

# Biocompatibility testing of tissue engineered products

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## Introduction

The discipline of Tissue Engineering has developed over the past 15–20 years as a compilation of the emerging technologies in regenerative medicine and reconstructive surgery, holding the promise of surpassing the limitations imposed by the biomaterials currently available. By utilizing the regenerative capacity of tissues it is anticipated that functional tissue analogues having the desired dimensions, mechanical attributes (such as strength, compliance, etc.) and enduring biological function can be deployed giving device, organ or tissue integration that overcome the potential problems of inflammation, immune response, tumorigenicity and device failure that purely synthetic systems can cause.

Although many billions of dollars have been invested in tissue engineered products world-wide, viable products have so far only been marketed in the skin and skeletal tissues. Current research will soon bring to the market cardiovascular devices that, by their very nature hold greater risk to the patient. The specific issues relevant to cardiovascular tissue engineered products are described in detail below.

## History of tissue engineering

The term *Tissue Engineering* was first coined at a scientific workshop in 1987 [1], as a description for the multidisciplinary technologies starting to be exploited for controlling cell behaviour to allow their implantation, in combination with non-biological scaffolds, in place of traditional synthetic prostheses, in order to effect repairs and reconstruction. This has been a logical progression from the concept of attaining minimal rejection through inertness, to achieving maximal integration and ultimately to controlled repair or regeneration [2]. It is worth noting at this point that the ideas of repair and regeneration are now regarded as complementary concepts, rather than a description of the same phenomenon.

Evaluating the authenticity or success of implantation of synthetic materials from historical accounts is difficult to judge, but postrenaissance manuscripts relate the use of artificial teeth and legs. The first occurrence of proper biological transplantation, however, probably did not arise until skin grafting became a reality in the early 19th century, stimulated by an increased understanding of the role of the vasculature, with the first use of pedicled flaps. Elucidation of the role of cells of the immune system during the mid-20th century led to the first successful organ transplant in 1954. Through the late 20th century, researchers came to understand the nature of receptors, intracellular signalling and the role of extracellular matrix and mechanical stimuli in controlling the nature of cultured cells and tissues. Finally, at the start of the 21st century, the great mass of information supplied by unlocking the genome has provided the tools necessary for scientists to begin to unlock the puzzles of controlled cell differentiation, commitment and plasticity required for the full exploitation of tissue engineering technologies.

## Tissue engineering products

The promise of the 1970s that artificial prostheses would become available for every type of morbidity has faltered, even though biomaterials in recent years have been designed specifically for their medical application. Traditionally, biomaterials have been adapted from other industrial applications: Teflon (PTFE), silicone, polyurethane and polyethylene were taken from cooking utensils, lubricants, ladies girdles and industrial containers yet are still used in vascular grafts, catheters, artificial hearts and stents [3,4]. Yet even after many decades of research, approaches to improve surface permeability, thrombogenicity and coatings for improved endothelial binding, etc., have not resulted in the creation of an artificial graft for coronary bypass. So, while saphenous vein transplantation is the preferred option for surgeons, it is unavailable in up to 30% of patients [5], demonstrating the social and economic need for vascular tissue engineering, given that > 500 000 procedures are performed world-wide annually.

The other major cardiovascular application which is likely to reach the marketplace in the near future is heart valves. Patients who have had rheumatic heart disease have valve leaflets which have degenerated and currently can only be

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treated by implanting either a bioprosthetic heart valve constructed from glutaraldehyde-treated porcine tissue or a mechanical design, requiring life-long anticoagulation therapy. The bioprosthetic valves calcify and harden and thus have a lifespan no longer than 10–15 years [6], somewhat shorter in paediatric cases which are particularly susceptible to calcification [7]. The world-wide market is currently estimated at about 200 000 valve replacements per year [8].

It is anticipated that tissue engineered cardiac tissue, such as muscle-based assist devices, or even whole hearts may in the future be considered.

The products that are currently on the market which could be described as tissue engineered are either acellular scaffolds in which patients' cells and tissue subsequently infiltrate scaffolds seeded with differentiated autologous cells or scaffolds containing allogeneic cells which are quickly replaced with the patients' own cells.

### Biocompatibility testing of biomaterials

A device or biomaterial can be considered biocompatible if it performs with the desired host response in any particular application. For an artificial vascular graft, this may include not just a measurement of its ability to act as a passive conduit for blood, but also how it integrates into the surrounding tissues and prevents intimal hyperplasia.

Medicinal products and medical devices must, by law, be tested before they can be sold, to the satisfaction of the competent authority which, in the UK, is the Medicines and Healthcare Products Regulatory Agency (MHRA). In Europe, member states define regulations that implement directives that are developed by the European Commission. The main recommendations for testing a biomaterial or medical device in order to comply with these regulations are contained within the unwieldy document ISO 10993, which sets out some of the parameters that may be considered important for different classes of device.

A medical device is constructed from biomaterials and so the constituent parts are usually tested *in vitro*, for blood-contacting devices, for their effect on platelets (adhesion, activation, aggregation) and coagulation and complement cascade activation. This may help a medical device manufacturer to choose between more or less suitable materials for the device's construction, but is generally poor as a predictive test for the long-term efficacy of the device. Most manufacturers demonstrate safety and efficacy by testing in an animal model for some months prior to clinical trials.

### Regulatory aspects of tissue engineered devices

Tissue engineered products, especially for the cardiovascular system, cannot be considered the same as medical devices.

Manufacturers of medical devices must have manufacturing facilities conforming to Good Manufacturing Practice (GMP) with certified quality assurance procedures. These ensure, to the most part, that individual devices will produce reproducible performance and have stamps to demonstrate sterility and conformity to European directives (CE marked). They will also have a specified shelf life.

A tissue engineered vascular graft, however, cannot be sterilized, although it should be free from infectious organisms. Neither will it have a CE mark or, under likely practical considerations, a shelf life. Since the cells will be either autologous, allogeneic or possibly xenogeneic, it is unlikely that they will perform to the same standard as a *proof of concept* construct developed under ideal conditions within a laboratory, considering that, for autologous cell sources, the patient is likely to be unhealthy and of later years.

The framework for regulation of tissue engineered products, however, will not fall under the medical device regulations. Two new European directives are, at the time of writing, being prepared which will deal with procurement of tissues and cells, of relevance to tissue and cell banks, and the issues regarding the manipulation of these cells in the development of the various processes and products will be of relevance to product manufacturers. However, these directives will do no more than to provide the framework to ensure that cells stored in banks are free from microbial contamination, and that cell cultures are not exposed to any harmful environment during tissue maturation, along with issues regarding patient privacy and security of sample identity.

It is not clear therefore how the performance of a tissue engineered product can be assessed, or comparisons made between competitors' products, since the performance of a tissue engineered device is likely to be dependent on variability of donor cell function, as well as the effects of scaffold degradation aside from the generation of any inflammatory, immune or metastatic response.

The regulations have a very difficult task of defining what is a product and how it differentiates from a process. In the context of regulatory issues, therefore, measurement of the performance of a tissue engineered product is practically an impossible task. The principal danger for the manufacturers of tissue engineered products is a requirement to perform unnecessary tests and pass arbitrary milestones using non-predictive tests. For patients, the danger lies in the inability of scientists as yet to ascertain the key markers of cell or tissue phenotype or function that satisfactorily define the cell sources having appropriate levels of regenerative capacity to repair the patient's morbidity.

### Future perspectives

Tissue engineered constructs for simple tissues can be created from uncomplicated, degradable scaffolds seeded with

differentiated, autologous cells. For complex tissues of the cardiovascular system which have various functions (active anti-thrombogenicity, leucocyte signalling, etc.) as well as critical mechanical properties (compliance, strength, etc.), the product is likely to be constructed from undifferentiated cells seeded into hybrid scaffolds which may release growth factors over critical periods that have been subjected to a variety of environmental stimuli, and may include cell transfection as a preliminary step. The determination of the efficacy of these complex tissue engineered products will require much more data than a simple assessment of the host response to a synthetic biomaterial. The challenge will be to find ethically acceptable ways to perform prospective clinical trials of sufficient size to substantiate proof of principle concepts, rather than merely ensuring the validity of an up-scaling process.

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