D. 考察

鼓室形成術は、病変除去と伝音再建を目的にしたものであり、鼓室、乳突腔に病変が存在する重症例も対象となるが、いずれも、耳後部の皮膚切開と外耳道の剥離、自家組織の採取・移植は必須である。鼓膜穿孔が大きくなると、鼓膜の代用としての自家組織（側頭筋膜）移植も手術手技として難易度が上がる。すなわち、手術を施行しても確実に聴力が改善するとは限らない。術後の鼓膜再穿孔や耳周囲の違和感、耳鳴りなど多くの後遺症の可能性がある。さらに術後鼓膜が浅在化あるいは肥厚するなどして、本来の鼓膜とは程遠いものになり、聴力も低下する。加えてこれらの手術では、通常 1 週～数週間程度の入院加療を伴い、患者の精神的・肉体的・経済的負担も大きくなるなどの難点がある。

一方、われわれが開発した鼓膜再生療法によって再生された鼓膜は、組織学的にも正常なものが再生されることが確認されており、聴力も気骨導差がほとんどない理想的聴力回復が期待できる。しかしその反面、その適応症例が限られており、鼓室・乳突洞に病変のある症例や、鼓室・残存鼓膜が濡れた（軽微な耳漏）症例は対象外である。この両者の欠点を補い、利点を最大限に引き出すために考案されたのが、ハイブリッド鼓室形成術である。ハイブリッド手術の特徴を表２まとめた。

ハイブリッド手術の結果から、鼓膜再生の成功率は 90%で、このうち手術のみで再生したものが 3 例（30%）その後に鼓膜再生の追加処置を必要としたものが６例で、残り１例ついては 4 回までの処置で完全な再生はできなかった。この１例については術前の高分解能 CT 上、乳突蜂巣の発育が比較的よく、軟部組織陰影等はなく、軽度の粘液性耳漏を認めるのみであったので、乳突腔にコントロールホールを開け、洗浄液（イソジン希釈生食）による洗浄のみを行った。術後に持続的な耳漏を認めているので、これらの処置だけでは不十分であった可能性が考えられる。

E. 結論

１．慢性中耳炎に対して、従来の鼓室形成術と鼓膜再生とを組み合わせた新しい手術療法であるハイブリッド鼓室形成術を開発した。

２．ハイブリッド手術は、従来の鼓室形成術と比較して、その適応範囲は限られるが、聴力の改善に優れ、患者の精神的・肉体的・経済的負担が少ない新しい治療法である。

F. 健康危険情報

特記すべきことなし

G. 研究発表

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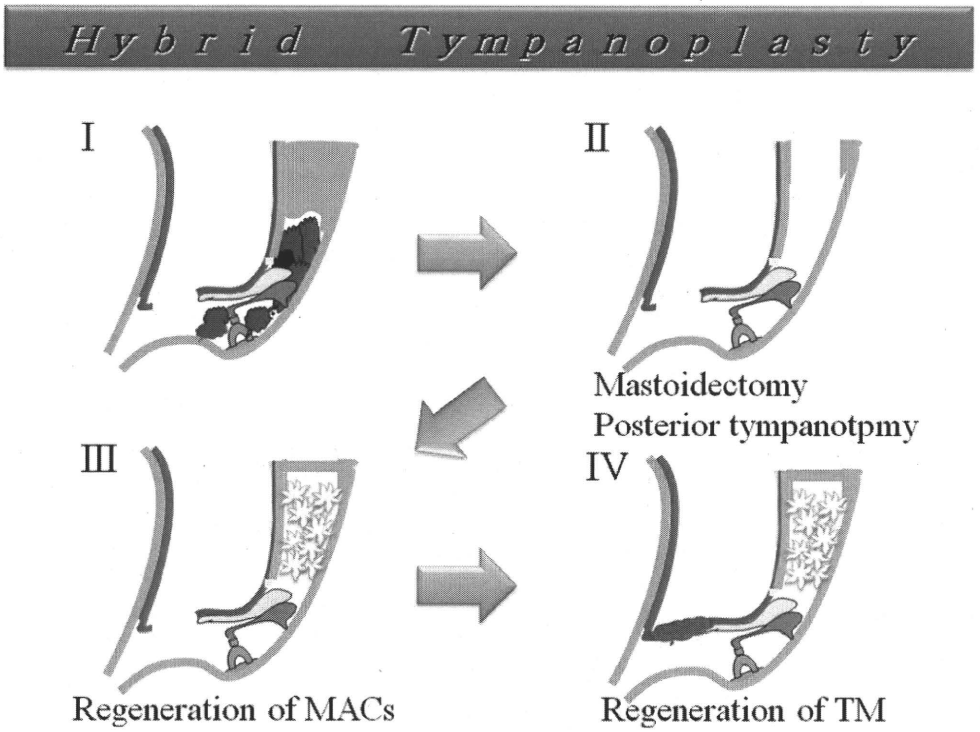
表 1

ハブリッド鼓室形成術の結果

症例	年齢	性別	PT/RMAC の有無	聴力改善 (3 分法)	鼓膜再生に要した回数	後遺症の有無
1.	30	M	+/+	20.0dB	1	鼓膜軽度陥凹
2.	62	F	+/+	21.7dB	2	-
3.	45	F	-/-	20.0dB	1	-
4.	78	F	-/+	11.7dB	2	一時的耳漏
5.	39	M	-/-	5.0dB	>4	持続的耳漏
6.	66	F	+/+	16.7dB	4	一時的耳漏
7.	71	F	-/+	15.0dB	1	-
8.	44	M	-/-	13.3dB	2	-
9.	16	F	-/-	10.0dB	3	-
10.	69	F	-/+	13.3dB	4	一時的耳漏

PT：後鼓室開放、RMAC：乳突蜂巣再生、>4：4 回までの鼓膜再生療法で再生なし

図1.



I. 鼓膜穿孔と鼓室・乳突洞に病変を有し、耳小骨連鎖に以上のない慢性中耳炎
II. 乳突削開、後鼓室開放術施行し、中耳腔の病変除去と清掃を図る
III. 乳突蜂巣再生施行
IV. 鼓膜再生施行
重症度や乳突蜂巣の発育の程度に応じて、後鼓室開放術、乳突削開、乳突蜂巣再生などを行う。

表2

ハブリッド鼓室形成術の特徴

長所
自然な鼓膜が再生され、最大限の聴力改善が得られる
鼓膜のための組織採取をしないので術後の後遺症や違和感が少ない
鼓索神経（味覚神経）損傷のリスクが低い
日常生活での制限がほとんどない
日帰り/短期入院（1泊あるいは2泊3日）ですむ

短所
鼓膜再生が不十分な場合がある
術野が狭い
耳小骨連鎖が保たれていることが必須
適応が慢性化膿性中耳炎に限られ、真珠腫性中耳炎、癒着性中耳炎などは適応外

研究成果の刊行に関する一覧表

原著論文

発表者氏名	論文タイトル名	発表誌名	巻号	頁	出版年
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Kanemaru S, Hiraumi H, Omori K, Haruo T, Ito J.	An early mastoid cavity epithelialization technique using a postauricular pedicle periosteal flap for canal wall down tympanomastoidectomy.	Acta Oto-Laryngologica	130	20-23	2010
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研究成果の刊行物

21回日本末梢神経学会学術集会　シンポジウム2「末梢神経の再生」3

人工神経の基礎と臨床*

中村　達雄[1)], 萩原　明於[1),2)], 稲田　有史[1),3)], 金丸　眞一[4)]

Key Words: artificial nerve（人工神経）, nerve conduit（神経チューブ）, regeneration（再生）, collagen（コラーゲン）, in situ Tissue Engineering（生体内再生）

Peripheral Nerve 2010; 21(2): 192–196

はじめに

19世紀に神経解剖学の基礎を確立したカハール（Santiago Ramón Y Cajal）がいみじくも指摘したように、中枢神経はほとんど再生しない。これとは対照的に末梢神経は旺盛な再生能がある。両者の再生能の違いはどこから来るのだろう。全く著者の想像ではあるが、中枢神経は記憶をはじめとする高度な機能をもった臓器であり、頑丈な頭蓋骨や脊椎で守られている。つまり傷つかないように厳重に守られているかわりに再生しないのではないか。というのも、脳が記憶機能に関与しているために、損傷を受けた後むやみに再生するとこの記憶が混乱してしまうからではないか。胸郭に守られている心臓や肺に再生能がなく、骨性胸郭に守られていない肝臓に旺盛な再生能があるのもこれに近い生体の設計戦略によるのかもしれない。中枢神経系では、損傷が起きるとグリア細胞がNOGOやNI-35をはじめとした種々の軸索再生阻害因子を分泌するシステムが存在する。つまり再生しない仕組みがあらかじめ中枢神経には組み込まれているのである。

これに対して、末梢神経は四肢の末梢と中枢を結ぶ情報の導線として全身に張り巡らされるという解剖学的な特徴がある。したがって構造上、全てを骨組織で守ることは不可能である。そこで切れた場合にも、回復するメカニズムが付与されているのではなかろうか。この末梢神経の自己再生（修復）能力を利用した治療法の1つとして、人工神経（神経連結管）が開発されている。ここでは、人工神経の基礎と臨床について紹介する。

1．末梢神経の再建法

事故や外傷で末梢神経が切れた場合、切断端間の距離が5mm以内であれば直接縫合が可能である。しかし、ギャップが5mm以上ある場合、末梢神経組織は長軸方向の伸展に弱いので吻合部に強い張力がかかるため、断端を引き寄せて吻合しても機能が戻らない。そういった5mm以上の欠損に対して、従来は自家

* Artificial nerve (Nerve conduit): Research and clinical application
1)Tatsuo NAKAMURA, M.D., Akio HAGIWARA, M.D. and Yuji INADA, M.D.：京都大学再生医科学研究所［〒606-8507 京都市左京区聖護院川原町53番地］; Institute for Frontier Medical Sciences, Kyoto University, Kyoto
2)Akio HAGIWARA, M.D.：同志社大学生命医科学部（京都府立医科大学）［〒610-0394 京田辺市多々羅都谷1-3］; Doshisha University, Kyoto
3)Yuji INADA, M.D.：稲田病院（奈良県立医科大学）［〒630-8131 奈良市大森町46］; Inada Hospital, Nara
4)Shin-ich KANEMARU, M.D.：（財）田附興風会北野病院（京都大学耳鼻咽喉科）［〒530-8480 大阪市北区扇町2-4-20］; Kitano Hospital, Osaka

神経移植がGOLD STANDARDの術式として行われてきた。

自家神経移植で使う神経は末梢神経の中でも切除しても比較的術後障害が少ない感覚神経が選ばれ、これを採取して、再建部に移植するものである。よく用いられるのは下肢の腓腹神経、耳鼻科領域では大耳介神経である。この自家神経移植という術式は「移植」という言葉が使われるが、肺臓移植や心臓移植など他の臓器移植とは概念が大きく異なる。すなわち自家神経移植では移植された自家神経片は神経軸索として機能するのではなく、移植片は神経再生の足場として働く。主な機能をつかさどる神経軸索は切断中枢端から伸展し、移植された神経片を貫通してWaller変性した末梢端に入り、さらにそこから最終標的器官である神経筋接合部や感覚受容器に到達して初めて神経機能が回復する。

マイクロサージェリー技術の発達により、微細な神経縫合が可能になり、自家神経移植は標準術式になった。しかしながら自家神経移植には必然的に犠牲にする神経がどうしても必要であり、また術後の機能回復成績も決して満足するものではなかった。さらには神経片採取部位に新たに疼痛を生じた症例も報告されている。

2．人工神経の歴史

人工神経が開発される以前は、自己の静脈管を用いて神経欠損部を補填する試みが1904年頃から行われた[1)]。1970年代から自家神経移植に代わる新しい術式として、チューブによる欠損部の補填が研究されてきた。切断された神経の両端をチューブで連結しておくと、両端から組織が伸びてきて、チューブ内に連続した索状組織ができる。そしてその新生組織内を中枢端から軸索が伸展する。この現象に注目して神経連結管（人工神経）の研究を大きく発展させたのは本学会でも平成18年8月に広島で特別講演をされたスウェーデンのGöran Lundborg博士と同僚のDahlin博士のグループである。Lundborg博士らはシリコン製のチューブを用いて神経を再生させることに成功し、さらに臨床に応用した[2)]。しかしながら、シリコンに代表される非吸収性材料のチューブは再生した神経にとっては周囲との隔壁であり、再生部位の疎血の原因となる。従って長い距離の再建に用いると機能的回復が遅れる。そこで神経がつながった後、チューブは手術で二期的に抜去する必要があるが、これは患者にとっても外科医にとっても大きな負担であった。

3．生体内吸収性人工神経

抜去手術の不要な神経連結管（人工神経）として分解吸収性チューブの開発が進められた。生体で安全に分解吸収する外科用埋植材料としてはポリグリコール酸（PGA）、ポリ乳酸やこれらの共重合体が1950年代から吸収性外科用縫合糸として使われている。これらの素材で作られた神経管が開発された。アメリカでは1990年代終わりにすでにNEUROTUBE（図1）という商品名のPGA製人工神経が開発され、FDAの承認を得て、30mm以内の欠損補填では自家神経移植に比べて良好な感覚機能の回復が得られることが、ランダムスタディで証明されている[3)]。また、

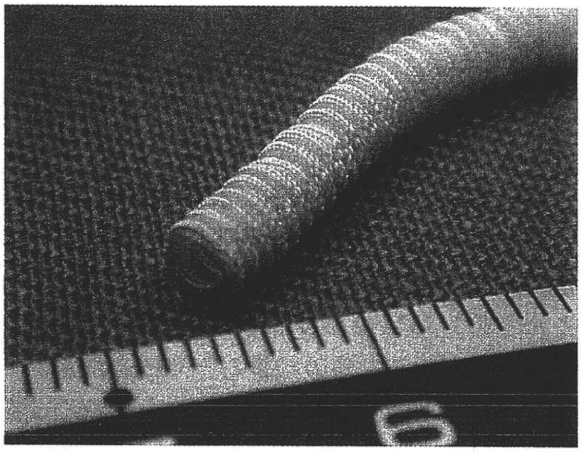

図1　アメリカで販売されているPGA製人工神経（Neurotube）

表1　Nerve Guide Tube commercially available

	Product Name	Company	Country	Material	FDA510k	φ (mm)	L (mm)
1	Neurotube	Synovis LT	USA, MN	PGA woven corrugated tube	1999	2.3～8	20～40
2	Neurolac	Polyganics BV	Netherlands	poly (DL-lactide-co-e- caprolactone)	2003	1.5～10	30
3	NeuroGen*	Integra NS	USA, NJ	Collagen	2001	2～7	20～30
4	Neuroflex	Stryker/Collagen Matrix	USA, NJ	Type I collagen	2001	2～6	25
5	Salu Bridge	Salumedica	USA, GA	Poly (vinyl alcohol) hydrogel	2000	2～10	64
6	Axo Gen	Cook Bioteck	USA, IN	porcine small intestine	2003	1.5～7	10

*Integra provides now NeuraGen tube, similar to previous NeuroGen.

生体内由来物質であるコラーゲンを架橋した素材で作られた人工神経管も、NeuraGenという商品名で市販されている。この他に同様の中空の連結管が著者らの知る限り全部で7種類ほどFDAの認可を得ている（表1）。

4．人工神経の内部構造

現在製品化されている人工神経はアメリカ製とオランダ製のものがあるが、日本では市販されていない。いずれも中空な構造である。使用に際しては内部に生理食塩水を充填して使うように使用説明書に記載されている。

神経管内部に神経の伸展を促進する物質を補填する研究が進められている。

神経組織は基底膜のIV型コラーゲンに沿って伸展する性格がある。そこでIV型コラーゲンやラミニンを充填したり、また神経成長因子（NGF）を応用したりする方法が考案されている[4]。さらに培養したシュワン細胞を神経管内に充填して軸索再生を促進させる試みも進められている[5]。さらに未分化な体性幹細胞をシュワン細胞に分化させてこれを用いる手法も研究が進められている。

神経管の内部では軸索が長軸方向に向かって伸びる必要があり、伸びるための足場となる線維束を長軸方向に充填する実験が行われた。この足場にはコラーゲン繊維や生体内分解性合成高分子材料の繊維などが使われる。

コラーゲンの繊維束を入れた人工神経は長い欠損においても良好な神経再生をもたらすことが判明した。しかしこのコラーゲン繊維は一定の品質の繊維を作るのが難しく、作製には大がかりな紡糸装置が必要で作成費が極めて高価になる。この点が人工神経を製品化するには大きな障害になっていた。ところが、充填物は繊維束でなくても凍結乾燥させたコラーゲンを充填しても同等の神経再生効果があることが判明して[6]、人工神経の開発は大きく進歩した。人工神経管内で凍結乾燥させたコラーゲン水溶液は薄フィルム多房構造（図2）となり、これが神経の再生に良好な環境を作り出すからである。

5．新しく開発された人工神経（PGA-Collagen Tube）とその臨床応用

米国製の人工神経管が中空であったのに対して、日本で開発された人工神経は内部にコラーゲンが充填されている点に特色がある。軸索の再生の場としての足場の利用である。

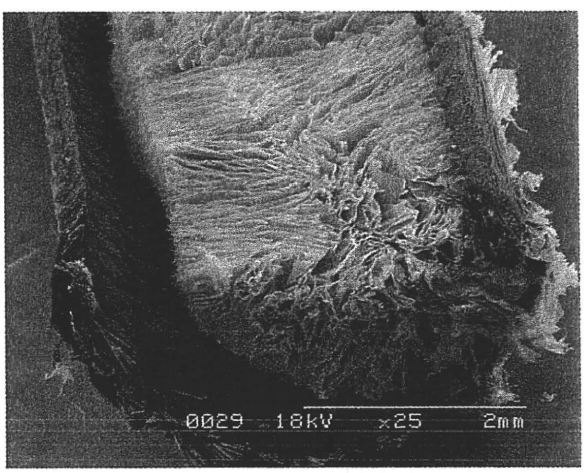

図2　PGA-Collagen Tube内コラーゲンの薄フィルム多房状構造（SEM所見）

組織工学では足場、細胞、増殖因子を駆使して組織を作製するが、このタイプの人工神経では再生の場を培養室のシャーレの中ではなく直接再生部位に置くことにより、局所にある生体由来の増殖因子、細胞などの力を使って神経を再生させるもので、生体内組織再生（in situ Tissue Engineering）の応用である。

ビーグル成犬を用いた動物実験で80mmの欠損で神経再生が確認され[7]、また自家移植との比較でも腓骨神経15mm欠損を補填すると、電気生理学的にも組織学的にも自家神経移植に比べて良好な回復をすることが判明した[8]。

そこで、このPGA-Collagen Tube（図３）は2002年より京都府立医科大学で学内の倫理委員会の承認のもと、初めて臨床応用が行われた[9]。さらに現在、奈良県立医科大学、稲田病院、京都大学病院、田附興風会北野病院、新潟大学歯学部でも臨床応用が続けられている。

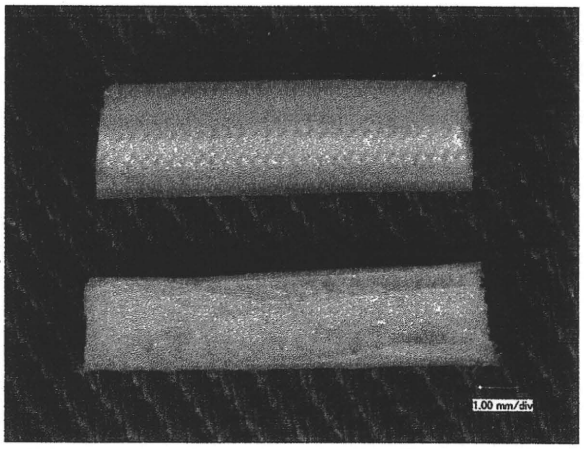

図３　PGA-Collagen Tube

６．人工神経の適応疾患と再生の場の理論

人工神経の臨床応用では、外傷や医原性の神経断裂が主なる治療対象になる。これ以外には悪性腫瘍切除後の神経再建にも使われている。人工神経は末梢神経の自己再生能力に依存しており、極端に血流が乏しい部位や、放射線照射が予定される部位では使用できない。生体内組織再生（in situ Tissue Engineering）では、その場に再生に適した環境（再生の場）を作り出すことが必要だからである。

受傷から神経再建までの時間は早いほど良いことは言うまでもない。しかし複雑骨折などを伴った外傷例などでは周囲の創が治癒するのを待って神経再建手術が行われることも多い。受傷後数年以上経った陳旧症例の再建にも用いられているが、やはり新鮮例に比べると回復は遅く、受傷後１～２年以内の症例が良い適応と考えられる。

７．人工神経の埋入手技

神経の両端が神経管内に内挿されるように8-0モノフィラメント糸で固定する。この際に神経の断端がチューブ内で向き合うように固定することが大切である。

何度も繰り返すが、周囲に血流豊富な軟組織があり、瘢痕を作らないような環境が望ましい。このために血管柄付組織移植などのマイクロサージェリーの技法を併用して、周囲の再生環境を整えることが必要になる症例もある。

８．人工神経（PGA-Collagen Tube）による臨床の実際

人工血管と人工神経とは名前は似ているが、臨床像が全くと言ってよいほど異なる。血管再建手術では、縫合が完了してクランプを外した瞬時に血流が再開し機能は回復する。人工神経では縫合が完了しても機能的には全く改善が見られない。軸索が伸長して目的組織に到着して初めて機能が回復する。そのため機能回復に数年かかることも珍しくない。また術後の神経再生過程で異常感覚や局所の再生痛が生ずることもある。

この点を術前に患者に説明し、理解の得られた患者にのみ治療を行うこと（patient selection）が鉄則である。神経再生治療は長い時間のかかるものであり、治療をスムーズ

に行う上で、充分な説明に基づく患者選択は何よりも重要なことである。

9．人工神経による新しい治療法の展開

末梢神経外傷の治療の中で人工神経を用いて神経を再生させると、神経因性疼痛が劇的に改善する症例がある[10),11)]。また口腔外科、歯科領域では舌神経の再建にも応用が進められている[12)]。この他、耳鼻科、頭頸部外科へも応用され、これまで治療できなかった症例でも機能回復がみられることが判明してきた[13)]。

人工神経の臨床応用に関しては、まず従来の治療法では治せない症例を対象に使用が開始された。このうちカウザルギーに代表される外傷後の神経因性疼痛は、従来のあらゆる治療に反応しないものも多く、外科的なアプローチは禁忌とされてきた。この新しい人工神経による治療は、こういった闇を切り拓く新しい治療法として、疼痛に苦しむ患者の大きな福音になるものと期待が高まっている。

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ORIGINAL ARTICLE

An early mastoid cavity epithelialization technique using a postauricular pedicle periosteal flap for canal wall-down tympanomastoidectomy

SHIN-ICHI KANEMARU[1], HARUKAZU HIRAUMI[2], KOICHI OMORI[3], HARUO TAKAHASHI[4] & JUICHI ITO[2]

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Abstract

Conclusions: Most ears that were treated with a new surgical method were rendered dry and safe, with cavity problems minimized by this simple technique. This technique is also valid in terms of medical economy because it shortens the hospitalization period and subsequent outpatient care is not required frequently. *Objectives:* Canal wall-down tympanomastoidectomy was a well established procedure for severe chronic otitis media, especially cholesteatoma. However, this procedure has some defects, so-called cavity problems, caused by non-epithelialized bony wall. The aim of this study was to evaluate the early epithelialized technique for the surface of widely formed external acoustic meatus after canal wall-down tympanomastoidectomy. *Methods:* Twenty-five patients who had been diagnosed with cholesteatoma were divided into two groups. Group I consisted of 15 patients who underwent a new method in which the open cavity was lined with a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts. As a control, 10 patients in group II underwent the standard operation that uses only free temporal fascia grafts. *Results:* A comparison of the two groups showed that it took only 1 month on average for the entire surface of the external auditory meatus of the patients in group I to epithelialize and dry up perfectly, although the same area in all the patients in group II was not dried up perfectly until over 80 days.

Keywords: *Cavity problems, non-epithelialized bony wall, cholesteatoma, free temporal fascia grafts, postoperative complications*

Introduction

The newly formed external acoustic meatus that results from canal wall-down tympanoplasty is a single cavity that joins the mastoid cavity. The volume of this cavity is much larger than that of the original external acoustic meatus, and it is difficult to completely cover the surface of the newly formed bony wall by free temporal fascia grafts alone. Even if the surface epithelialization of the bony wall is accelerated by postoperative treatments, a bone-exposed area is often observed. Furthermore, the remaining non-epithelialized bony wall is difficult to dry, gets crusty, and is easily infected, which consequently causes the recurrence of otitis media [1,2]. We attempted to completely epithelialize and thereby expedite healing of the surface of the newly formed bony wall using a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts.

The aim of this study was to assess the ability of this new operative technique to reduce postoperative complications in canal wall-down tympanoplasty.

Material and methods

Twenty-five patients ranging in age from 16 to 78 years who had been diagnosed with cholesteatoma

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underwent a canal wall down tympanomastoidectomy operation. Fifteen of these patients underwent a new method in which the open cavity was lined with a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts (group I), and as a control, 10 patients underwent the standard operation that uses only free temporal fascia grafts (group II).

All procedures are shown in Figures 1–4. In all cases, T-shaped external auditory canal plasty was done during the first stage of the operation. After the canal wall-down mastoidectomy had been performed,

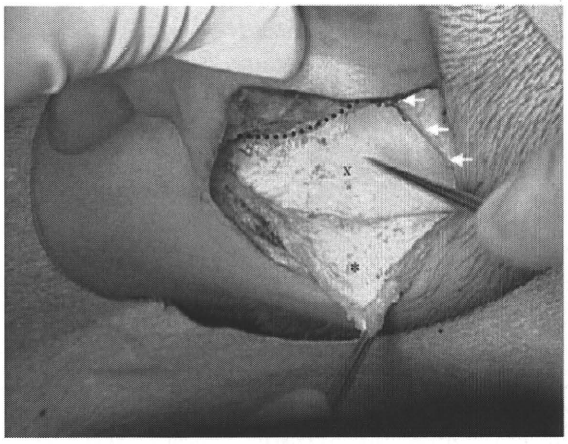

Figure 1. Skin incision and flap. White arrows, temporal line; dotted line, posterior wall of the external auditory meatus; X, mastoid cortex; asterisk, subcutaneous tissue over the mastoid cortex.

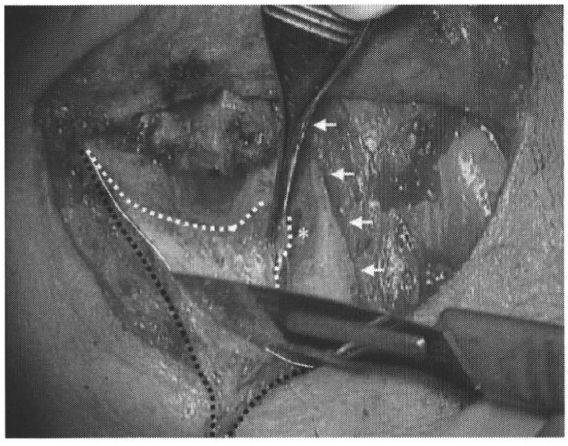

Figure 2. Preparation of the upper and lower layers. Subcutaneous tissue over the mastoid cortex is separated into two layers with a scalpel along the white line. Black and white dotted lines indicate the border of the upper and the lower layers of the subcutaneous tissue, respectively. White arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity.

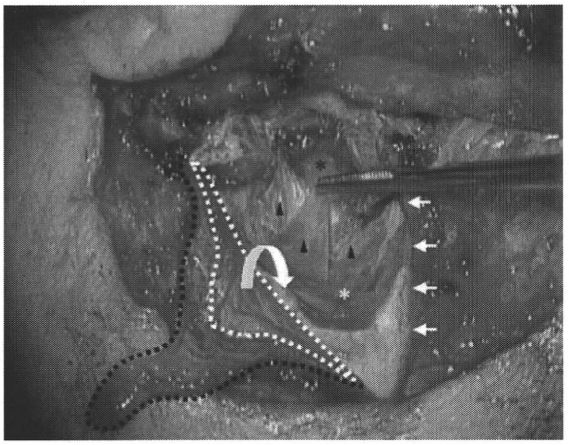

Figure 3. Combination of the periosteal flap and the free temporal fascia grafts. White dotted line, lower layer (periosteal flap); black dotted line, upper layer; white arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity; black arrowheads, free temporal fascia grafts.

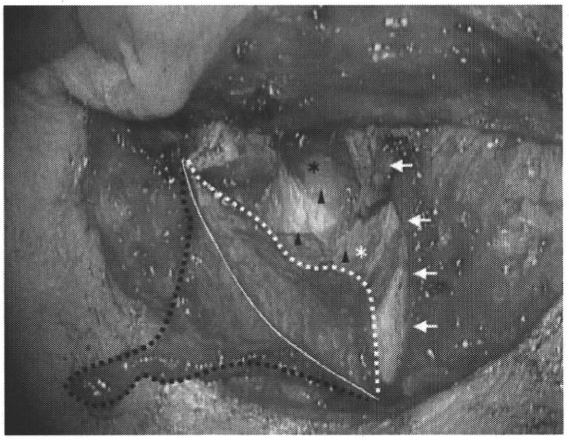

Figure 4. No bone exposed in the region of the opened mastoid cavity. The posterior wall of the open cavity was lined with the periosteal flap. There is no bone exposed in the region of the tympanomastoid cavity. White dotted line, lower border of periosteal flap; black dotted line, upper layer; white line, border of the upper and the lower layer; white arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity; black arrowheads, free temporal fascia grafts.

the tympanic membrane was repaired with temporal fascia grafts. In group I, the subcutaneous tissue over the mastoid cortex was separated into two layers with a scalpel (Figure 2). The upper layer was composed of the subcutaneous tissue and the lower layer was the periosteal flap. Ablation of the subcutaneous tissue beyond the posterior boundary line of the mastoidectomy had to be performed before this procedure to obtain a sufficient periosteal flap and to improve its

mobility. The posterior wall of the open cavity was lined with this periosteal flap and the remaining bone-exposed region was completely lined with the free temporal fascia grafts, which were fixed by fibrin glue (Figure 4). The upper layer was used to cover the open cavity of the postauricular region.

The results of both groups were compared as regards three items, as follows. Item 1: were any bone-exposed regions present in the newly formed external auditory meatus on the first day that the tampon gauze was exchanged postoperatively? Item 2: how many days did the entire surface of the external auditory meatus take to dry? Item 3: for how many days did the patients remain in the hospital after the operation?

Patients were discharged from hospital when they no longer needed to change the tampon gauze in their ears and only needed to apply eardrops.

Results

Table I shows the results of the comparison between groups I and II. For item 1, the tampon gauze was first exchanged an average of 8.5 days after the procedure in all patients. In group I, a bone-exposed region was observed in only one case, which occurred because the transplanted temporal fascia was out of place. This dislocated fascia was immediately returned to its original position. In contrast, bone-exposed regions were observed in all patients in group II. For item 2, it took an average of 30.8 days for the entire surface of the external auditory meatus to dry completely in group I. In group II, it took an average of 81.4 days for it to dry in six patients. Since the remaining four patients had bone-exposed regions, the external auditory meatus could not dry during the observation period. As regards item 3, group II patients remained hospitalized for an average of 10 days longer than patients in group 1.

Table I. Results of the comparison between groups I and II.

Item	Group I (n = 15)	Group II (n = 10)	p value (Student's t test)
1	1/15 (6.7%)	10/10 (100%)	< 0.001
2	30.8 days	81.4 days*	< 0.001
3	18.5 days	29.4 days	< 0.005

Group I, the open cavity was lined with a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts; group II, the standard operation that uses only free temporal fascia grafts. Item 1: bone-exposed rate in the newly formed external auditory meatus on the first day that the tampon gauze was exchanged postoperatively. Item 2: how many days did the entire surface of the external auditory meatus take to dry perfectly? Item 3: for how long did the patients remain in the hospital after the operation?
*In group II, it took an average of 81.4 days for the entire surface to dry in 6 of 10 patients. In the remaining four patients, the external auditory meatus did not dry during the observation period.

Discussion

There are two major approaches for tympanomastoidectomy: canal wall-up and canal wall-down. The former is divided into canal wall-up alone and temporary canal wall-down accompanied by reconstruction of the posterior wall. Each approach has advantages and disadvantages [3–5]. Canal wall-down tympanomastoidectomy is a well-established surgical procedure for the treatment of chronic otitis media and severe cholesteatoma in particular [3,6]. However, the newly formed cavity is far larger than that of the original external acoustic meatus, and consequently, this approach has negative sequelae called cavity problems [1,2]. Subsequent outpatient care is required frequently. Some postoperative complications occur because the bony wall of the open cavity remains partially non-epithelialized, and physiologically abnormal states such as bone-exposed regions make the ear susceptible to infection and relapse. Therefore, early epithelialization of the entire surface of the bony wall is essential for quick healing and for prevention of cavity problems.

It is necessary to provide a sufficient blood supply for the free flap on the bone surface to keep it alive and to prevent infection. If the entire surface of the open cavity is covered with only free temporal fascia, its fascia is too large to prevent necrosis. The maximum boundary that can supply blood from the original external auditory skin may be a fascia graft from the tympanic membrane and its very near surroundings. In this case, we have no choice but to leave the bone exposed in the newly formed posterior wall.

In this study, we epithelialized the entire surface of the bony wall very early in the procedure by using the pedicle periosteal flap of the postauricular region. As this flap has a wide pedicle, it can be supplied with blood by a postauricular artery. It is located in an anatomic region that can supply blood to its surrounding free fascia grafts. Since the periosteum is the contact surface with the bone wall of this flap, a flap covering the bone is more suitable than other flaps. Moreover, this technique is very simple and easy and it does not take so much time for operation.

Most ears were rendered dry and safe, with cavity problems minimized by this simple technique. This technique is also valid in terms of medical economy because it shortens the hospitalization period and subsequent outpatient care is not required frequently [7].

Conclusions

We attempted to completely epithelialize and thereby expedite healing of the surface of the newly formed bony wall using a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts in canal wall-down tympanoplasty. This new operative technique is efficacious to reduce postoperative complications in canal wall-down tympanoplasty and is also valid in terms of medical economy.

Acknowledgment

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ORIGINAL ARTICLE

A tissue-engineering approach for stenosis of the trachea and/or cricoid

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Abstract

Conclusion: This new regenerative therapy shows great potential for the treatment of stenosis of the trachea and/or cricoids (STC). *Objectives:* To estimate the potential of tissue-engineered artificial trachea (AT) for treatment of STC in clinical applications. We previously reported that AT was a useful material for implantation into a tracheal defect after resection of cancer. There are many causes of stenosis of the respiratory tract and STC is particularly difficult to treat. *Methods:* The AT was a spiral stent composed of Marlex mesh made of polypropylene and covered with collagen sponge made from porcine skin. Three patients with STC were treated by this tissue-engineering method. All of them suffered from STC caused by long endotracheal intubations. They underwent a two-stage operation. In the first operation, after resection of the stenotic regions, the edge of the tracheal cartilage was sutured to the edge of the skin. The tracheal lumen was exposed and a T-shaped cannula was inserted into the large tracheostoma. At 3 weeks to 2 months after the first operation, the trachea and skin were separated. The trimmed AT with venous blood and basic fibroblast growth factor (b-FGF) was then implanted into the cartilage defect. *Results:* Postoperatively, all patients were able to breathe easily and had no discomfort in their daily activities. Six months after the second operation, we observed enough air space in the trachea and cricoid by computed tomography (CT) imaging and fiber endoscopy.

Keywords: *Respiratory tract, artificial trachea, basic fibroblast growth factor, regeneration of the trachea, staged operation*

Introduction

Stenosis of the trachea and/or cricoids (STC) is a fibrotic narrowing of the airway at the level of the cricoid and/or tracheal cartilage, which can result in severe dyspnea. There are many causes of STC. Post-intubation and tracheostomy are the most common causes of acquired STC. In spite of technological improvements and more skilful patient care in intensive care units, STC still constitutes a serious iatrogenic sequela after intubation and tracheostomy [1–4]. Although management varies according to different concepts and techniques, there is no well established treatment. Following STC, it is very difficult to recreate an airway structure that allows enough space to breathe.

Progressive tissue-engineering techniques have made it possible to regenerate various tissues and/or organs [5]. According to the concept of tissue engineering, three elements – cells, scaffold, and regulatory factors – are essential to regenerate tissues and/or organs. Depending on the in vivo condition,

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these three elements are chosen and can be applied in combination [6]. On the basis of this in situ tissue engineering, our group has developed a new artificial trachea (AT) scaffold made from a Marlex mesh tube covered with collagen sponge and has used it successfully to repair tracheal defects after resection of thyroid cancer. In this clinical study, we investigated the application of the new AT scaffold for the treatment of STC post-intubation.

Material and methods

Scaffold

The AT scaffold was composed of a spiral stent and a single sheet mesh (Marlex mesh; CR Bard Inc., Billerica, MA, USA) made of polypropylene and covered with collagen sponge made from porcine dermal atelocollagen (Nippon Meatpackers Inc., Ibaraki, Japan) consisting of type I (70%) and type III (30%) collagen dissolved in a hydrochloric acid solution, pH 3.0, at a concentration of 1.0% (Figure 1). A single sheet mesh has a pore size of 260 μm. After collagen coating, the scaffold was freeze-dried with a freeze dryer (FDU-810; Tokyo Rikakikai Co. Ltd, Tokyo, Japan), and cross-linked with a vacuum dry oven (VOS-300SD; Tokyo Rikakikai Co. Ltd).

Patients and surgical procedures

Three patients with STC were treated by this tissue-engineering method. All of them had suffered from STC caused by endotracheal intubations. Table I shows the patient profiles. This new tissue-engineered treatment for STC was applied to a human in accordance with the IRB guidelines of Kyoto University and Medical Research Institute, Kitano Hospital.

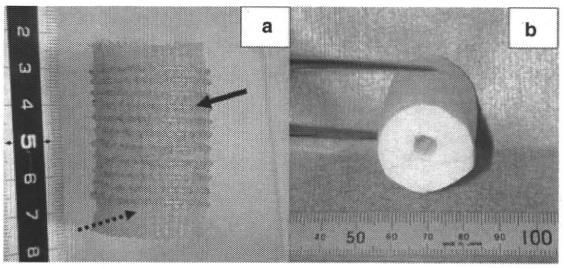

Figure 1. Tissue-engineered artificial trachea (AT) scaffold. (a) Framework of the AT scaffold. Black arrow indicates a spiral stent. Black dotted arrow indicates a single sheet mesh. These materials are made of polypropylene. (b) Framework of the AT scaffold is covered with collagen sponge made from porcine dermal atelocollagen.

All subjects underwent the same two-stage operations. In the first operation, after resection of the stenotic regions, the edge of the tracheal cartilage was sutured to the edge of the skin. The tracheal lumen was exposed and a T-shaped cannula was inserted into the large tracheostoma. At 3 weeks to 2 months after the first operation, the trachea and skin were separated and the AT was trimmed. Venous blood and basic fibroblast growth factor (b-FGF) (Fibrast; Kaken Pharmaceutical Co. Ltd, Tokyo, Japan) was then implanted into the cartilage defect. The artificial material was sutured to the edge of the trachea. Figure 2 shows the operative procedures.

Assessment

Endoscopic examination was performed periodically to observe regeneration of the AT implanted site with a video-endoscope system (BF typeIT 240, CV240, CLV-U40D, Olympus Co., Tokyo, Japan). Whole images of the reconstructed site were estimated by computed tomography (CT) 6 months after the second operation.

Results

Postoperatively, all patients were finally able to breathe easily and had no discomfort in their daily activities. Six months after the second operation, we observed enough air space in the trachea and the cricoid by CT imaging and a fiber endoscope (Figure 3).

Discussion

There are many causes of STC. The most frequent cause of STC is post-intubation. The long-term mechanical stress of intubation causes a deficiency of blood and necrosis of tracheal cartilage. This leads to cicatrization of the necrotic region and results in cicatricial stenosis. Once this chain reaction begins in the tubular trachea, it is very difficult to stop its progression. The rates of STC following tracheostomy and laryngotracheal intubation have been reported to range from 0.6% to 21% and 6% to 21%, respectively [7].

Tracheal resection followed by end-to-end anastomosis is a well-established technique performed under well-established indications. High success rates of over 70% have been reported [1,2,4,7]. However, in cases where long tracheal segments are to be resected, end-to-end anastomosis cannot be adapted. Also, lesions that involve the infraglottic larynx and the upper

Table I. Patient profiles.

Case no.	Age (years)	Sex	Cause of STC	Adverse conditions
1	71	F	EI after traffic accident	Infection (MRSA) Asthma
2	39	F	EI after status asthmaticus	Infection (MRSA) Atopic dermatitis Asthma
3*	45	M	EI after inhalation burn	Infection (MRSA) Autoimmune renal failure

EI, endotracheal intubation; MRSA, methicillin-resistant Staphylococcus aureus; STC, stenosis of the trachea and/or cricoids.
*Patient no. 3 also had stenosis of vocal fold as a complication of inhalation burn.

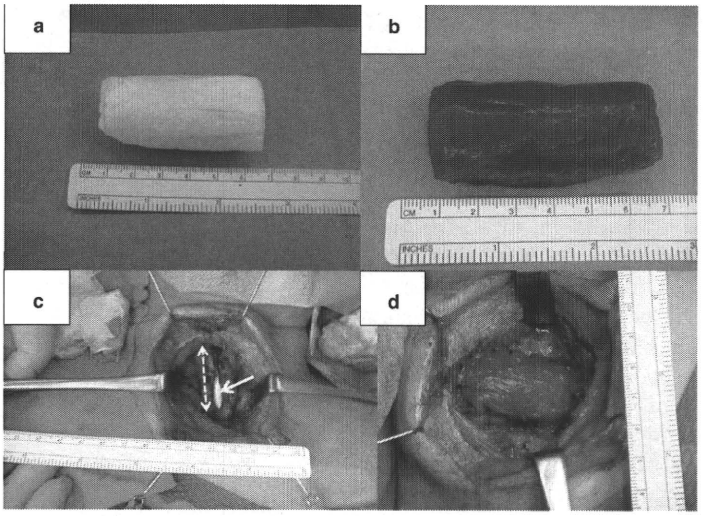

Figure 2. Operative procedure of the second-stage operation. (a) Trimmed artificial trachea (AT) scaffold: AT was trimmed for the size and the shape of the tracheal defect. (b) Trimmed AT scaffold with venous blood and basic fibroblast growth factor (b-FGF). Venous blood and b-FGF were added to the AT immediately before suturing to the trachea. (c) After separation of the trachea and skin. White arrow indicates intubation tube. White dotted arrow indicates the defective region of tracheal cartilage. (d) Trimmed AT scaffold with venous blood and b-FGF was sutured to the defective region of tracheal cartilage with 3-0 absorbable thread.

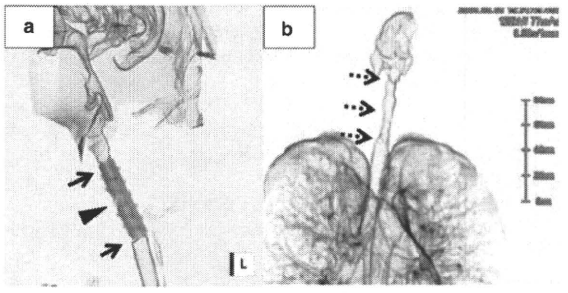

Figure 3. CT scan air-emphasized images of trachea before and after operation. (a) Case no. 2 before operation. A cannula was inserted in the region of STC (black arrows); black triangle indicates the stomal orifice. (b) Case no. 2 after operation. Although the region of STC remains, the inner space of the trachea (black dotted arrows) is maintained without the cannula.

trachea are much more difficult to repair surgically [1–3,7]. In this study, all cases involved long resections of the trachea with cricoid. In case no. 2, it proved particularly difficult to maintain the inner space of the trachea because of necrosis of a long tracheal segment.

Even now, there is no definite and successful treatment for severe cases like those presented here. Major variations in the treatment of STC consist of reconstructive material, the way the operation is performed, and postoperative sequelae. Although autologous tissues such as cartilage are the best reconstructive materials available, it is impossible to obtain them in enough volume and of adequate shape. On the other hand, artificial materials are inferior to autologous tissues as regards affinity and anti-infection. Considering these points, the AT used here is near

to an ideal biomaterial. We have previously reported that our AT had high affinity, sufficient volume, good shape, and mechanical strength equal to normal trachea. Moreover, we confirmed by histological examination that the luminal surface of reconstructed trachea had a normal mucosa with cilia [8–11].

After successful outcomes of animal experiments, we applied AT as a regenerative biomaterial for repairing tracheal defects after resection of cancer in humans [12,13]. These clinical applications succeeded in all cases because most of the trachea itself was normal, except for small cancer invasive regions. However, most patients with STC have adverse conditions, including infections such as methicillin-resistant Staphylococcus aureus (MRSA), asthma, and systemic diseases. The cases presented here have these unfavorable conditions, which prevent the trachea from regenerating.

According to the concept of in situ tissue engineering, for regeneration of tissues/organs it is necessary to arrange three elements – cells, scaffolds, and regulatory factors – in favorable conditions [5,6]. In this clinical study, we arranged two of the elements, scaffold and regulatory factors, because cells were supplied from the host tissue. In addition, to create the best regenerative conditions, we selected a two-staged operation. The purpose of the first operation was to enlarge the region of STC, allow early epithelialization of its luminal side, and prevent infection. After creating these suitable conditions, we implanted AT with b-FGF in the second operation. This two-staged operation may be a good strategy for severe cases of STC.

Basic FGF is a regulatory factor that plays an important role in regeneration of the trachea. In normal tissues, b-FGF is present in basement membranes and in the subendothelial extracellular matrix of blood vessels [14]. It stays membrane-bound as long as there is no signal peptide [15]. It has been hypothesized that during wound healing of normal tissues or during tumor development, the action of heparan sulfate-degrading enzymes activates b-FGF and mediates the formation of new blood vessels, a process known as angiogenesis [14–16]. Administration of b-FGF may therefore be effective in early stages of the regenerative process [17–19]. Collagen sponge, which is a component of AT, could provide not only inducer for cell migration from host tissue but also sustained release of b-FGF [20]. Basic FGF may be not suitable to repair tracheal defects after resection of cancer as it may contribute to tumor recurrence, but there is no such risk in the treatment of STC caused by prolonged intubations [16].

Conclusions

We applied tissue-engineered AT and b-FGF for the treatment of STC [2]. A two-stage operation for the treatment of severe STC may provide better regenerative conditions [3]. This new regenerative therapy shows great potential for the treatment of STC.

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