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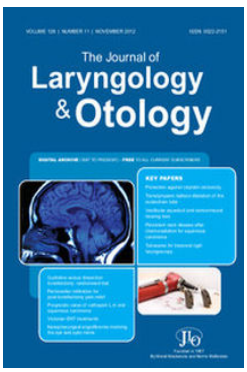

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Review Articles

Tissue engineering and ENT surgery

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Abstract
Tissue engineering is the development of biological substitutes for the repair and regeneration of damaged tissues. We explain the principles of this emerging field of biotechology. The present and potential applications of tissue engineering technologies in ENT surgery are then reviewed.

Key words: Tissue Extracts; Biomedical Engineering; Otolaryngology

Introduction

A large part of modern medical practice is aimed at the restoration of function by replacement or repair of damaged tissues or organs. This is achieved either by using artificial implants or by transplantation of tissues. Factors such as immune rejection, limited supply, donor site morbidity and infection risk, are a constant risk. Specifically in Otorhinolaryngology–Head and Neck surgery, disease processes and therapeutic interventions result in destruction or malformation of tissues and organs such as the trachea, pinna, nasal cartilages, ossicles and mandible. Reconstruction of these and other head and neck structures remain a great challenge. Tissue engineering, which represents an alternative philosophy, offers the scope to repair and replace damaged tissue with specifically constructed living tissue.

Tissue engineering may be defined as a multidisciplinary approach combining principles of engineering and life sciences for developing living prefabricated replacement tissue and organs tailored to the needs of the individual. Broader definitions include regeneration and repair by cell transplantation, insertion of acellular bioactive scaffolds and even *in vitro* induction of cellular regeneration.[1,2] Tissue engineering approaches have been pursued for decades,[1,3–5] and by the 1990s tissue engineering developed into a cross-speciality discipline in its own right.[5]

With an estimated total market potential of tissue-engineering products in the USA being $80 billion per annum it is not surprising that the tissue engineering industry is burgeoning.[1,5,6] The number of patent applications alone bear astonishing testament to the commercial interest in the discipline.[7] However, there are some who would question the ethics of granting patents for many of these 'discoveries'.

Scientific interest in the field is escalating, with articles appearing in leading scientific and clinical journals.[1,8–10] The journal *Science* featured research on embryonic stem cells (a core research area in tissue engineering) as their 'breakthrough of the year 1999'.[11] The field has also attracted media interest; *Time* magazine in a series of articles on life in the 21st century predicted 'tissue engineer' as the number one job of the future.[12]

The tissue engineering process

Tissues are composed of cells and extracellular matrix. Most tissue engineering processes, therefore, involve the replacement of cells affiliated with a matrix of scaffold to form a construct for implantation. However, some techniques are reliant solely on cells, others solely on matrices.

The steps involved in the creation of living tissues and organs for replacement illustrate the need for a wide-ranging skill mix for successful tissue engineering. The primary step is cell harvest. A suitable donor source for the cells is identified and a viable sample of the appropriate cell type is separated from the donor tissue. The cells usually require a scaffold onto which they are seeded and may survive. Figure 1 shows cells attached to a scaffold. The sample cell population must then be nurtured to proliferate to at

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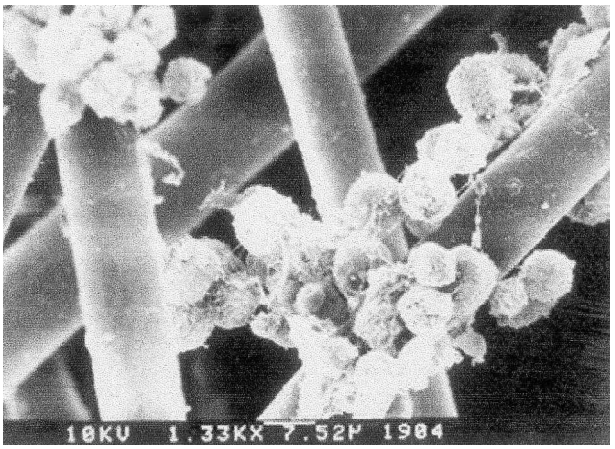

FIG. 1

Scanning electron micrographs of chondrocytes attached to a polymer scaffold (1 centimetre = 20 micrometres).

least a viable cell number. Maintenance or development of specialist characteristics ie. cellular differentiation is the next essential step towards a prefabricated replacement construct which is then transplanted to the recipient patient. This process is outlined in Figure 2.

During these processes there are three fundamental issues that need to be addressed before any tissue-engineering therapy becomes a clinical reality.

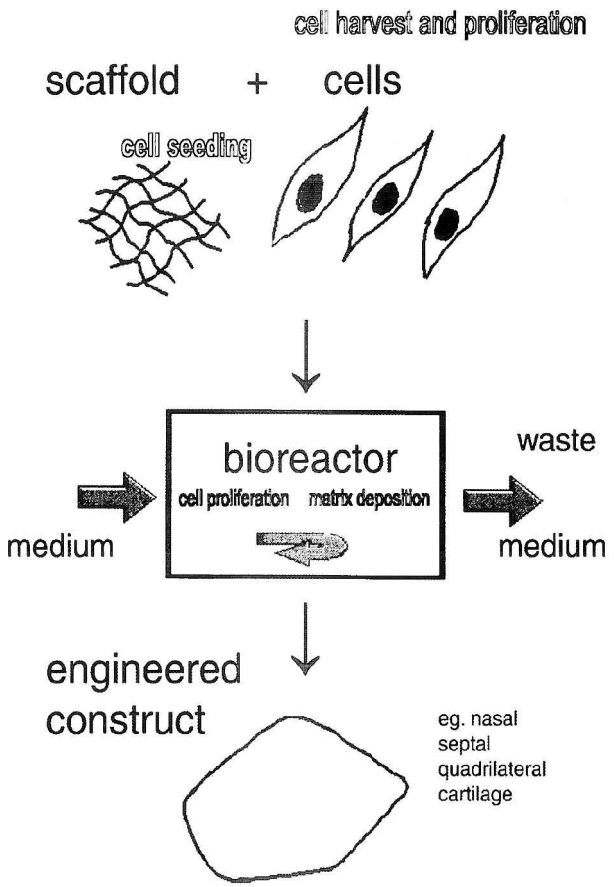

FIG. 2

Diagram illustrating the process of tissue engineering a construct.

These are the size, complexity and stability of tissue constructs.

Animal cell culture and indeed human cell culture techniques are readily available to grow two dimensional colonies of cells *in vitro* up to sizes of a few square millimetres. Indeed techniques have now been developed to grow three dimensional colonies *in vitro* and *in vivo*.[13–15] To overcome the limitations of diffusion for cellular nutrition and excretion to allow cell survival to be maintained inside large three-dimensional constructs there is extensive interest in neovascularization[11] and the development of prevascularized engineered solid organs.[16]

Tissues and organs are composed of multiple types of cells and cellular matrices. This complexity cannot be overlooked, a tissue-engineered construct requires a similar level of multi-cellular and three-dimensional structure.

As with transplantation of allograft or xenograft donor organs the long-term stability of a tissue-engineered construct is of paramount importance. Factors affecting the long-term stability of the cells, the scaffold and the cell-scaffold unit are relevant. Scaffold degeneration may be due to an immune response, the scaffold's inherent physicochemical nature and due to the physicochemical stresses of the newly implanted organ, for example the potential mechanical forces acting upon an implanted articular cartilage construct within a joint. Different methods are being explored to avoid host rejection of the cellular elements of the construct, these include the use of cells that are modified to be immunotolerant, or the use of pluripotent embryonic stem cells after transfer of the genetic code of the patients' own nucleus.[17]

The cells of the tissue-engineered construct

The donor cells that are used may be adult or embryonic. Adult cells may be committed or stem cells. Embryonic cells are pluripotent stem cells.

Committed adult cell lines from a variety of tissues have been cultured and investigated for their tissue-engineering applications. In some tissues limitations exist however, relating to aspects of cell harvest, the ability of cells to proliferate and/or the maintenance of cellular differentiation. Autologous cells are by definition immunoprivileged, however, the ability to utilize allogenic cell sources would expand the scope of tissue engineering in part by the creation of therapeutic cell banks. In addition, these cells could be engineered by genetic manipulation to produce proteins of therapeutic or morphogenic importance. In allogenic transplantation immunomodulation will be necessary, and this may well be possible through gene manipulation. A donor cell's age has implications upon its ability to divide. Telomeres cap chromosomes and are lost when cells divide, this process being strongly implicated in cellular senescence. An approach to overcome this problem is the introduction of telomerase by gene transfection into cells. This enzyme maintains the telomeres and thus would allow cells to continue dividing.[18]

The benefit of pluripotent stem cells is that they may be directed into different cell types as required. This offers an enormous variety of applications. Human embryonic stem cells have been isolated from human embryonic blastocysts.[19] These stem cells were proliferated in an undifferentiated state and then directed into cell types representing three embryonic germ layers. Controversy exists regarding the ethical implications of such work, which is currently carried out under the strictest guidelines and controls.

The scaffold of the tissue engineered construct

The matrix component of a 'construct' may be synthetic or natural, biodegradable or non-biodegradable. They may be described as being closed or open cell delivery systems and, as such, exist in fibrous, hydrogel, foam, or capsular forms.

Synthetic matrices can be broadly divided into polymeric compounds (for example; polylactic acid, polyglycolic acid, co-polymers of polyglycolic and polylactic acid, polyethylene oxide) and bioactive glasses (for example, Bioglass 45S5™, US Biomaterials Corp, USA). The synthetic polymers can be shaped and moulded relatively easily and have physicochemical characteristics that may be manipulated,[20] but they do not themselves contribute greatly to cell growth and differentiation or synthesis of extracellular matrix. Investigation into bioactive materials promises the development of resorbable scaffolds that enhance growth of engineered tissue.[21] There is also exploration into the impregnation of scaffolds with growth factors that would be released in a controlled way to modulate growth and differentiation including angiogenesis within the implant.[22]

Natural scaffolds include alginates,[8] autologous fibrin polymers[23] and collagen. The naturally occurring scaffold materials that are common to the matrix of the actual tissue being replaced have the advantage of being inherently interactive with the implant cells.

In closed cell delivery systems the cells are separated by semipermeable membranes from the recipient tissue through which the cells cannot pass. These systems have limited applications except in the case of microcapsules made of hydrogels such as alginate. Minimally invasive technologies can exploit the ability of hydrogels to readily change from liquid to gel in the development of injectable systems.

Open cell implant systems such as porous three dimensional synthetic polymer scaffolds offer large surface areas for diffusion, cell-cell and cell-matrix interactions and even the ingress of new vasculature.

Bioreactors

The tissue creation process is usually carried out in a bioreactor, which is an *in vitro* culture system designed specifically to develop tissue-engineered constructs. These systems specifically aim to (1) establish spacially uniform cell distributions on three dimensional scaffolds, (2) maintain desired concentrations of gases and nutrients in culture medium, (3) provide efficient mass transfer to growing tissue and (4) expose developing tissue to appropriate physical stimuli (for example, pulsatile flow to engineer vessels).[24,25]

Applications in otorhinolaryngology–head and neck surgery

Reconstruction of the soft tissues and skeletal elements of the face offer particular functional and aesthetic challenges to the surgeon. Good results may be obtained with modern techniques of plastic and reconstructive surgery including local flap reconstruction, implantation of synthetic materials, tissue expansion and even free tissue transfer. However, such techniques often demand specialist skills, risk implant extrusion, cause donor site morbidity and may require multiple operative procedures.

Tissue engineered allograft skin is commercially available but currently most products act only as biological dressings. Autologous cells are cultured and used as skin substitutes, but unfortunately this technique is flawed by the time taken for the cells to grow. Although no commercially available skin substitute currently has the ideal properties of a conventional autograft, this is a realistic expectation for the near future.[26]

Human nasal septal cartilage[27] and a variety of chondrocytes from other human and animal sites[15,28] have been used as donor sites for the production of three-dimensional cartilage reconstructions. None have so far been used in the head and neck in clinical trials. However, well publicized *in vivo* animal studies show great promise.[23] The potential for reconstruction of the damaged nose including augmentation rhinoplasty, reconstructive rhinoplasty after rhinectomy and repair of nasal septal perforations exist. Reconstruction of the auricle for microtia is another attractive potential application.

Laryngotracheal reconstruction techniques in the adult and child currently include; resection and end to end anastomoses, muscular augmentation flaps, stenting, rib cartilage grafts and tracheal allograft or xenograft transplantation. Vacanti and Vacanti report tracheal reconstruction in rats using polyglycolic acid/chondrocyte engineered constructs.[20] The constructs remained patent for a number of weeks. Work by the same group continues using co-culture techniques to develop organs with cartilage and luminal mucosal lining to ensure long-term stability and patency.

Much has been written about reconstruction of the mandible following resection for oral cavity malignancy. The currently favoured technique is vascularized free tissue transfer using composite-grafts centred around the radius, fibula or iliac crest. There have been successful implants of Bioglass™ in humans as a matrix-only implant for small mandibular defects.[29,30] Current research is aimed at larger reconstructions with viable cell-scaffold constructs.

Oncological surgery of the head and neck often results in the resection of neck viscera. Grower *et al.*[31] and Vacanti *et al.*[20] have replaced segments of

resected oesophagus in dogs and rats respectively with co-polymer tubes that became epithelialized and colonized by connective tissue allowing resumption of a semi-solid diet. Thus it may be feasible to engineer pharyngo-oesophageal tissue even if only as passive food conduits. Following glossectomy various techniques of reconstruction are employed, often with significant donor site morbidity. Although there have been no reports of tongue reconstruction in animal or human subjects with prefabricated implants, the basic science of myocyte growth and replication is being unravelled, such that tissue-engineered reconstruction of the tongue may one day become a reality.

Endocrine tissue engineering is well advanced. Initially focused on pancreatic islet cell replacement for diabetic mellitus, research has now broadened to consideration of organs such as the parathyroid glands. Experiments are ongoing with parathyroid cells encapsulated in selectively permeable membranes for eventual transplantation in hypoparathyroid patients.[32] This provides the possibility of freedom from calcium replacement therapy.

Materials science has long been involved in ossicular reconstruction and indeed bioactive ceramics are established materials for implants,[33] however if seeded with osteoblasts thus subsequently ossifying into living bony ossicles, implant survival may be assured.

Closure of cranial vault defects has been carried out in rats.[20] In the future these methods may be usefully employed to obliterate mastoid cavities or defects such as those left after approaches to the skull base.

Progress is being made in our understanding of vestibulocochlear hair cell biology and the regeneration potential of avian hair cells has been documented.[34] As the mechanisms governing this process are unravelled and with the possibility of exploiting embryonic stem cell technology it may well be that organs with the differentiation commitment of the cochlea are within the scope of repair or replacement.[35]

TABLE I
POTENTIAL APPLICATIONS OF TISSUE ENGINEERING IN ENT SURGERY

Facial plastics	Nasal reconstruction
	Auricular reconstruction
	Chin augmentation
Rhinology	Closure of septal perforation
Paediatric surgery	Engineered trachea for stenosis
Head and neck surgery	Engineered parathyroid glands
	Mandibular reconstruction
	Anterior skull base reconstruction
	Engineered pharyngo-oesophagus
Otology and neuro-otology	Engineered ossicles
	Closure of lateral skull base defects
	Mastoid obliteration
	Hair cell regeneration
	Facial nerve repair

By stem cell or supporting cell implantation to initiate axonal regeneration and directed neuronal networking, neuronal tissue engineering developments offer the prospect of repairing peripheral nerve[36] and central nervous system lesions.[37]

The areas where tissue engineering has entered clinical use to date are; autologous chondrocytes for articular cartilage repair,[38] engineered skin substitutes and bioactive materials for bony repair. Many other technologies are approaching clinical trials. Table I summarizes some of the areas in which tissue engineering may be applied to the ENT surgery.

Conclusion

Significant advances have been made in the basic sciences that underpin tissue engineering. Since the recognition of tissue engineering as a biotechnological process in its own right, progress has been made in drawing together much of this basic science research. Construct implantation has been performed *in vivo*, but largely in non-primate animals. The obvious future stages are further *in vitro* studies followed by *in vivo* work on primates and then human clinical trials. Other future challenges might include novel applications for reconstructive surgery, the further union of genetic engineering and tissue engineering, novel delivery approaches and developing functional complex artificial organs.[5]

Tissue engineering is a multidisciplinary field in the truest sense, involving materials science, matrix science, embryology, gene therapy, histochemistry, cell biology, immunology, molecular biology, transplant biology and clinical medicine. Although young and largely experimental, it appears that progress in tissue engineering has been rapid and its potential applications vast, such that tissue engineering is sure to change the way we practise medicine in the future.

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