Revitalization of Biostatic Tissue Allografts: New Perspectives in Tissue Transplantology

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ABSTRACT

Background. Biostatic (nonvital) tissue allografts have been used for temporary replacement as well as to trigger, stimulate, and ensure space for the regeneration of a recipient’s own tissues. Examples of biostatic allografts routinely used in clinic are bone, tendons, skin, and amniotic membrane. A characteristic feature of biostatic allografts is the lack of living cells. In the recipient’s body, biostatic allografts function as scaffolds as well as sources of growth, differentiation, and chemotactic factors. After implantation, recipient cells migrate onto the graft, colonize it, and initiate synthesis of extracellular matrix, thereby regenerating the structure of the lost or damaged tissue. The allograft gradually degrades before being remodeled and substituted by the recipient’s new tissue. However, this process is not always effective due to a lack of reaction by recipient cells. New concepts have proposed seeding recipient cells onto the allograft prior to implantation, that is, biostatic allografts that are revitalized ex vivo. The aim of this presentation was to review scientific publications to provide essential information on the revitalization of biostatic allografts, as a rising trend in tissue transplantology.

Results. Biostatic allografts show the following advantages: they are human-derived, non-toxic, biocompatible, and, in some cases, already display the desired shape. The process of introducing cells into the biostatic graft is described as “revitalization.” The cells used in the process are recipient autologous elements that are either differentiated or progenitor elements. Cells are seeded onto the graft directly after retrieval or after propagation in culture. Revitalized biostatic allografts can be used orthotopically for the regeneration of the same tissue they have been retrieved from or heterotopically wherein the graft retrieved from a different tissue is used as a carrier for cells typical for the tissue to be regenerated. Examples of orthotopic use include revitalized trachea, tissue-engineered blood vessels, urinary bladder wall, and revitalized trabecular bone cubes. Examples of heterotopic use include: amniotic membrane as a carrier of limbal stem cells to treat corneal defects, or for chondrocytes to treat articular cartilage defects. Various requirements set by law must be met by tissue banks performing cell seeding of grafts. In Europe, the requirements are described in directives: 2004/23/EC, 2006/17/EC, 2006/86/EC), and in the regulation 2007/1394/EC. Revitalization of biostatic allografts gives new, promising tools for creation of functional parts of organs; brings the methodology used in tissue banks closer to tissue engineering; places the enterprise in the mainstream of advanced biotechnology; allows the full potential of tissue allografts; and opens a new, large area for clinical and laboratory research.

BIOSTATIC (NONVITAL) tissue allografts are used as temporary replacement to trigger, stimulate, and ensure space for the regeneration of a recipient’s own tissues. Examples of biostatic allografts routinely used in the clinic are bone, tendons, skin, and amniotic membrane. A characteristic feature of biostatic allografts is the lack of living cells.
cells, although they are not crucial for their function. Biostatic grafts can therefore be sterilized, stored for long periods (years), and do not require immunosuppressive treatment. Three elements are necessary to induce tissue regeneration: a scaffold, growth and differentiation factors, and living functional cells capable of synthesizing extracellular matrix typical for the tissue to be regenerated. In the recipient’s body, biostatic allografts function as scaffolds as well as sources of growth, differentiation, and chemotactic factors. The third necessary element—living functional cells—is not present in biostatic allografts at the moment of implantation. Cell death and cell loss take place because of the long ischemic period, the sterilization, or the monitored process of decellularization.1 In response to factors released from the graft, recipient cells migrate onto the graft and colonize it. After implantation, recipient cells synthesize extracellular matrix, regenerating the structure of the lost or damaged tissue, so that the allograft gradually degrades being remodeled and substituted by the recipient’s new tissue. However, this desired process is not always effective due to a lack of reaction by recipient cells. New concepts have been proposed in which recipient cells are seeded onto the allograft prior to implantation, that is, biostatic allografts revitalized ex vivo. Experimental and clinical results show promising positive effects on graft remodeling. Cell seeding also makes it possible to create complex, patient-designed, multitissue living structures ex vivo.

Revitalization of tissue allografts is one of the latest developments in tissue transplantology. So far, reports on these procedures have referred to particular experiments with or applications of a particular type of biostatic allograft. Revitalization as a general trend has not been presented in the existing literature. The authors of this short review sought to highlight this emerging approach of tissue allografts as scaffolds to end users in the clinic.

**METHODS**

This presentation is based on a review of scientific publications with the aim to provide essential information on revitalization of biostatic allografts as a rising trend in tissue transplantology.

**RESULTS**

The new approach to biostatic allografts as natural scaffolds for cell seeding brings transplantology closer to tissue engineering. Tissue engineering, as in the understanding of the European Commission, is the regeneration of biological tissue through the use of cells with the aid of supporting structures (scaffolds) and/or biomolecules. Scaffolds used in tissue engineering are made of nonorganic or processed organic substances of natural or synthetic origin. These materials require careful testing of biocompatibility; their features do not equal those of natural tissues. Biostatic allografts show the following advantages: they are human-derived, nontoxic, biocompatible (chemical composition, mechanical features, surface providing molecules for cell attachment and adhesion), and in some cases, already have the desired shape (eg, meniscus). The process of introducing cells into the biostatic graft is described as “revitalization” since the dead structure of extracellular matrix becomes revived.2 Autologous recipient cells are seeded onto the graft directly after retrieval or after propagation in culture. The cells can be differentiated, with the characteristics typical for the tissue to be regenerated, or they can be progenitor cells, which differentiate in culture or during graft incubation after seeding. The source of differentiated cells is the appropriate recipient tissues, for example, the source of osteoblasts is bone. The source of progenitor cells is bone marrow (bone marrow mesenchymal stem cells [BMSC]), adipose tissue (adipose tissue stem cells, cord blood, or corneal limbus (limbal stem cells).3 After seeding, complex interactions between the cells and the graft surface involve extracellular matrix ligands present in the graft and integrin receptors of the cells. The main phases are: cell attachment, adhesion, and flattening. The aforementioned interactions trigger signals that direct cell proliferation, differentiation, and synthesis of specific proteins by the cells. It is recommended to incubate seeded grafts under static conditions for the first few hours to allow effective attachment and adhesion. Further incubation can be continued under dynamic conditions that stimulate cell differentiation. The process can be optimized by the use of bioreactors that, except for stabilizing culture conditions, can also provide stimulation by generating mechanical load imitating in vivo conditions; this process of incubation with mechanical stimulation is referred to as “graft preconditioning.”4 Graft cell seeding enables creation of simple constructs, for example, revitalized trabecular bone cube, as well as more complex, multitissue constructs. Revitalized biostatic allografts can be used orthotopically for the regeneration of the tissue from which they have been retrieved or heterotopically as a carrier for cells typical of the different tissue to be regenerated. Examples of orthotopic use include: The revitalized trachea represented a landmark where in seeded autologous BMSC-derived chondrocytes and bronchial epithelial cells were cultured onto both sides of an acellular matrix obtained from a deceased donor trachea ex vivo. The revitalized construct was successfully implanted as a replacement for the left main bronchus in a patient suffering from bronchomalacia. A previous clinical attempt to use an allograft for tracheal replacement was reported in 2000. Irradiated tracheal allografts have been used without cell preseeding with satisfactory effects.5 Other studies have been performed in animal and in vitro models.6–12

Tissue-engineered blood vessels (TEBV) of small diameter (<6 mm) target vessels where blood flow conditions negatively affect graft survival. Allogenic, decellularized vessels are seeded with autologous endothelial cells and smooth myocytes obtained from BMSCs. Reepithelialization and revitalization of the vessel wall is expected to prevent bad remodeling of the graft such as intimal overgrowth, thrombosis, restenosis, aneurysms, or intensified
Atherosclerosis as observed when standard allogenic or artificial grafts are used for small-vessel replacement. Reconstructed urinary bladder wall originally used intestinal autografts. Because of the unfavorable characteristics of intestinal absorptive epithelium, allogenic acellular fragments of urinary bladder are currently used as nonseeded grafts.

Comparison of the results of the implantation of seeded and nonseeded grafts revealed seeded

Table 1. Examples of Laboratory and Clinical Trials With the Use of Cell-Seeded Allografts

<table>
<thead>
<tr>
<th>Tissue or Organ Regenerated</th>
<th>Biostatic Allograft Used</th>
<th>Cells Used</th>
<th>Year and Reference</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea (humans)</td>
<td>Cryopreserved, irradiated tracheal grafts</td>
<td>No cells preseeded</td>
<td>2000⁶</td>
<td>Follow-up 18 to 20 mo. Four patients. Three patients successfully underwent decannulation. The postoperative tracheal lumen appeared to be near normal, with histological evidence of normal respiratory epithelium at the graft site.</td>
</tr>
<tr>
<td>Main bronchus (humans)</td>
<td>Fresh, decellularized donor trachea</td>
<td>Mesenchymal stem-cell-derived chondrocytes; epithelial cells</td>
<td>2008⁵</td>
<td>The graft immediately provided the recipient with a functional airway. Normal appearance and mechanical properties at 4 months. No antidonor antibodies. No immunosuppression required.</td>
</tr>
<tr>
<td>Bone (humans)</td>
<td>Highly washed morselized allograft</td>
<td>Bone marrow-derived autogenous progenitor cells</td>
<td>2006⁷¹</td>
<td>Two patients. Uncomplicated clinical recovery. Imaging confirmed defect filling with graft incorporation. Histochemical and alkaline phosphatase staining confirmed osteogenic activity within the graft.</td>
</tr>
<tr>
<td>Bone (mice)</td>
<td>Allograft bone discs from tibia and femur</td>
<td>Bone marrow-derived osteoprogenitor cells</td>
<td>2008⁷²</td>
<td>Bone marrow-derived OPCs adhered to and produced new bone on corticocancellous allograft in vitro.</td>
</tr>
<tr>
<td>Bone (pigs)</td>
<td>Porcine hemimandibles</td>
<td>Adipose stem cells</td>
<td>2010⁷⁰</td>
<td>Constructs implanted to pigs, in two locations: (1) an intercostal-based peristomal envelope (thoracic) and (2) within the rectus abdominis muscle. New bone in both groups contained Haversian systems, but only thoracic constructs contained marrow elements and blood vessels resembling normal bone.</td>
</tr>
<tr>
<td>Cornea, epithelium (mice)</td>
<td>Amniotic membrane</td>
<td>Limbal stem cells</td>
<td>2011³⁴</td>
<td>Amniotic membrane proved to maintain the limbal-like environment for the transplanted area of cornea.</td>
</tr>
<tr>
<td>Cornea, epithelium (rabbits)</td>
<td>Amniotic membrane fixed by a biomembrane-fixing device</td>
<td>Limbal stem cells</td>
<td>2011³⁷</td>
<td>The corneal reepithelialization. The histological sections at different time points proved that the delivered cells adhered to the wounded corneal surface and proliferated well.</td>
</tr>
<tr>
<td>Articular cartilage (rabbits)</td>
<td>Decellularized human amniotic membrane</td>
<td>Rabbit articular chondrocytes</td>
<td>2007³⁸</td>
<td>The defect area in rabbits was successfully regenerated with hyaline cartilage after 8 wk of implantation.</td>
</tr>
<tr>
<td>Articular cartilage (humans)</td>
<td>Human amniotic membrane</td>
<td>Human articular chondrocytes</td>
<td>2010³⁹</td>
<td>In vitro repair experiments (44) showed formation on human osteoarthritis cartilage of new tissue expressing type II collagen, typical for a hyaline cartilage. Integration of the new tissue with trial cartilage was excellent.</td>
</tr>
<tr>
<td>Blood vessels (sheep)</td>
<td>Decellularized arterial scaffold</td>
<td>Autologous bone marrow-derived mesenchymal stem cells differentiated in vitro to endothelial cells and smooth muscle cells</td>
<td>2010¹⁴</td>
<td>The vessels were antithrombogenic, and mechanically stable for 5 months in vivo. Nonseeded grafts occluded within 2 wk. Histological, immunohistochemical, and electron microscopic analyses demonstrated the existence of endothelium, smooth muscle, and the presence of collagen and elastin.</td>
</tr>
<tr>
<td>Blood vessels (dogs)</td>
<td>Decellularized aortic canine artery</td>
<td>Endothelial and smooth muscle cells</td>
<td>2009¹⁵</td>
<td>Good functional performance demonstrated by regular Doppler ultrasonography at 1, 3, and 6 mo postoperatively. Histological and immunohistochemical analyses indicated the presence of high cell density and development of a highly organized structure.</td>
</tr>
<tr>
<td>Urinary bladder (rabbits)</td>
<td>Bladder acellular matrix grafts</td>
<td>Autologous adipose-derived stem cells</td>
<td>2010⁷¹</td>
<td>Adipose-derived stem cells promoted regeneration of smooth muscle and nervous tissue regeneration in a rabbit model. This compound graft was more suitable for bladder reconstruction than bladder acellular matrix graft alone.</td>
</tr>
</tbody>
</table>

OPC, osteoprogenitor cells.
grants, to produce regeneration of a three-layer wall of proper histological structure. Nonseeded grafts maintain only up to half of the bladder’s capacity, while the seeded ones show up to and above 100%. Revitalized trabecular bone cubes have been seeded with osteoblasts and/or endothelial cells to stimulate the vascularization process. Seeded cubes have been used for surgical treatment of bone defects in orthopedics, dental implants, and surgery, as well as maxillofacial surgery as alternatives to standard bone cubes. Examples of heterotopic use include: amniotic membrane as a carrier for limbal stem cells to treat corneal defects and for chondrocytes to treat articular cartilage defects. Examples of laboratory and clinical trials are shown in Table 1. The wide range of potential applications of seeded biostatic allografts is beyond the scope of this paper.

DISCUSSION

Modern tissue banking and tissue transplantation dates to the mid-20th century when cell cultures were not yet advanced. Since then, much progress has been made in this field. Combining traditional solutions for allograft processing with biological technologies sets new directions for the development of tissue transplantology. The experimental results suggest that revitalization of biostatic allografts will speed and modify positively the processes of graft remodeling, thereby improving treatment effectiveness. The range of use of allografts can be broadened first of all due to the possibility of heterotopic applications. Revitalization can be especially beneficial for massive bone allografts, for example, the distal part of the femur, that in practice are not or are only to a limited degree remodeled. Recipient cells do not effectively settle the graft and new tissue is not formed, which severely affects the mechanical features of the graft.

The use of revitalized allografts will be limited as is the case of unseeded allografts by the number of donations, which is a general problem in transplantology. Transplantation of a human tissue carries the risk of disease transmission, which is minimized by sterilization of the allografts. Another critical point is the security of cultured cell application due to potential mutations that can take place in the culture environment. Such mutations have been observed in cases of adult stem cells after several passages. Culture processes for graft revitalizations are limited to shorter safe periods. From a technical point of view, cell seeding of allografts is a relatively simple procedure, but certain requirements have been set by law and must be met by tissue banks. In Europe, the requirements are described in the “tissue directives” (2004/23/EC, 2006/17/EC, 2006/86/EC), in the Regulation 2007/1394/EC (on advanced therapy medicinal products), and in local/national legal acts. One requirement is clear rooms of the highest class in compliance with good manufacturing practice. Meeting criteria set by law means considerable financial outlay to adapt facilities, which can be a limiting factor for tissue banks. On the other hand, many tissue banks already meet these criteria due to their involvement in the process of preparation of material for cell therapies, for example, chondrocytes or keratinocytes.

General conclusions for this subject are that revitalization of biostatic allografts: (1) gives new promising tools for therapeutic treatment, including the creation of functional parts of organs; (2) brings the methodology used in tissue banks closer to tissue engineering; (3) places tissue banking in the mainstream of advanced biotechnology; (4) changes the approach to allografts, allowing us to use the potential of tissue allografts in a more complete way—as natural, biocompatible scaffolds; and (5) opens a new large area for clinical and laboratory research.

REFERENCES


