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內文：

Introduction

- Salivary gland impairment resulting in xerostomia can occur as
  1. **irradiation therapy to the headand neck cancer patients,**
  2. **Sjogren's syndrome (SS)**
  3. **other medical conditions mainly the usage of xerogenic medications**

  (Atkinson and Fox, 1992; Fox, 1998; Shipet al, 2002).
- Xerostomia is an important clinical concern in oral health and is known to induce various problems including
  1. **dental caries,**
  2. **periodontitis,**
  3. **denture problems,**
  4. **mastication and swallowing problems,**
  5. **burning sensations,**
  6. **dysgeusia (Atkinson et al, 2005).**
- Muscarinic agonist medications such as **pilocarpine** and **cevimeline** induced salivary secretion from the residual functional tissue (Fox, 2004). However, they
  1. **only provided temporary relief of symptoms**
  2. **had a limited effect on the recovery of damaged tissue.**

Recently, concepts of regenerative medicine and tissue engineering have drawn much attention (Langer and Vacanti, 1993; Baum et al, 1999a; Alsberg et al, 2001;Kaigler and Mooney, 2001; Bu‥cheler and Haisch, 2003).

- In humans, the potential for regeneration is limited except for organs such as the **liver**, which can regenerate from 10% of the residual tissue (reviewed by Chamuleau and Bosman, 1988; Taub, 1996, 2004; Fausto, 2000).
- Clinically, these concepts have been reported as successful in regenerating
  1. **skin** (Hefton et al, 1983;Gallico et al, 1984),
  2. **corneal epithelium** (Germain et al,1999),
  3. **cartilage** (Mow et al, 1991; Vacanti and Vacanti,1994)
  4. **bone** (Syftestad et al, 1985; Caplan, 1987;Reddi and Cunningham, 1991; Crane et al, 1995).
- Although the regeneration of more complex organs is still underway, successful regeneration of the human **bladder** has been reported recently (Atala et al, 2006).
- Currently, considerable efforts have been made for the regeneration of
  1. **pancreas (beta cells),**
  2. **liver**

3. **kidney**
4. **heart**
5. **tooth**
6. **central nervous system (CNS).**

specific stem cells and **induction to a favorable phenotype** are the major goals of most of these studies.

- **In this review, the possibility of restoring salivary gland function was discussed in relation to novel approaches including tissue engineering and gene therapies.**
- The concept of gene therapy has proved useful for various diseases such as **severe combined immunodeficiency (SCID)** ,but some potential side effects have recently been reported such as the development of leukemia in those patients with SCID who have been **treated using retroviral-mediated gene transfer** (Hacein-Bey-Abina et al, 2003).

*Salivary gland regeneration*唾液腺再生

- The three fundamental components in regenerative medicine include
  1. graft cell
  2. growth factors,
  3. scaffold (支架)(Cima et al, 1991; Reddi and Cunningham,1991).
- Regeneration is a physiological function of living organisms, which enables the repair of lost or damaged tissue. Regenerative capacity differs among species and organs.

  For example, a newt (蠑螈.巴西火龍.中國火龍)is known for its surprising ability to regenerate a complete eye or leg after resection. In contrast, humans have a much more limited ability for regeneration. The liver is known to have an amazing ability for regeneration, enabling the organ to regain its normal size after a 90% hepatectomy (reviewed by Chamuleau and Bosman, 1988; Taub, 1996, 2004; Fausto, 2000).
- The concept of regenerative medicine based on the body's naturally existing capacity for regeneration can be deliberately enhanced by
  1. the manipulation of cells and growth factors
  2. providing growing space using scaffolds.

  This concept has been proven feasible in the tissue engineering of skin (Hefton et al, 1983; Gallico et al, 1984).
- Early clinical trials for tissue regeneration have also been reported in the field of dentistry.
  1. The regeneration of periodontal tissue using a **barrier membrane** (Nyman et al, 1982a,b; Gottlow et al, 1984)
  2. **enamel matrix-derived protein (Emdogain®)** has been successfully applied clinically (Hammarstrom, 1997; Hammarstrom et al, 1997; Heijl, 1997).
- Stem cells can be found in the bone marrow, fat and possibly in most of the tissues in the human body. Mesenchymal stem cells, one of the most well-characterized somatic stem cells, are usually obtained by bone marrow aspiration (reviewed by Burry and Murphy, 2004; Gregory et al, 2005; Risbud and Shapiro, 2005).
- In organs such as the liver and the pancreas, the presence of tissue-specific stem cells/precursors has been suggested (reviewed by Matthews and Yeoh, 2005;

Otonkoski et al, 2005; Soria et al, 2005; Theise, 2006).For example, the pancreatic and hepatic cell types have shown remarkable plasticity（可塑性）, which can de- and transdifferentiate into each other under appropriate conditions(Otonkoski et al, 2005).

- Salivary gland stem cell and possibility of cell transplantation To date, Research into the development of the salivary gland has revealed that **cells in the duct close to the acini** are believed to provide all the cell types required for the formation of acini and ducts (reviewed by Redman, 1987; Cutler, 1989).Accordingly, the stem cell population of salivary glands is considered to be present in the **intercalated duct** (Man et al, 1995, 2001).
- Presumably, such regeneration processes are not antagonistic(不相容的) to the presence of stem cell populations in the intercalated ducts. The stem cell/ precursor cell population may usually provide cells during the normal cell renewal process. When severe damage to the gland occurs, the differentiated cells may de-differentiate and proliferate to induce a rapid recovery of the gland.
- Does a salivary gland-specific stem cell actually exist? If so, what is the nature of this kind of stem cell?
- Most of the established cell transplantation (a.k.a. cell therapy) requires
  1. a donor able to provide a sufficient number of cells.
  2. A dearth of such donors and the possible immunoreaction against the allogeneic cells.

Markers	*Phenotype*	*Species*	*Reported by*
ND	Duct/acinar	Rat	Horie *et al* (1996), Sugito *et al* (2004)
ND	Duct/acinar	Human	Bücheler *et al* (2002)
Sca-1$^+$/C-kit$^+$	Liver/pancreas	Mouse	Okumura *et al* (2003)
ND	Duct	Human	Tran *et al* (2005)
ND	Acinar	Mini-pig	Sun *et al* (2006)
ND	Duct/acinar/myoepithelial	Rat	Kishi *et al* (2006)

ND, not determined.

If the cultured cells from autologous tissue can be used for cell therapy, these shortcomings could be overcome.

- For cell transplantation to be feasible, the transplanted cells would have to
  1. attach and survive in the transplanted damaged/atrophic region.
  2. be integrated into the native structure and be able to differentiate into a salivary gland cell lineage.
- Our group has investigated the fate of the transplanted cultured salivary epithelial cells in the regenerating submandibular gland in rats (Sugito et al, 2004).

  Fluorescent-labeled salivary epithelial cells were injected into normal and atrophic rat submandibular glands. Our results showed that the transplanted cells **could attach and remain in the regenerating gland for at least 4 weeks**.
  However, such cells were not observed when they were transplanted to normal glands, suggesting that both cell attachment and survival are significantly

affected by the environment of the host organ.

- More recently, the potential of mesenchymal stem cells to regenerate salivary glands was reported using a radiation-damage model (Lombaert et al, 2006).
- The incorporation of stem cells into the atrophic or damaged tissue will open up the possibility of an alternative treatment in the future.
  1. more potent stem cells

     【bone marrow-derived stem cells】
  2. tissue-specific stem cells

     【salivary gland stem/progenitor cells】
- In both human- and rat-cultured submandibular gland epithelial cells, **basic fibroblast growth factor** accelerated cell proliferation (Hiramatsu et al, 2000).Ohlsson et al (1997) reported the effect of systemic administration of epidermal growth factor (EGF) on the pancreas and salivary glands. It was concluded hat EGF increased the labeling index of serous and ductal cells in the parotid gland.
- For example, **hepatocyte growth factor** is a well-known protein which promotes the regeneration of liver and even protects tissue from damage (Nakamura et al, 1986, 1989; Kinoshita et al, 1991; Ishiki et al, 1992).
- It would be significant to determine the specific factors for salivary gland regeneration. We have examined the gene-expression profile in a regenerating submandibular gland after ductal ligation and removal. Total RNA was extracted from the gland, and the gene-expression profile was compared with 12 h to 6 days and also 36 h to 6 days. Gene-expression profiles were independently analyzed using DNA microarray and fluorescent differential display (FDD) technique. From a preliminary analysis using FDD, **16 clones have been identified** (Sugito et al 2004). Using the microarray analysis, **genes related to inflammation, regeneration, and adhesion molecules were mainly detected** (Sugito et al 2004). More precise study of the roles of those genes during regeneration may lead to improving our understanding of their possible mechanisms.

Tissue engineering of salivary gland

- If the gland damage is severe and the residual tissue can no longer be restored, an alternative approach is required. purpose is tissue engineering, which utilizes cells, biodegradable scaffolds, and signals to regenerate tissues.

CELLS

- In this review, we focused on this topic aside from salivary gland regeneration, as the ultimate goal of studies in this field is to generate neo-salivary glands. Possible cell sources can be divided into **(1) progenitor/stem cells from salivary glands and (2) pluripotent stem cells from other tissues (such as bone marrow or even embryonic stem cells).** Although the embryonic stem cell has a significant potential to generate various tissues, it is difficult at present to apply it to salivary gland tissue engineering out of ethical and safety concerns.
- In early studies of artificial salivary glands, a **human salivary cell line, known as HSG**, was used (Wang et al, 1999; Aframian et al, 2000). **HSG cells** were useful in evaluating the characteristics of the biomaterials used as a scaffold for an artificial salivary gland. As HSG cells lack tight junctions essential for the formation of polarized epithelial monolayers and unidirectional liquid-salt secretion, the application value of this cell line is limited (Aframian et al, 2002).

CULTURE

- A pioneer work on culturing salivary gland epithelial cells was reported by Brown (1974).
- Since then, several culture procedures have been published, initially by use of feeder cells (Horie et al, 1996; Aframian et al, 2004), and **more recently using a serum-free medium for epithelial cells** (Joraku et al, 2005; Tran et al, 2005).
- **Scaffold materials** for salivary gland tissue engineering Another important factor in salivary gland tissue engineering is the usage of appropriate scaffold material. So far, a simple combination of cultured salivary gland epithelial cells and biodegradable materials has been used (Wang et al, 1999; Aframian et al, 2000, 2002; Bu·· cheler et al, 2002; Chen et al, 2005; Joraku et al, 2005; Sun et al, 2006).
- The materials consisted of
  1. a denuded rat tracheal preparation (Wang et al, 1999),
  2. poly-L-lactic acid (PLLA),
  3. polyglycolic acid (PGA) and
  4. PGA/PLLA (Aframian et al, 2000; Joraku et al, 2005),
  5. chitosan (Chen et al, 2005) and
  6. poly (ethylene glycol)- terephthalate (PEFT)/poly (butylene terephthalate (PBT) (Sun et al, 2006).
- Importantly, most of the polymers must be precoated with matrix proteins such as **fibronectin** and **collagen I** (Aframian et al, 2000; Chen et al, 2005).
- The results of our recent study using miniature pig parotid gland-derived cells showed that the cells adhere and grow on biocompatible materials, maintaining an acinar cell phenotype and showing a-amylase activity (Sun et al, 2006).
- Recently, a simple tissue engineering approach using isolated cells and scaffold has been proved feasible to generate more complex structures such as tooth germ (Young et al, 2002).
- **previous studies have shown similarities in epithelial–mesenchymal interaction between salivary gland primordium & tooth germ.**
  Although the potential of epithelial–mesenchymal interaction using adult salivary gland-derived cells has not yet been reported, the discovery of potent stem cells could be sufficient to generate a neo-salivary gland.

Gene therapy and therapeutics in salivary glands

- Salivary glands are connected to the oral cavity via ducts. This anatomic structure enables easy access to the gland per-orally (O'Connell et al, 1995, 1996; Kagami et al, 1996). Conventional cannulation techniques can be applied to introduce viral or non-viral vectors into the gland. Furthermore, cannulation-mediated gene transfer to the gland is beneficial in limiting the extension of the vectors systemically compared with that using drip infusion to a vein (Kagami et al, 1996; Delporte et al, 1998).
- An adenovirus-mediated water channel (aquaporin-1, AQP1) gene transfer into irradiated submandibular glands showed **increased saliva flow** in a rat model (Delporte et al, 1997).
- A study evaluated the efficacy of a single administration of AdhAQP1 to the parotid glands of adult rhesus monkeys. In this study, a single parotid gland of rhesus monkeys was irradiated with a single dose of 10 Gy and AdhAQP1 was administered intraductally at 19 weeks postirradiation and salivary secretion examined 3, 7, and 14 days later.
  The results, however, were inconsistent, and only two of the four

AdhAQP1-treated monkeys displayed **increased salivary flow rates** compared with the animal administered an irrelevant virus (O'Connell et al, 1999).

- Rats and mice are the most frequently used animal models in the studies of salivary gland gene transfer. Recently, the miniature pig has been increasingly used as a large animal model in a variety of biomedical studies (Hainsworth et al, 2002; Screaton et al, 2003). The parotid glands of miniature pigs are almost identical to those of humans in terms of their volume and morphology (Wang et al, 1998).
- **Luciferase and b-galactosidase genes** were administered to miniature pig parotid glands by a recombinant adenoviral vector. Luciferase assays indicated that gene transfer to miniature pig salivary glands could be readily accomplished using **rAd5 vectors**. The results from **X-Gal staining** have shown that the b-galactosidase expression was observed in both acinar and ductal cells. Thus, the results of salivary gland gene transfer from rodent studies can be extended to a larger animal model, and support the value of using miniature pigs for preclinical applications of gene transfer to these tissues (Li et al, 2004).
- The effects of a solitary mega-dose protocol of ionizing radiation (IR) on the structure and function of miniature pig parotid glands was evaluated by our group. Our results showed that the structural changes induced by single, regional mega-doses of IR were generally identical to those induced by the fractionated radiation dose protocol, and similar to those found in humans. At the 16-week time point, the salivary flow rates had decreased approximately 60% in the 15-Gy group and by around 80% in the 20-Gy group. These findings indicated that **the parotid glands of miniature pigs locally irradiated with a single dose 20 Gy may be useful as a large animal model for the studies of gene transfer into irradiationdamaged salivary gland** (Li et al, 2005).
- A study was performed to evaluate whether AdhAQP1 would be effective in improving the salivary secretion of irradiated miniature pig salivary glands, which are _100-fold larger than those of rats. Subsequent administration of the AdhAQP1 vector resulted in a dose-dependent increase in parotid salivary flow (Shan et al, 2005). Three days following administration of the highest dose used **herein, 2.5 · 105 pfu AdhAQP1/ll infusate (109 pfu total/gland), a marked increase in parotid salivary secretion was observed, reaching on average _80% of pre-IR levels.**
- Conversely, administration of the same dose of control Ad vector encoding **luciferase** showed **no** significant effect on salivary flow.

  The effective dose of AdhAQP1 was comparable to that confirmed in the reporter transgene expression analysis in both murine and miniature pig salivary glands. Importantly, this effective dose in miniature pig was only 20% of that required to be effective in irradiated rats (Shan et al, 2005) (Table 2). **Localized delivery of AdhAQP1 to IR-damaged salivary glands is useful in transiently increasing salivary secretion in both small and large animal models with no significant risk of general adverse effects.**
- Based on these results, Baum et al (2005) have developed a clinical trial to determine whether the hAQP1 cDNA transfer strategy will be clinically effective in increasing salivary flow in patients with IR-induced parotid hypofunction.
- Gene therapy for Sjo··gren's syndrome impaired salivary gland function

  At present, although the exact pathogenesis of SS is unclear, several possible immunologic mechanisms have been proposed which might play roles in the tissue destruction of salivary glands (Delaleu et al, 2004; Hjelmervik et al, 2005).

Potential target genes in gene therapy for SS-damaged hyposalivation include

1. inflammatory mediators,
2. cytokine inhibitors,
3. apoptotic molecules,
4. cell–cell interaction, or intracellular molecules.

- Interleukin 10 (IL-10) is a homodimeric protein with a wide spectrum of immune activities. One study showed that vector-encoded hIL-10 was biologically active in vivo by challenging rAAVhIL10-treated IL-10 knockout mice with lipopolysaccharide to induce endotoxic shock 8 weeks after systemic delivery (Yamano et al, 2002).
- A recombinant AAVhIL10 vector was administered to the salivary glands of **non-obese diabetic (NOD)** mice and its effects on the stimulated salivary flow rate were measured (Kok et al, 2003).
- The animals receiving the rAAVhIL10 showed markedly higher salivary flow rates than those observed in the sham group of animals. In addition to the effects on salivary function, rAAVhIL-10 administration led to marked improvements in histologically assessed inflammatory changes in the submandibular glands.
- Vasoactive intestinal peptide (VIP), initially discovered as a gastrointestinal hormone, exhibits abundant functions, ranging from neurotransmitter, vasodilator, and bronchodilator effects to acting as a trophic agent, secretagogue, and immunomodulator (Said, 1986; Delgado et al, 2002; Voice et al, 2002; Gozes and Furman, 2003). A recombinant serotype 2 adeno-associated virus encoding the human VIP transgene (rAAV2hVIP) was administered into the submandibular gland of a female NOD mice to examine its ability to alter the progressive SS-like dysfunction in NOD mice. While it led to higher salivary flow rates, there were no differences in focus scores or apoptotic rates. In the experimental group, increased expression of VIP in submandibular gland and serum, and a reduction in cytokines IL2, IL10, IL12 (p70), and tumor necrosis factor-a in submandibular gland extracts were observed compared with the control vector results. The results indicated that local delivery of rAAV2hVIP can have disease-modifying and immunosuppressive effects in submandibular gland of the NOD mouse model of SS (Lodde et al, 2006).
- Furthermore, a key study reported that the treatment of acute and chronic sialadenitis in B6-gld/gld mice with local fasL gene transfer resulted in a significant reduction in the number of inflammatory foci and in the level of tissue destruction in salivary glands (Fleck et al, 2001).
- Gene transfer to salivary glands Many reports hypothesize that a gene transfer to salivary glands can lead to stable long-term secretion of a therapeutic protein into the bloodstream or the saliva for therapeutic purposes.
- Investigations clearly demonstrated the potential of salivary glands as a systemic gene therapeutic target. It was shown that rat salivary glands, after being administered the rAd5 vector encoding human a-1-antitrypsin (ha1-AT), were able to secrete the transgene protein into the bloodstream (Kagami et al, 1996).
- This potential was extended in subsequent studies using another rAd5 vector encoding human growth hormone (hGH), also administered to rat salivary glands. These results provided the first demonstration of systemic biologic activity from an endocrine transgene product secreted into the bloodstream from salivary glands (He et al, 1998).
- Following rAAV2 vector encoding human erythropoietin (hEPO) gene transfer to mouse salivary glands, the concentration of hEPO in serum was stable

throughout the experiment from 10 to 54 weeks. Furthermore, the transgeneencoded hEPO was functional, because the hematocrit levels in all infected animals followed a similar pattern and remained elevated throughout the experiment (Voutetakis et al, 2004).

- Most recently, an adenoviral serotype 5 (Ad5) vector encoding hEPO cDNA or an adeno-associated virus serotype 2 (AAV2) vector encoding either the hEPO or hGH cDNA was administered to individual submandibular salivary glands of Balb/c mice (Voutetakis et al, 2005). AAV2 vectors led to a stable gene transfer, unlike the results with the Ad5 vectors. Indeed, hEPO production in one mouse was observed for a period of 2 years after administration of AAVhEPO to the salivary glands. hEPO, which is a constitutive pathway secretory protein, was readily secreted into the bloodstream from the salivary glands, yielding therapeutically adequate serum levels.
- Conversely, hGH, a regulated secretory pathway protein, was preferentially secreted into saliva. Salivary glands may be an attractive candidate target tissue for gene therapeutics of some monogenetic endocrine deficiency disorders. At present, AAV2 vectors seem particularly useful for such applications, and transgenes encoding constitutive secretory pathway hormones are more suitable for this application with salivary glands than those encoding regulated secretory pathway hormones (Baum et al, 1999a; Voutetakis et al, 2005).
- These studies demonstrated that gene delivery to salivary glands might not be limited to the treatment of salivary gland disorders, but may also be an attractive approach to cure certain cases of major systemic diseases such as hemophilia and diabetes.
- Salivary glands normally produce and secrete into the saliva a variety of beneficial proteins that play important roles in maintaining the oral cavity and upper gastrointestinal tract tissue homeostasis and integrity.
- Investigations have demonstrated that transgenic proteins can be effectively secreted into saliva for therapeutic purposes. The cDNA for histatin 3, an anti-candidal peptide normally found in the saliva of Old World primates and humans, was expressed in rat salivary glands using a rAd5 vector (O'Connell et al, 1996). The transgenic histatin 3 produced in rat saliva was highly effective for killing azole-resistant Candida albicans.
- Moreover, many other naturally occurring antimicrobial peptides such as defensins and magainins have been identified and those peptides might be clinically useful against resistant microorganisms. The therapeutic potential of antimicrobial peptides appears to be in their effectiveness as target genes for gene therapeutics in salivary glands (O'Connell et al, 1996). Another valuable potential application of local salivary gland gene therapeutics is to deliver growth factors or cytokines, such as EGF, keratinocyte growth factor, and IL-11, to promote mucosal wound healing (Palomino et al, 2000; Sonis et al, 2000; Dorr et al, 2001; Baum et al, 2004). In clinical or preclinical protein therapeutic studies, the above mentioned substances have shown considerable potential.
- Transient local expression of these genes after salivary gland gene transfer might be more effective and less expensive in promoting mucosal wound healing in patients with delayed wound healing such as diabetics.

Conclusion and future prospects

- The replacement of damaged or lost tissue is a challenge, especially if the replacement can be achieved using autologous graft cells, requiring no special considerations such as mechanical degradation or immunologic reaction.

- Regenerative medicine and tissue engineering may thus provide new treatment modalities for atrophic salivary gland. However, such efforts are still in a very early stage, and a more basic understanding of salivary gland tissue regeneration and stem cells is required. Furthermore, understanding of the detailed mechanisms of salivary gland development is critical for the exploitation of salivary gland regeneration therapy.
- Initial clinical trials (i.e., a phase I/II, dose escalation studies) using **adenoviral vector encoding hAQP1 gene** in patients with IR-induced parotid gland hypofunction can test the safety and efficacy of this strategy. Should this strategy prove useful, a longlived vector with a persistent expression of hAQP1, e.g., a serotype 2 or 5 adeno-associated viral vector, may be used in the future for long-term correction of a salivary gland hypofunction induced by irradiation. Moreover, this strategy may be easily expanded to the treatment of SS and for both systemic and local (upper gastrointestinal tract) gene therapeutics.

題號	**題目**
1	下列何者情況不會造成口乾症 (A) 薛格倫氏症候群 (B) 服用methydopa藥物 (C) 腹式呼吸法 (D) 糖尿病
答案(C)	出處：Oral and Maxillofacial Pathology, Neville 2nd. P.399-340
題號	**題目**
2	
	(A) **(B)** **(C)** **(D)**
答案( )	**出處：**