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REVIEW ARTICLE

Current concepts and advances in the application of tissue engineering in otorhinolaryngology and head and neck surgery

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Abstract

Objective: This paper reviews the progress in the rapidly expanding scientific discipline of tissue engineering, which may have an integral role in the future of otorhinolaryngology. This article seeks to inform on the current concepts and principles of tissue engineering, and describe the state of the art research and developments in this exciting field as applied to ENT and head and neck surgery.

Method: In order to carry out a comprehensive review of the literature spanning the past 30 years, a search of relevant publications was performed using the Web of Knowledge, Medline and PubMed databases.

Results: This search identified 85 scholarly articles, which were utilised as the basis of this review.

Conclusion: Given the current rate of development of tissue engineering research, it is likely that tissue-engineered implants will be widely used in surgical practice, including ENT and head and neck surgery.

Key words: Tissue Engineering; Rhinoplasty; Trachea; Tissue Scaffolds; Stem Cells; Mandible; Salivary Gland

Introduction

Tissue engineering is a rapidly developing field, which combines the disciplines of materials science and biotechnology to develop tissue constructs that can be implanted into the human body. Surgeons frequently have to remove damaged and diseased tissue. The advent of bioengineered tissues heralds a new era of restorative surgery, allowing the surgeon to implant a tissue construct that aims to replicate the proper form and function of the diseased tissue.

The use of engineered tissue constructs can provide numerous benefits for the patient. These include the reduction of donor site morbidity compared with autogenous grafting techniques, and the absence of immune rejection which can occur when using allografts.

The purpose of this review is to provide an insight into the latest techniques currently being used in tissue engineering, and to describe the many potential applications of tissue engineering in ENT and head and neck surgery.

Fundamental concepts in tissue engineering

Tissue engineering is defined as 'an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological tissues that restore, maintain, or improve tissue function'.[1] The primary approach to tissue engineering can be summarised as 'the regeneration of biological tissue through the use of cells, with the aid of supporting structures/biomolecules'.[2]

The many techniques used in tissue engineering have emerged from the advent of mammalian cell culture *in vitro*.[3] The replication of form and function of animal tissues is termed biomimetic design. By combining cells with an appropriate scaffold, an engineered tissue construct with a biomimetic design can be achieved. The goal of biomimetic design is to replicate the *in vivo* interactions of regulatory factors in space and time, thus allowing cells to differentiate at the right time, in the right place and into the right phenotype. This requires three main components: cells, a tissue scaffold and cell signalling factors. It is important to note that one does not have to artificially engineer all three components. For example, producing a tissue construct in a suitable local environment *in vivo* will expose it to native signalling factors.

Cells used in tissue engineering

It is critical that the most appropriate cell type for scaffold seeding is selected. The most obvious choice would be the differentiated cell type appropriate to the tissues being replicated by the construct. These

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autogenous primary cells can be harvested by way of biopsy and expanded *in vitro* using standard cell culture techniques[4] before being used to seed the scaffold.

Another potential cell source that has elicited great interest are stem cells. Stem cells are characterised by their ability to self-renew and to differentiate into a variety of other cellular phenotypes.[5] There are two main sources of stem cells: embryonic[6] and adult.[7]

Embryonic stem cells, which are derived from the inner cell mass of blastocysts, are truly pluripotent and can differentiate into cell types derived from all three germ cell layers.[8,9]

Non-embryonic stem cells, which are found in concentrations called niches in adults and children, are mainly thought to be multipotent[10] (i.e. differentiation of cell type is limited according to the organ for which the cells originate), but some have been shown to be pluripotent.[11,12] The main source of non-embryonic stem cells has been from bone marrow, but recent work has revealed their presence in more readily accessible locations such as adipose tissue, dental pulp, circulating blood and amniotic fluid (the latter is considered to be an ethically sound source as extraction does not harm the embryo).[13–17]

The advantage of using stem cells lies in their potential for differentiation into any tissue type. This would be particularly useful in situations where a biopsy of differentiated autogenous cells might not yield sufficient cells, as in end-stage organ failure, or when an organ has been extensively affected by pathology.

Cell signalling factors

The ability of cells to perceive and respond to changes in their microenvironment in a co-ordinated and organised manner is vital to tissue repair *in vivo*. Cell signalling factors are bioactive substances that alter the behaviour of cells. These signalling factors can be used to manipulate the behaviour of cells when delivered appropriately.[18] Examples of signalling factors include vascular endothelial growth factor,[19] transforming growth factor beta,[20] epidermal growth factor,[21] mixed metalloproteinases[22] and bone morphogenetic protein 2.[23]

A key challenge in the field of tissue engineering is the effective delivery of cell signalling factors. This has become a much studied area that has resulted in the development of systems for the delivery of these substances. Examples include gelatine microspheres,[24] chitosan microparticles[25] and polylactic acid nanoparticles.[26] These delivery systems enable the localised control of a given microenvironment.

By understanding how the cells respond to these cell factors, scaffolds can be engineered to accommodate and incorporate these cell factors and thus influence and direct cell growth.

Tissue engineering scaffolds

Tissue scaffolds are three-dimensional structures used in tissue engineering to provide mechanical support, physical protection and conduits for cells and signalling factors. These scaffolds have been found to play an important role in the three-dimensional growth of tissues *in vitro*.[27] Two key considerations when engineering a scaffold are the choice of material and the processing technique used.

A variety of natural materials have been used for the construction of these matrices. These include collagen,[28] fibrin[29] and chitosan.[30] Novel biomaterials such as polylactic acid[31] and polyglycolic acid[32] have also been developed for this purpose. These porous structures can be manufactured using a range of techniques such as the electrospinning of nanofibres.

One key feature of these scaffolds is their porosity. The porous nature of scaffolds allows good cell penetration into the scaffold and results in uniform tissue distribution in the construct.[33] Porosity can be achieved using a variety of techniques, which dictate the ease of processing and final structure of the scaffold. For instance, the electrospinning of polymers results in fibrous meshes with fibre diameters on the scale of nanometres.[34] Gas foaming and solvent casting can produce porous structures with interconnected pores, which improve cell penetration and total surface area for cell adhesion; however, these techniques offer little control over the pore dimensions and location. The variation in processing has a direct effect on the spatial organisation of the matrices, which may conform to a porous sponge structure, a semisolid hydrogel or a finely spun mesh.

The concept of a mesh scaffold can be used to illustrate the way scaffold morphology can affect cell behaviour and growth. Randomly aligned fibres will result in the random attachment and spread of cells, whereas fibres aligned in one direction will prompt the cells to attach and spread in the same direction, which is invaluable when attempting to mimic tissues such as tendons. A mesh scaffold is composed of fibres and voids; manipulation of void volume, fibre diameter and directionality can dictate cell behaviour. The two most promising techniques developed for fibre deposition are three-dimensional fibre deposition and electrospinning. Three-dimensional deposition allows for more closely regulated extrusion to conform to the size of the defect being addressed, enabling the maximisation of contact between the scaffold–cell composite and the margins of the defect.

Hydrogels are networks that have been engorged with water. They are an alternative method for the delivery of cells and signalling factors. A key advantage conferred by hydrogels is that they are semisolid, thus enabling them to be injected into the required site using minimally invasive techniques. They support the transport of nutrients and waste to

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and from the cells. Furthermore, they are capable of supporting limited mechanical loading, thus allowing mechanical stress fields to drive the differentiation of individual cells. This feature replicates physiological conditions that stimulate cellular differentiation. These unique abilities have led to the use of hydrogels as support frameworks, particularly for cartilage engineering.

Role of bioprinting

Tissue engineering is not constrained to a solid scaffold seeded with cells. Manufacturing processes are constantly being adapted and introduced into the field. A key example of this is the use of bioprinters to deposit and pattern cells.

Bioprinting uses a variety of devices to deposit biological materials onto a substrate. The advantage of these devices is the ability to print two-dimensional and three-dimensional tissue constructs for implantation. This could potentially include the manufacture of complete organs.

Two groups of technologies are currently in development for this purpose. One is inkjet printing (Figure 1), which prints individual cells or clusters of cells onto a surface.[35–43] This method has the advantage of being rapid, versatile and inexpensive. However, its primary disadvantage lies in the fact that it is difficult to assure the high cell densities required for the manufacture of solid tissue constructs.

The alternative approach to printing involves the use of mechanical extruders, which expel pre-constructed multicellular particles known as 'bio-ink' onto a supportive substrate for the development of the construct.[44,45] These particles then fuse to form the desired structure. The primary advantage of this technique is that the bio-ink particles represent tissue fragments and thus replicate the microenvironment that occurs *in vivo*. Currently, this method is time consuming and expensive, making it difficult to produce clinically useful tissue constructs in large quantities.

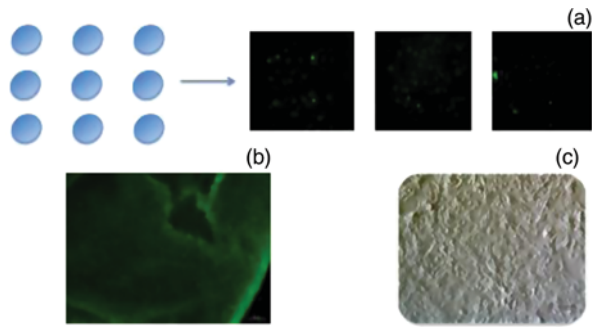

FIG. 1

(a) Fluorescence micrograph of printed oral keratinocytes, showing an array of micro droplets of keratinocytes and cell media printed onto a thermoresponsive surface. (b) Live/dead stain of harvested cell sheet of printed keratinocytes (×10). (c) Light micrograph of cell sheet derived from printed keratinocytes (×10).

Bioreactors

A bioreactor is a device that uses mechanical means to induce biochemical reactions under controlled conditions. The conditions that can be controlled include: pH, temperature, partial pressure of oxygen, nutrient supply and partial pressure of carbon dioxide. In situations where cells are grown in several layers, or where they are seeded onto scaffolds, access to substrate and signalling molecules, growth factors and nutrients (oxygen, glucose, amino acids and proteins), and clearance of the end products of metabolism (carbon dioxide, lactate and urea), are critical to cell survival.[46]

Bioreactors also allow the application of mechanical stimuli to *in vitro* cultures using a range of techniques, including ultrasonic stimulation,[47] which have been shown to influence the tissue phenotypes produced.[48] These devices are employed to accelerate and improve tissue culture growth *in vitro* by optimising conditions. Bioreactors can be classified as either open systems, such as basic culture dishes, or closed systems, which offer a more controlled environment by using ports and filters.

Examples of bioreactors currently in use include the perfusion bioreactor system for the production of autogenous cartilage grafts,[49] a stretching bioreactor for the production of muscle tissue[50] and a rotating wall bioreactor used to simulate a microgravity environment;[51] the latter of which is advantageous in that it reduces the effect of gravity on cellular interactions.

Applications of tissue engineering in ENT

Airway surgery

The principles of traditional airway reconstruction have been recognised and developed since the 1890s.[52–54] The current treatment for tracheal stenosis involves endoscopic treatments such as sequential dilatations, or open procedures such as anterior or posterior cricoid split[55] with or without cartilage grafting.[56] Other treatment options include segmental resection, tracheal mobilisation and end-to-end anastomosis.[57] The advent of tissue engineering has potentially offered an alternative approach.

This was demonstrated recently by Macchiarini and colleagues.[58] Specifically, they obtained a tracheal cartilage 7 cm in length from a deceased transplant donor. This was decellularised and immunohistochemistry was performed to confirm the complete absence of major histocompatibility complex antigen-positive cells. The team then used the decellularised donor trachea as a scaffold, onto which they seeded epithelial cells and mesenchymal stem cell derived chondrocytes which were obtained from the recipient. The seeded scaffold was then incubated in a novel bioreactor, which rotated it at regular intervals, allowing it to be bathed in culture media for a total of 96 hours. Following surgical implantation, the recipient was found to have a functional airway with no anti-donor

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antibodies. More recently, this same team have reported producing and implanting a neotrachea *de novo* using a nanocomposite material seeded with mesenchymal stem cells.

A different, scaffold free approach was adopted by Wiedenbacher *et al.*,[59] who created a neotrachea in an animal model. In this experiment, the auricular cartilage of rabbits was used as a source of chondrocytes, and these were grown to produce confluent cell sheets. A skin graft from the rabbit was then wrapped around a silicone tube, which in turn was wrapped in the engineered cartilage sheets. The entire construct was then implanted in the anterior abdominal wall of the rabbits, using the muscle belly of the external oblique as a source of vascularisation. After 10 weeks, the neotrachea was harvested and found to be comparable to the native trachea of the rabbit. In this example, the animal itself was used as a bioreactor.

Plastic and reconstructive surgery

Reconstructive surgery of the nose and ear often require autologous cartilage.[60] This cartilage is harvested from the nasal septum, auricle and ribs.[61,62] However, there are significant risks associated with donor site morbidity.[63] Significant research efforts have been dedicated to cartilage tissue engineering.

The globally publicised and pioneering work by Cao *et al.*[64] (Figure 2) resulted in the production of neocartilage using a polyglycolic acid scaffold, which was seeded with chondrocytes and implanted into athymic mice.

More recently, Yanaga *et al.*[65] developed a two-stage implantation technique for the reconstruction of congenital microtia. In this technique, they harvested auricular cartilage chondrocytes, expanded their number *in vitro* and allowed the cultured chondrocytes to form an extracellular matrix of immature cartilage. This was used as the scaffold to which fibroblast growth factor was added. The matrix was then implanted by injection into a subcutaneous pocket on the fascia of the lower anterior abdominal wall. The implant was then allowed to mature for six months, producing a construct of mature cartilage, before being removed. The cartilage was then sculpted to the required shape and implanted into the temporal area to reconstruct the missing ear. This procedure was performed on a total of four patients, with a follow up ranging from two to five years. None of the reconstructed ears demonstrated cartilage re-absorption, and all of them supported subsequent split-thickness skin grafts after ear elevation.

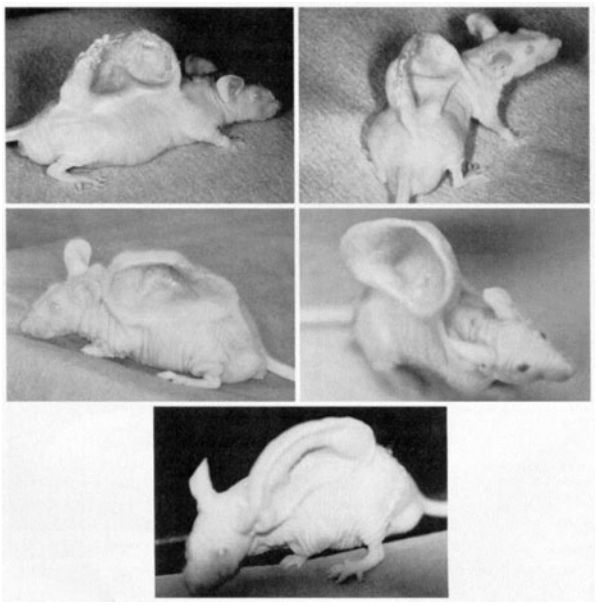

FIG. 2

Photographs of an athymic mouse implanted with a polyglycolic acid scaffold seeded with chondrocytes. Reproduced with permission.[64]

A further tissue construct has been produced by a team in Shanghai,[66] who developed a polylactic acid and polyglycolic acid scaffold moulded by computer-aided design which was based on a computed tomography scan of an actual human ear. This scaffold was then seeded with mature porcine chondrocytes and cultured for 12 weeks. This resulted in the formation of an ear shaped construct, which demonstrated a tissue structure with abundant cartilage extracellular matrices. The final construct also demonstrated excellent elasticity and good mechanical strength.

Nasal reconstructive surgery and augmentation rhinoplasty utilise a wide range of materials. These include synthetic substances such as silicone,[67] polytetrafluoroethylene[68] and high density porous polyethylene (Medpor). Although widely used, these artificial materials have been shown to have an increased risk of graft extrusion and infection compared with autogenous cartilage. The most frequently used autogenous cartilage materials have been autologous costal cartilage,[69] auricular cartilage[70] and nasal septal cartilage.[71] In addition to donor-site morbidity, there is the additional drawback of limited availability of materials, particularly in revision surgery.

An adaptation of the above-mentioned method reported by Yanaga *et al.*, using cultured autologous chondrocytes from the auricular cartilage, has been used to culture a matrix of immature cartilage, which was subsequently injected into a subcutaneous pocket on the nasal dorsum of the patient.[72] This construct was harvested after three weeks and aesthetically carved, before being used for surgical augmentation rhinoplasty. The authors of this paper have reported their 6-year experience with 75 cases, which showed promising results.

Dobratz *et al.*[73] recently reported an interesting, alternative method of producing shaped autologous tissue constructs. In that experiment, human nasal chondrocytes were harvested and suspended in an alginate hydrogel scaffold. The gel was then injection-moulded into a circular shape and implanted subcutaneously into nude mice (which have inhibited

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immune systems). When harvested after 38 weeks, the chondrocytes had retained their overall size and shape and showed a hydroxyproline content, which did not differ significantly from the control of native septal cartilage.

Head and neck surgery

Salivary glands

Xerostomia has a significant impact on an individual's quality of life, predisposing them to dental infections, dysphagia and oral mucosal infections. The loss of salivary gland function can result from surgical resection, radiotherapy[74] and autoimmune diseases,[75] or it may be a side effect of pharmacological treatment. Current treatment strategies rely on symptomatic relief using supplements.

Salivary glands are exocrine glands and as such present a unique set of challenges to tissue engineers. It is the ability of these glands to secrete fluid, modify its content and propel it in a unidirectional manner that increases the level of complexity beyond that of producing constructs analogous to other tissues.

Of the cell sources investigated so far, human ductal epithelial salivary gland cells were initially thought to be the most promising. They were found to demonstrate growth in monolayers on a poly-L-lactic acid scaffold[76] and to be able to generate an osmotic gradient necessary for the production of saliva.[77] However, this cell line is unable to produce unidirectional fluid flow.[78] More recently, subsets of autologous stem cell populations have been utilised, which have revealed promising findings.[79]

Approaches that involve the use of scaffolds are centred on the construction of a permeable, bio-absorbable material in a blind-ended tube-like conformation with branching side ducts.[80] The materials investigated for these scaffolds have included polyglycolic acid coated with poly-L-lactic acid,[77] chitosan[81] and collagen with Matrigel.[82]

In a recent promising study, Joraku *et al.*[82] used primary human salivary gland cells obtained from the parotid and submandibular glands and seeded them onto a polyglycolic acid scaffold. This construct was then implanted subcutaneously into athymic mice; the retrieved samples were shown to contain human alpha-amylase.

Mandibular reconstruction

Mandibular defects may arise from trauma, osteoradionecrosis, or benign or malignant disease. The reconstruction of mandibular defects presents a challenging problem for head and neck surgeons. Current treatment strategies have focused on free flaps with microvascular re-anastomosis. Sources of these flaps include the fibula and the radius.[83]

In addition to the usual features of a tissue construct, the ideal engineered mandibular tissue construct should be capable of surviving in an environment with a compromised vascular bed such as those found in sites exposed to adjuvant radiotherapy or previous infection.

An alternative to the *in vitro* culture of mandibular defect constructs has been to use cell-signalling factors to stimulate new bone growth *de novo*. This technique has been successfully demonstrated by recent research using bone morphogenetic protein 2.[84]

Another study used stem cells in an animal model to show the growth of tooth-like structures on tooth bud stem cell seeded scaffolds,[85] which revealed promising prospects for the development of functionally specialised tissue composites such as that required of engineered mandibular tissue constructs.

Conclusion

Considerable progress has been made in the field of tissue engineering over the past few years, most notably in the use and application of stem cells and the design and delivery of cell signalling factors. The advent of bioprinting techniques has opened up the possibility of microscale, patterned deposition of cells onto scaffolds and intelligent culture surfaces.

However, significant challenges in the development of viable autogenous cartilage construction remain. One of the main hurdles is the issue of construct vascularisation, although there have been promising results in the search for a solution, most notably with the development of nanoscale scaffolds.

Future directions of tissue engineering are likely to involve further investigation into the fate of stem cells, which entails a better understanding of the signalling cues that govern their differentiation.

Given the current rate of development in tissue engineering research, it seems likely that tissue-engineered implants will be used widely in surgical practice, including ENT and head and neck surgery.

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