi T, Suzuki K, Kazui T. Complete replacement of tracheal epithelia by the host promotes spontaneous acceptance of orthotopic tracheal allografts in rats. Transplant Proc 2004;36:2406—12.