

**Table 4. Radiological Measurements (Degrees)**

	Reconstruction Failures (6)	No Complication (24)	P
C2–C7 lordotic angle	13.5 ± 17.1	13.5 ± 10.9	n.s.
Fused area lordotic angle	9.6 ± 13.1	7.4 ± 9.1	n.s.
PO C2–C7 lordotic angle	15.6 ± 10.2	13.3 ± 8.5	n.s.
PO fused area lordotic angle	15.6 ± 8.4	8.6 ± 6.4	P < 0.05
C7 horizontal angle	22.7 ± 7.0	22.0 ± 6.8	n.s.
Change in C2–C7 lordotic angle	2.1 ± 11.8	-0.23 ± 7.4	n.s.
Change in fused area lordotic angle	3.1 ± 9.0	0.9 ± 8.9	n.s.

dislodgement and graft migration.<sup>5,6,13</sup> Figure 2 shows a typical case of graft subsidence and migration with screw pullout that occurred in the early postoperative period. Figure 3 shows images from another patient who also experienced early reconstruction failure. The original cervical curve of this patient was slightly lordotic, and we tried to change the alignment of the cervical spine more lordotic to decompress the spinal cord. However, screw pullout and fracture of C7 vertebra occurred during the early postoperative period. Reformatted CT views showed that the C2–C7 lordotic angle had increased after surgery and that C7 fracture had occurred.

In this study, 6 of 30 (20%) patients who underwent multilevel corpectomy and reconstruction without the use of a halo brace experienced reconstruction failures. The postoperative fusion area lordotic angle of the patients who

experienced reconstruction failure was significantly larger than that of the patients who did not experience reconstruction failure (Figure 1). As seen in the scatter graph (Figure 4), 3 of the 6 patients who experienced early reconstruction failure exhibited postoperative fusion area lordotic angles of >15°. Although it is not easy to determine a cut-off with regard to the fusion area lordotic angle at which it is no longer advisable to perform this procedure, the risk of reconstruction failure may increase in patients with postoperative fusion area lordotic angles of >15°. In our study, this value (15°) was equal to the mean postoperative fusion area lordotic angle of the patients who did not experience reconstruction failure plus 1 standard deviation. Hyperlordosis of the cervical spine yields to high shear stress at the bottom of the fused segment, which can lead to anterior slippage of the construct. Herrmann and Geisler has pointed out that both device failure and pseudarthrosis have been observed with greater frequency at the inferior end of long-segment constructs than the top of the fused segment.<sup>13,14</sup> They speculated that the long lever arm of the anterior plate transmitted unacceptably high forces to the inferior level of the fusion area. They observed that shear stress increased at the bottom of the fusion area, especially in patients with a hyperlordotic alignment and continuing micromotion of their cervical spines, both of which can lead to reconstruction failure. These findings were similar to our results.

Additional factors that affected the 3 patients who experienced early reconstruction failure but did not have excessive lordosis of their cervical spines were not elicited in this study. We postulate that overdistracted during grafting could have been one of the causes of recon-

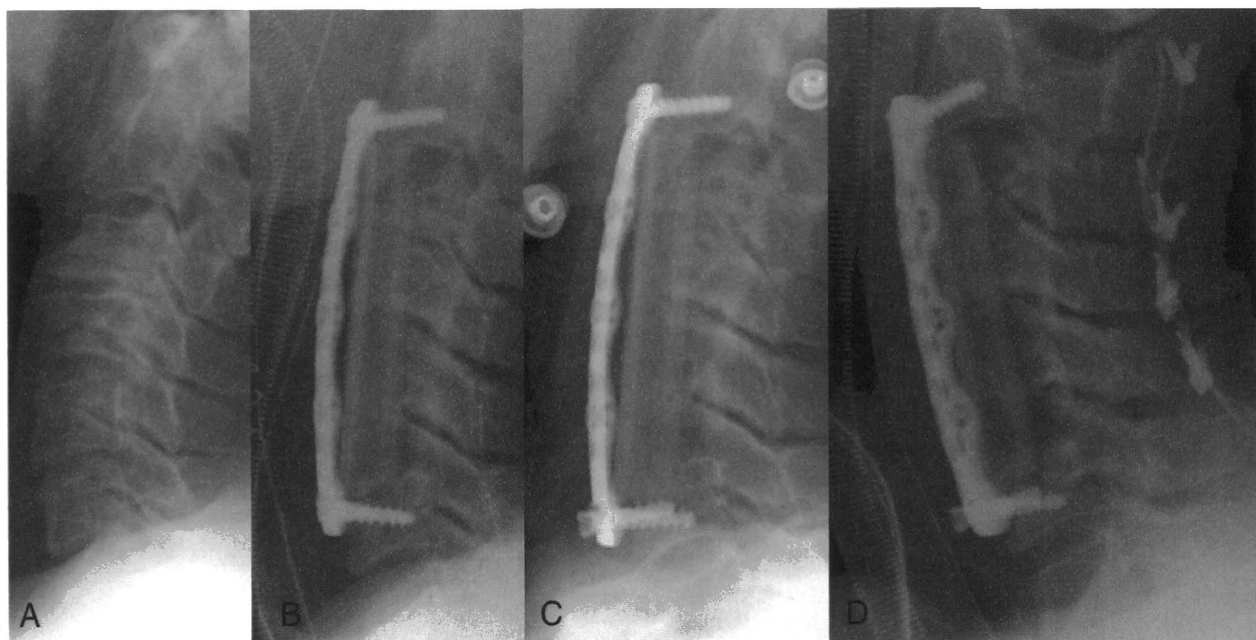


Figure 2. Preoperative radiograph of a 61-year-old man shows mixed-type OPLL with a fusion area lordotic angle of 25.8° (A). C2–C6 fusion was performed using an autologous fibular graft and a rotationally dynamic plate. Note the retention of the C6 endplate just after the operation (B). Graft subsidence and migration with screw pullout occurred 3 weeks after surgery (C). After in situ spinous process wiring, the degree of the patient's cervical lordosis decreased. However, solid fusion was observed (D).

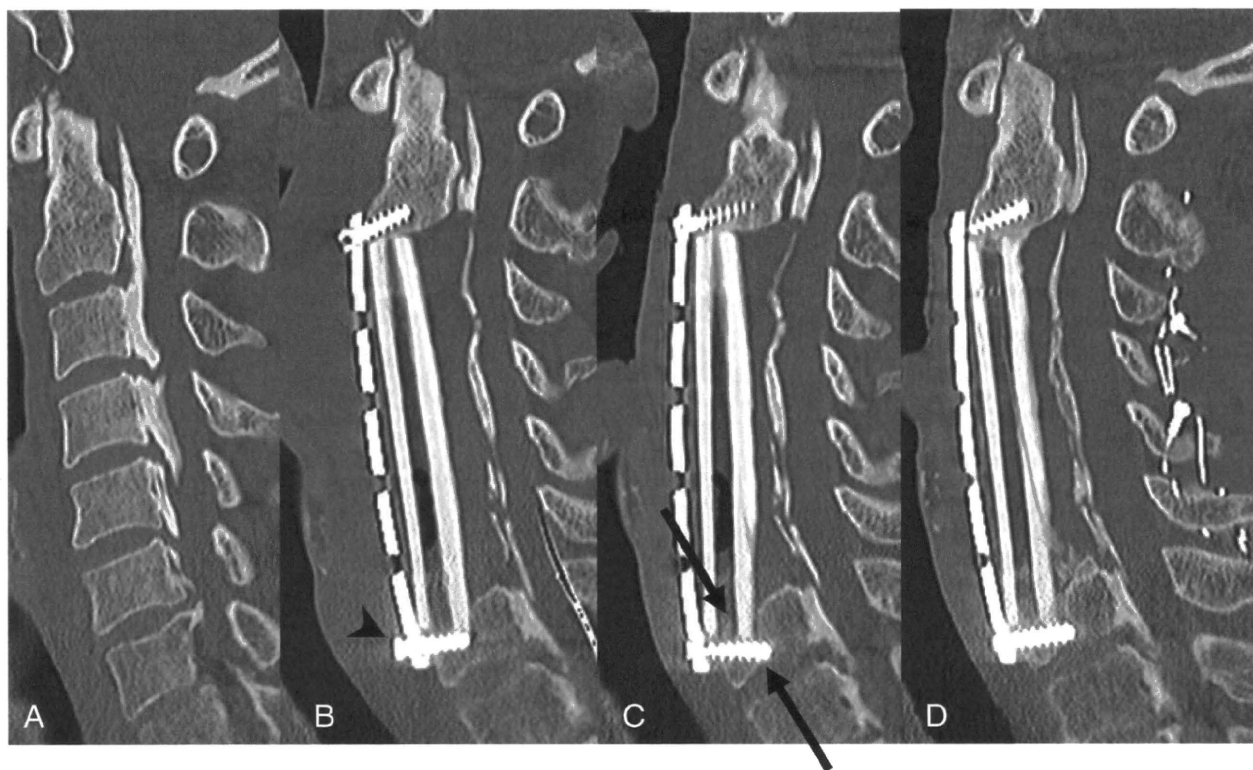


Figure 3. Preoperative radiograph of a 51-year-old man shows mixed-type OPLL with a slight lordosis (A). Postoperative scan taken 3 weeks after the surgery revealed screw pullout (arrow head) (B). Note cervical lordosis seemed to increase after the surgery. The screw was retightened, but graft subsidence and C7 fracture occurred (arrows) (C). After *in situ* spinous process wiring, the degree of the patient's cervical lordosis decreased. However, solid fusion was observed (D).

struction failure in these patients. We distracted the neck carefully while the graft was being inserted, but the distraction force was not controlled precisely.

The following factors were not associated with reconstruction failure: age, gender, preservation of the vertebral endplate, screw type, the occurrence of postoperative graft subsidence within 1 week of surgery, and the

use of intermediate screws for fibular grafting. However, preservation of the vertebral endplate is thought to be important in patients with osteoporosis. In a biomechanical study, Lim *et al* confirmed that load to failure tended to decrease with incremental removal of the endplate and the risk of failure tended to increase.<sup>15</sup>

When the alignment of the cervical spine is lordotic, laminoplasty is normally performed. However, the clinical outcomes of patients with massive OPLL or with a kyphotic cervical alignment treated by anterior cervical decompression and fusion have been previously shown to be better than those of patients treated with posterior decompression.<sup>16</sup> Thus, some patients with cervical myelopathy respond well to multilevel corpectomy and reconstruction.<sup>17</sup> Cervical lordosis is also normally thought to be important for preventing long-term fusion-related problems such as adjacent segment degeneration. However, we hypothesized that postoperative cervical hyperlordosis may adversely affect graft stability in the early postoperative period.

When graft migration is discovered, posterior wiring can provide solid union. When the graft dislodges completely, reinforcement by screw fixation is needed. Because some authors recommend simultaneous anterior and posterior fixation for patients with hyperlordotic cervical spine alignments,<sup>18,19</sup> a combined surgical plan should be used to decrease the rate of early reconstruc-

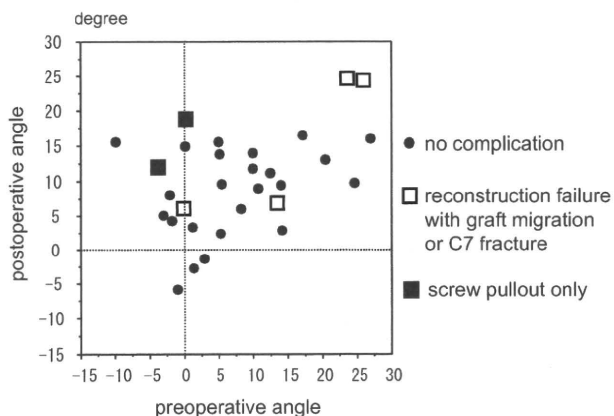


Figure 4. The preoperative and postoperative fusion area lordotic angles of the included patients have been plotted. Of the 6 patients, the 4 who experienced early reconstruction failure exhibited postoperative fusion area lordotic angles of  $>10^\circ$ . Additional factors that affected the remaining 2 patients without excessive lordosis of their cervical spines were not elicited in the current study.

tion failure in the limited number of patients this type of alignment.

The limitations of this study include its retrospective nature and the fact that only a small number of cases are included. A prospective study is, therefore, needed to further examine this paradoxical hypothesis that we generated based on the results of our study.

#### ■ Key Points

- A retrospective study was conducted to investigate risk factors for early reconstruction failure of multilevel cervical corpectomy and reconstruction with dynamic plate fixation.
- Six of 30 cases experienced reconstruction failures, including graft migration at the bottom of the graft in 2 cases, fracture of the C7 vertebral body in 2 cases, and pullout of a screw in 2 cases.
- On radiologic measurement, the fusion area lordotic angle after surgery in patients with reconstruction failures was significantly larger than that of the patients with no complication.

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## 特 集

# 脊椎インストゥルメンテーションのリスクとベネフィット

## プレートを併用した頸椎広範囲前方除圧固定手術における留意点

大川 淳\* 川端 茂徳 加藤 剛  
富澤 将司 榎本 光裕 四宮 謙一

要旨：前方プレートの使用で、術直後であっても厳密な頸椎安静は不要となり、周術期の急性呼吸不全は減少した。また、頸椎カラー程度の外固定の併用で術後早期から起立歩行が開始可能となった。プレートには、スクリューとの間に動きがない constrained type と、上下あるいは回旋の動きをわずかに許容しつつ、抜去される方向には動かない semi-constrained type があり、最近では semi-constrained type での成績が良いという報告が多い。しかし、プレートによる内固定といっても絶対的な固定力が即時に得られるわけではない。3椎間以上の広範囲前方除圧固定術 40 例を対象として、周術期の合併症に関して調査したところ、移植腓骨の転位が 2 例、母床椎体骨折が 2 例、スクリュー突出単独が 3 例の計 7 例 (17.5%) に再建合併症がみられた。頸椎の前弯が強いことが危険因子と考えられたが、移植骨が完全に脱転する前に棘突起ワイヤリングを追加することで対処可能であった。

### はじめに

圧迫性頸髄症の手術治療は、脊髄圧迫の除去を目的として行われる。頸椎の不安定性が併存する場合に固定術が追加されるが、不安定性がなくても局所の動きをなくす方が神経回復にはよいとして、固定術が併用されることもある。頸椎の骨性固定を意図したときには、かつては前方固定術しか方法がなかったが、最近のインストゥルメンテーションの進歩により後方からの椎弓形成術にも固定術を組み合わせたことが可能になった。

ただ、すべての圧迫性脊髄症が後方除圧固定術

で対処できるわけではなく、頸椎後縦靱帯骨化症 (OPLL) で骨化が厚く狭窄率が 60% を超える場合や嚙状に局所突出がみられる場合、横断面において偏在性の場合には、広範囲前方除圧固定術の適応となる<sup>1)2)</sup>。広範囲前方除圧固定術では脊髄の前方に位置する骨化を浮上させるか、あるいは摘出することで脊髄を原位置に戻すことが可能である。また、頸椎症性脊髄症 (CSM) においても、後弯が限界を超えると前方法の成績が良いことが報告されている<sup>3)</sup>。

こうしたことから、頸髄症の一部の症例において広範囲前方除圧固定術の必要性があり<sup>4)</sup>、ハローベストを利用した広範囲前方除圧固定術では良好な術後成績が報告されている。特に、池永ら<sup>5)</sup> は 4 椎間以上の 118 例において移植骨の脱転はなかったとして、すばらしい成績を報告している。しかし、ハローベストには、術直後の後咽頭腔の腫脹 (図 1) による急性呼吸不全にも気管挿

\* Atsushi OKAWA et al, 東京医科歯科大学大学院医歯学総合研究科, 整形外科

Complication of cervical corpectomy and fusion with plate fixation

Key words : Anterior cervical corpectomy and fusion, Reconstruction failure, Anterior cervical plate

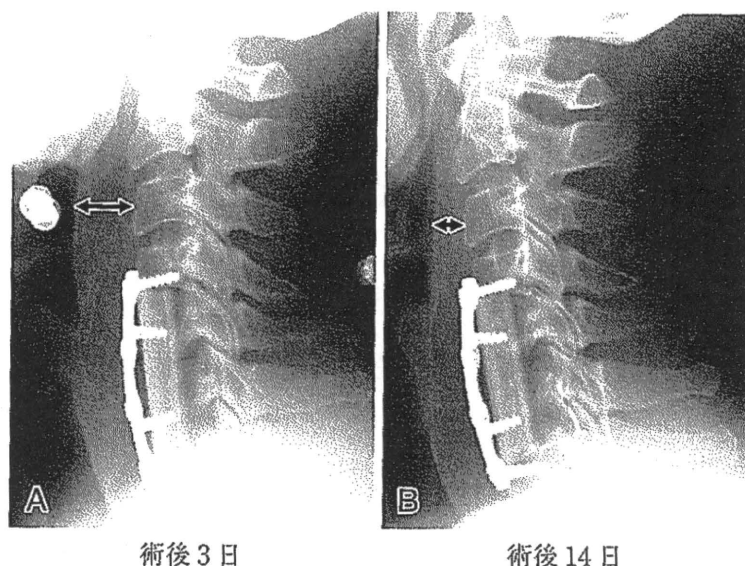


図1 術直後の後咽頭腔の腫脹

40歳男性のOPLLに対して、プレートを使用したC4-7椎体亜全摘前方除圧固定術を施行した。術後3日の頸椎X線側面像では、第3椎体高位での軟部腫脹が目立つ(A)。プレートがなくても同様の腫脹は発生し、急性の吸気困難となることがある。腫脹は時間がたてば自然軽快するので、初期数日の呼吸管理が重要である(B)。ハローベストでは、再挿管が困難なことが多いが、プレート固定であれば頸椎の伸展も可能である。

管による迅速な対処がしにくいこと、骨癒合まで2~3カ月間にわたり装着を必要とすることなどの問題点があり、次第に前方プレートが利用されるようになった。

前方プレートの使用で、術直後であっても厳密な頸椎安静は不要となり、周術期の急性呼吸不全は減少した。また、頸椎カラー程度の外固定の併用で術後早期から起立歩行が開始可能となった。しかし、プレートによる内固定といっても絶対的な固定力が即時に得られるわけではない。Sasso<sup>6)</sup>はプレートを併用した前方除圧固定術中、術後早期の再建合併症が2椎体切除では33例中2例であったものが、3椎体切除では7例中5例(71%)にみられたとしている。Daubs<sup>7)</sup>は、複数椎体切除後の再建をメッシュケージとプレートで行った場合に、術後12週間以内に8例中6例が再建合併症を生じたと報告している。これらの報告はいずれもconstrained typeのプレートを用いたものであるが、再建合併症をできるだけ減らすために

はプレートの種類に限らず、いくつかの注意が必要と考えられる。本稿では、プレートを併用した頸椎広範囲前方除圧固定術における留意点について述べる。

### I. 手術適応と術前準備

広範囲前方除圧固定術の上下方向の到達可能範囲は、C2下縁から1cm程度が上限で、胸骨縦割をしなければ通常T1上縁が無難であろう。厚い骨化(狭窄率60%以上)や嘴状の骨化、横断面で偏在する骨化を持つOPLLや後弯を形成するCSM・筋萎縮症が適応である。頸椎全体の前弯が保たれている場合には、後方からの除圧が基本である。また、頸椎症で広範囲にわたり除圧と固定が必要と考えられる場合には、椎体高位の除圧は不要であることがほとんどなので、椎間部のみを除圧する多椎間前方固定術を行う。

術前には患者の全身状態のチェックが重要であり、特に呼吸機能低下は術後の後咽頭腔の腫脹が

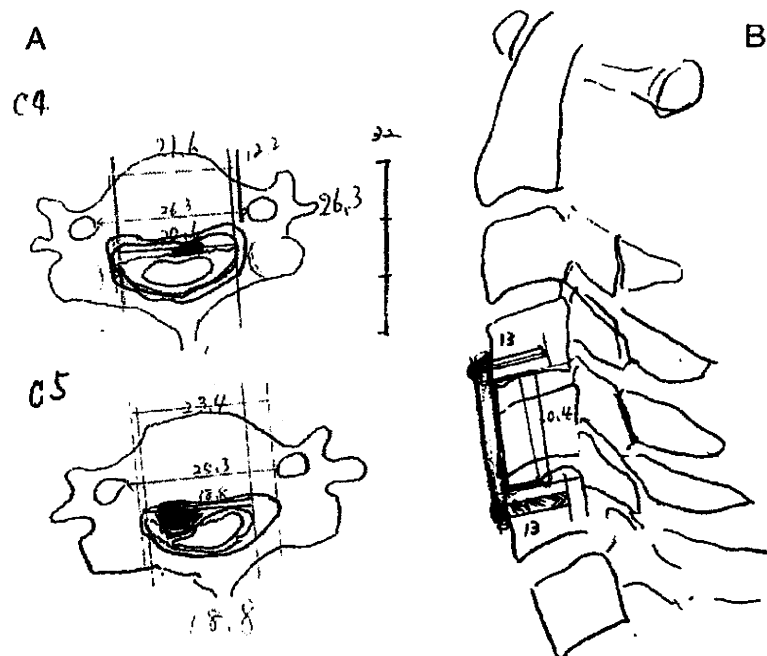


図 2 実際の paper surgery

C4-6 の前方除圧固定を予定した患者の CT の横断面像をトレースし、左右の椎弓根間距離、椎骨動脈間距離、硬膜管横径などを計測する (A)。側面像も同様にトレースし、移植骨長、使用するプレート長、スクリュー長の計画を練る (B)。

必発であることから致命的となり得る。喫煙は神経症状の回復や骨癒合も遅延させる可能性もあり、広範囲前方除圧固定術を適応する場合には最低でも術前 4 週間は絶対的な禁煙とし、守られない場合には手術を延期している。

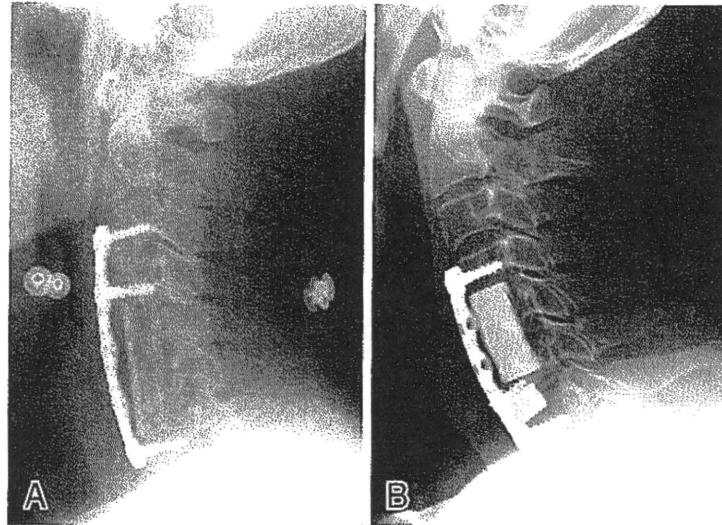
また、頸椎局所の準備として重要なのは、神経症状の誘発される肢位をあらかじめ把握しておくことである。特に C2 にアプローチする場合には若干伸展気味になるので、意識のある状態で同じ肢位を 10 分間程度とらせ、神経症状が悪化しないことを確認する必要がある。これをしないと、除圧完了前の術中肢位による神経症状悪化を予測できない。同様に、OPLL に対する広範囲前方除圧固定術における術中の脊髄機能モニタリングは、現在のわが国の医療安全に関する環境を考慮すればほぼ必須の検査となりつつある<sup>8)</sup>。脊髄機能モニタリング設備がなく、過去に十分な症例経験がない場合には、より専門的な医療機関に紹介することも考慮すべきであろう。

## II. 手術計画

### — 除圧固定範囲 —

広範囲前方除圧固定術では、上下方向だけでなく、特に OPLL では術前の画像診断により、骨化の成熟度や脊柱管の程度、椎骨動脈の位置に留意しなければならない。側面前後屈 X 線像では骨化の動きに注目する。骨化の切れ目の動きが高位であることが少なくない。MRI での脊髄圧迫の程度を参考にしつつ、CT 水平断像および再構成像の正面および側面像で骨化の正確な範囲と形状を把握した上で、paper surgery によって手術計画を立てる (図 2)。MRI で見られる靭帯肥厚部も除圧範囲に含める必要があり、椎体切除部位は脊髄圧迫がない位置に予定する<sup>9)</sup>。

広範囲前方除圧固定術は脊髄が同時に広範囲に除圧できるという点で、後方椎弓形成術と同等の効果を持ち得る。骨化浮上術では骨化の前方移動自体に 4~8 週間必要である<sup>10)</sup>が、神経組織に対する除圧効果は骨化の切り離しの直後から得られ



C2-6 固定 (腓骨移植)

C3-7 固定 (人工骨移植)

図 3 ハイブリッド固定

上下方向の固定範囲に関しては、OPLLが存在する範囲は連続除圧するものの、アライメント調整や不安定性の予防によって固定範囲に含めざるを得ない隣接椎間に関しては、別に椎間固定のみを追加するようになった。

る。その効果を維持するために安定した脊柱の再建が重要となる。

上下方向の固定範囲に関しては、かつては可及的に広範囲としていたが、現在ではできるだけ分節固定を併用するよう心がけている。除圧操作自体は広範囲である方が容易だが、固定に関して除圧範囲の間に椎体を残すことでスクリューのアンカーポイントを増やすことが可能となり、術直後の固定性は増す。そのため、OPLLが存在する範囲は除圧するものの、アライメントや不安定性によって固定範囲に含めざるを得ない隣接椎間に関しては、別に椎間固定のみを追加するハイブリッド固定を行うようになった(図3)。

### III. 除圧操作

CSMに対して前方法を選択するときには、多椎間であっても基本的に分節固定を行う。椎間板の廓清のち、顕微鏡下に後方の骨棘を削る。

OPLLでは鉤椎関節を目安に最低でも骨化横径から1~2mmの余裕を持たせて、除圧の横径は椎体部で20mm、椎間板部では20mm以上を目安にする。その後、椎間板と椎体を交互に切除し

て、次第に骨溝を深めていく。椎体前面から垂直に掘っていくことが重要であり、掘削が斜めになると術前に計画した除圧横径が確保できないか、骨化が片浮きとなって脊髄の偏在圧迫を起しBrown-Séquard syndromeを惹起する可能性がある。

### IV. 脊柱再建術

#### 1. 移植骨

1椎間の分節固定および1椎体の椎体亜全摘後の2椎間固定には、既成のハイドロキシアパタイトの人工骨を用いている<sup>11)12)</sup>。3椎間以上の脊柱再建には通常、遊離腓骨移植を行う(図4)。

椎体母床はできるだけ終板を温存することで、subsidenceを防止する。多椎間の椎体亜全摘後の母床作成でも終板のトリミングは行わないが、腓骨の前方部分に作成したpeg(くい)を差し込む溝を作成することが多い。

移植骨長は、術野の外から頸椎をわずかに伸張させ、腓骨を軽くたたきこむことで挿入できる程度としている。挿入時に頸椎を伸張しすぎると、脱転の可能性が高まる印象がある(定量的な検討

はなされていない)。

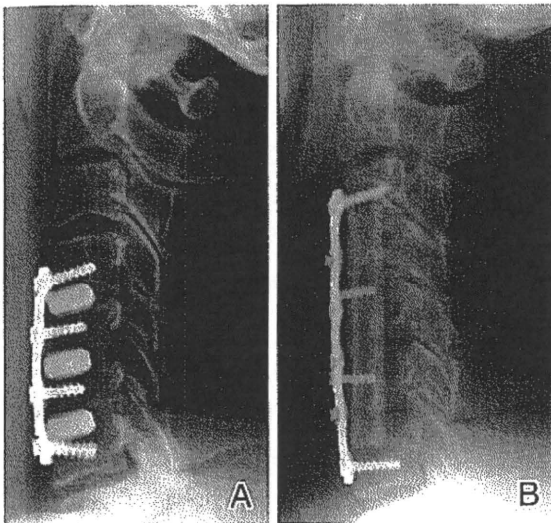
## 2. プレート固定

腓骨を設置した後に、プレートによる内固定を行う。プレートには、スクリューとの間に動きがない constrained type と、上下あるいは回旋の動きをわずかに許容しつつ、抜去される方向には動かない semi-constrained type がある。最近では semi-constrained type での成績が良いという報告が多い<sup>13)~16)</sup>。われわれは使用するスクリューの種類によって constrained type にも semi-constrained type にも利用できるプレートシステムを主として用いている。スクリューは下位端を

fixed type, 上位端を variable type としているが、メーカーのデータによれば上位で 2° 程度の回旋が許容される。Semi-constrained type のプレートには、これ以外にも上下方向の translation が数 mm にもわたって許容されるタイプもあるが、プレートの種類による臨床成績の差異は明らかになっていない。

## V. 合併症 (自験例)

過去 10 年間に行われた 3 椎間以上の広範囲前方手術のうち、術後 1 年以上経過観察が可能であった 40 例 (男性 36 例, 女性 4 例) を対象として、周術期の再建合併症と subsidence に関して調査した。手術術式は、椎体亜全摘を行わず椎間ごとに骨移植を行う分節固定 (segmental fusion : SF 群), 椎体亜全摘除圧固定 (long fusion : LF 群), 両者の併用 (hybrid fusion : HF 群) の 3 群に分類した。SF 群は 3 椎間 6 例, 4 椎間 1 例の計 7 例, LF 群は 3 椎間 14 例, 4 椎間 10 例, 5 椎間 1 例の計 25 例, HF 群は 3 椎間 2 例, 4 椎間 4 例, 5 椎間 2 例の 9 例であった (表 1)。疾患別には CSM 10 例, 頚椎症性筋萎縮症 3 例, 後縦靭帯骨化症 27 例で、頚椎症では 7 例が SF 群, 6 例が LF 群, 後縦靭帯骨化症では 19 例が LF 群, 8 例が HF 群であった。後療法は術後 2~4 日以内に顎つきカラーで起座歩行を開始し, 3 カ月間外固定を継続した。手術時間は 3 時間 25 分~9 時間 15 分 (平均 5 時間 53 分), 出血量は 78 ml~2,885 ml (平均 375 ml), 入院期間は 15 日~79 日 (平均 29 日) であった。神経合併症は C5 麻痺が 2 例, 脊髄麻痺悪化 1 例で, うち 1 例が移植腓骨の移動によるものであった。



C4-7 分節固定 C3-7 椎体亜全摘固定

図 4 分節固定と椎体亜全摘固定

1 椎間の分節固定および 1 椎体の椎体亜全摘後の 2 椎間固定には、既成のハイドロキシアパタイトの人工骨を用いている (A)。3 椎間以上の脊柱再建には通常、遊離腓骨移植を行う (B)。

表 1 対象患者の背景

固定方式	計	3 椎間	4 椎間	5 椎間	移植骨 (腓骨 : 人工骨)
分節固定群 (SF)	7	6	1	0	7 (1 : 6)
亜全摘固定群 (LF)	25	14	10	1	25 (25 : 0)
ハイブリッド群 (HF)	8	2	4	2	8 (5 : 3)
計	40	22	15	3	

SF : 分節固定, LF : 椎体亜全摘固定, HF : 分節固定 + 椎体亜全摘固定



### 1. 術後早期の再建合併症

固定に伴う合併症は移植腓骨の C7 での転位が 2 例、C7 椎体の母床骨折が 2 例、スクリュー突出単独が 3 例で、計 7 例 (17.5%) であった (表 2)。再建合併症は、SF 群 7 例にはみられず、HF 群 2 例と LF 群 5 例に起きていた。特に移植骨脱転と母床骨折の 4 例はいずれも移植骨下端の C7 に認めた。また、髄液漏・感染・食道損傷はなかったが、1 例では術後長期に嚥下困難が継続した。術後の再手術は神経症状悪化 3 例と移植骨転位あるいは母床骨折 4 例の 6 例 (1 例に C5 麻痺と再建合併症が重複) に行われた。神経症状悪化例に対しては除圧追加を、再建合併症に対しては固定範囲の後方ワイヤリングを行った。スクリュー突出では直後に締め直しが 1 例、骨癒合後の抜去が 2 例であった。平均術後 3 年の最終観察時の移植骨偽関節は 4 例にみられたが、再建合併症による再手術例ではなかった。後方ワイヤリングが行われた 4 例ではすべて骨癒合が得られた。この間の JOA スコアの改善率は疾患、偽関節、再建合併症で差はなかった (表 3)。

画像計測によれば、再建合併症がみられた群で

表 2 合併症

神経症状の悪化 (例数)	
C5 麻痺	2
脊髄症悪化	1
再建合併症	
移植骨転位	2*
C7 母床骨折	2*
スクリュー突出	3 (5)**

移植腓骨の C7 での転位が 2 例、C7 椎体の母床骨折が 2 例 (それぞれ 1 例ずつにスクリュー突出合併\*)、スクリュー突出単独が 3 例で、計 7 例 (17.5%) であった\*\*。

は、頸椎の前弯が強い傾向があり、特に術直後の固定範囲の前弯が有意に強かった。それ以外の危険因子として可能性のある終板の侵襲、移植骨の種別、中間スクリューの使用などに関しては、統計学的に再建合併症の有無とは関連がなかった<sup>17)</sup>。

### 2. 移植骨の subsidence

次に、再建合併症の発生に関して、移植骨の術後早期の subsidence の程度を retrospective に調査し、スクリューの pullout への影響を検討した。対象は 40 例中、術後早期に移植骨の転位または母床骨折のみられた 4 例を除いた 36 例で、術後 1 カ月の時点で 3 例にスクリューの突出がみられた。

術後 1 週間前後の起坐開始直後と 1 カ月程度経過したのちに撮影された頸椎単純 X 線中間位側面像において、プレート長を基準として、固定上下端椎体の終板間距離を計測して比較した。その結果、術後約 1 週間と約 1 カ月後で、平均 1.8% の subsidence が認められた。3 椎間では  $1.3 \pm 1.3\%$ 、4 椎間では  $2.4 \pm 1.2\%$ 、5 椎間では  $3.0 \pm 2.2\%$  であり、絶対値では 1~3 mm 程度にあたる。術式別には SF 群  $1.9 \pm 0.7\%$ 、LF 群  $1.6 \pm 1.5\%$ 、HF 群  $2.3 \pm 1.1\%$  で群間に統計学的に有意差はなかった。使用したスクリューにより constrained type のプレートとして機能するはずの 5 例でも 1.5% 程度の subsidence がみられ、semi-constrained type のプレート固定と有意差はなかった。また、C7 椎体の水平面に対する角度が小さいと subsidence が大きい傾向がみられた。

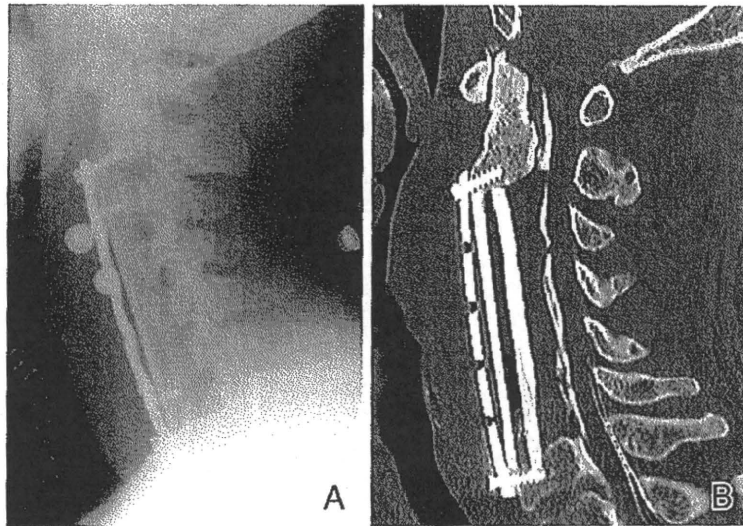
## VI. 考 察

### —プレート固定の利点と留意点—

広範囲の前方除圧の適応は、疾患と頸椎アライ

表 3 術後成績

比較	JOA スコア改善率
疾患別 (CSM 13 例 : OPLL 27 例)	60% : 63%
偽関節 (骨癒合完成 36 例 : 偽関節 4 例)	62.4% : 59.5%
再建合併症 (なし 33 例 : あり 7 例)	62.4% : 60.8%



術後7日の単純X線側面像

同日のCT再構成側面像

図5 CT再構成側面画像による再建合併症の評価

術直後にルーチンで撮影されるポータブルX線像では、移植骨の転位やスクリーウの突出は正確に把握できないことが多いが(A)、CTの再構成像でC7スクリーウの突出も明瞭にわかる(B)。

メント、脊髄に対する圧迫様式により決定され、後方除圧よりも前方から除圧を試みた方がよい症例が一定程度存在する。その後の再建に関して、従来はハローベストによる固定が行われたが、現在の医療環境からすれば、6~8週間にも及ぶハローベスト装着は困難になりつつある。また、術直後の呼吸不全への対応も外固定がない方が容易であることは自明であろう。さらに、頸椎の固定で、術後成績が安定する可能性もある。われわれの経験では、前方固定と後方除圧(非固定)を比較したところ、CSMでもOPLLでも術後4~5年で前方法の方が安定した術後成績を保っていた<sup>18)19)</sup>。

一方、前方法では固定隣接椎間板の変性加速が長期の合併症として知られている。しかし、広範囲前方除圧固定術では、あらかじめ相対的な狭窄や不安定性がある隣接椎間を固定範囲に含めていたためか、今回のシリーズでは神経症状再発はみられなかった。河村ら<sup>20)</sup>の4椎間以上の前方除圧固定術において固定隣接椎間障害発生がそれ以下よりも少なかったという報告に合致する。しかし、松岡ら<sup>21)</sup>によれば、術後平均13年のOPLLに対

する頸椎広範囲前方除圧固定術(プレート非使用)57例の長期成績では、隣接椎間障害は6例に認められ再手術が行われたとされる。今後、経過年数が長くなるとC6/7やC7/T1など下位椎間障害が問題となるかもしれない。

自験例からは、プレートを使用しても早期再建合併症は一定頻度で発生し得ることがわかった。移植骨の転位や母床の骨折などの再建合併症は術後1週間以内に開始される起坐より以前、あるいはその直後に発見されていた。固定下位端に起きるために、術直後にルーチンで撮影されるポータブルX線像では、移植骨の転位やスクリーウの突出は正確に把握できないことが多いが、CTの再構成像で明瞭となる(図5)。本シリーズでは再建合併症に対して安静臥床の長期化やハローベストによる外固定追加は行わなかったが、移植骨の転位が進行性の場合に棘突起ワイリングを追加した(図6)。その結果、移植骨が完全に脱転した例はなかった。また、1例には突出したスクリーウの締め直しを行ったが、いずれも術後1カ月以内に最初の処置が行われた。画像による検討では、再建合併症群で頸椎の前弯が大きい例が多く、移

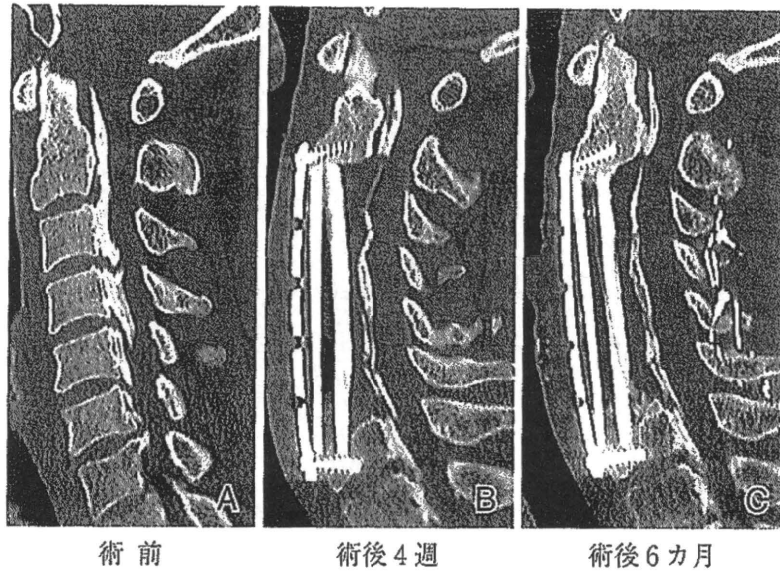


図6 Salvage手術としての棘突起ワイヤリング

図5と同一症例で、51歳のOPLLに対してC2-7前方除圧固定術を行った(A)。術後4週で移植腓骨の明らかな subsidence とともにC7母床の骨折がみられた(B)。棘突起ワイヤリングを追加し、術後6カ月で骨癒合を得た(C)。

植下位端において発生する剪断力が再建合併症の発生に影響している可能性が示唆された。一般に脊髄の除圧には頸椎の前弯が望ましいと考えられているが、広範囲前方除圧固定術においては過度の前弯にならないようにすべきであろう。

一方、術後早期に移植骨転位や母床骨折がみられなかった36例では、移植骨の subsidence は1~3mm程度みられたのみで、その後のスクリーンの突出とは関連性がみられなかった。Hughesら<sup>22)</sup>は、プレートを用いない2~3椎体の亜全摘後の腓骨移植で、術後2年で $6.7 \pm 5.7$  mmの subsidence が起きていたことを報告した。本シリーズではそこまでの subsidence は発生していなかったが、constrained type を使用しても subsidence は生じており、細江ら<sup>23)</sup>の術後1年で4mm程度の短縮を認めたとしている報告と同様であった。広範囲前方除圧固定術では、一定程度の subsidence は不可避と考えられる。終板切除により subsidence がより大きくなることが予想されるが、本シリーズでは明らかな影響は見いだせなかった。

#### おわりに

以上のように、広範囲頸椎前方除圧固定術は、前方プレートにより術後管理が容易となり、安静臥床やハローベストによる外固定も不要になった。しかし、再建合併症が一定頻度でみられ、特に頸椎の前弯が強いケースで固定下位端での移植骨トラブルが目立った。移植骨が完全に脱転する前に棘突起ワイヤリングを追加することで対処は可能であったが、ケースによっては最初から前後同時固定を考慮するなどの対処が必要かもしれない<sup>24)25)</sup>。

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# Effects of Gamma-Ray Irradiation on Mechanical Properties, Osteoconductivity, and Absorption of Porous Hydroxyapatite/Collagen

Yuichi Kawasaki,<sup>1</sup> Shinichi Sotome,<sup>1,2</sup> Toshitaka Yoshii,<sup>1</sup> Ichiro Torigoe,<sup>1</sup> Hidetsugu Maehara,<sup>1</sup> Yumi Sugata,<sup>1,3</sup> Masahiro Hirano,<sup>4</sup> Naomi Mochizuki,<sup>4</sup> Kenichi Shinomiya,<sup>1,3,5,6</sup> Atsushi Okawa<sup>1</sup>

<sup>1</sup> Department of Orthopaedic and Spinal Surgery, Graduate School, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519, Japan

<sup>2</sup> Department of Regenerative Therapeutics for Spine and Spinal Cord, Graduate School, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519, Japan

<sup>3</sup> Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Disease, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519, Japan

<sup>4</sup> PENTAX New Ceramics Division, HOYA Corporation, Itabashi-ku, Tokyo 174-8639, Japan

<sup>5</sup> Hard Tissue Genome Research Center, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan

<sup>6</sup> Core to Core Program for Advanced Bone and Joint Science, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan

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**Abstract:** In this study, the effects of gamma-ray irradiation on the mechanical properties, absorbability, and osteoconductivity of porous hydroxyapatite/collagen (HAp/Col) were investigated. Porous HAp/Col was exposed to 16, 25, 35, or 50 kGy of gamma-ray irradiation. The compressive elastic modulus showed irradiation dose-dependence, with a particularly pronounced decrease in the 50-kGy treatment group. An *in vitro* enzymatic digestion test showed that gamma-ray irradiation of porous HAp/Col resulted in accelerated degradation by collagenase. For *in vivo* studies, porous HAp/Col was transplanted into the back muscles or bone defects in the femoral condyle of rats. Specimens were obtained at 2, 4, and 8 weeks postoperatively. Absorption of the implants in the muscle was time- and irradiation dose-dependent, with notable absorption for the 35- and 50-kGy groups at 2 weeks. At the skeletal sites, porous HAp/Col demonstrated high osteoconductivity in all irradiation treatment groups. Interestingly, not only implant absorption but also bone formation was irradiation dose-dependent at early time points. © 2009 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 92B: 161–167, 2010

**Keywords:** gamma ray irradiation; osteoconduction; bioabsorption; hydroxyapatite composite; collagen

## INTRODUCTION

Artificial bone substitutes are widely used to reconstruct bone defects caused by tumors, trauma, and other adverse events. Sintered calcium phosphates and their composites have been the materials most widely used as bone substitutes,<sup>1–4</sup> although a variety of composites composed of

inorganic and organic materials such as hydroxyapatite/collagen (HAp/Col) have also been developed recently.

HAp/Col has a nanostructure similar to that of natural bone; it is composed of nanoscale needle-like HAp crystals with *c*-axes aligned along type I atelocollagen fibers derived from porcine skin. The HAp/Col weight ratio is 80:20.<sup>5</sup> Previous reports using HAp/Col implants with different macrostructures (dense or porous HAp/Col or as a composite with alginate) have demonstrated satisfactory osteoconductivity and bioabsorption when the implants were used as a bone substitute.<sup>6–9</sup> Above all, porous HAp/Col is a promising bone substitute for clinical use

Correspondence to: S. Sotome (e-mail: sotome.orth@tmd.ac.jp)

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because it has a sponge-like elasticity that makes it easy to handle during surgery. It can also be used as a drug delivery carrier for bone reconstruction.<sup>9</sup>

For clinical implementation of HAp/Col, sterilization is necessary. However, available sterilization methods are limited because of the distinctive composition of this material. Ethylene oxide gas, one of the most popular means of sterilization, stably adsorbs onto HAp crystals because of their broad adsorptive area.<sup>10</sup> Autoclaving also cannot be used for HAp/Col sterilization because the high-pressure steam severely disrupts the collagen fibers while leaving HAp crystals intact.<sup>11,12</sup> Therefore, these common sterilization methods are inappropriate for preparing HAp/Col for clinic use.

Although gamma-ray irradiation also disrupts collagen fibers to some degree, the severity of this disruption is much lower than that caused by autoclaving.<sup>13</sup> However, the specific effects of gamma rays on collagen fibers or collagen-containing composites have not been established. A previous study reported that 25 kGy of gamma-ray irradiation affected not only resistance to enzymatic digestion and the porous structure of a collagen sponge but also *in vitro* cell migration into the sponge.<sup>14</sup> Another group reported that 15-kGy irradiation of a bone graft did not compromise its efficacy.<sup>15</sup> There are many other conflicting reports about the effects of gamma rays on collagen or collagen-based composites.

In this study, the effects of a variety of doses of gamma-ray irradiation on the mechanical properties, bioabsorbability, and osteoconductivity of porous HAp/Col were examined.

## MATERIALS AND METHODS

### Preparation of Porous Hydroxyapatite/Collagen

HAp/Col nanocomposite fibers were synthesized from a Ca(OH)<sub>2</sub> suspension and H<sub>3</sub>PO<sub>4</sub> solution, which contained atelocollagen derived from porcine skin, using a previously reported coprecipitation method.<sup>5</sup> Briefly, 400 mM Ca(OH)<sub>2</sub> and 120 mM H<sub>3</sub>PO<sub>4</sub> containing dissolved collagen (atelo-type I collagen from porcine dermis; Nitta Gelatin, Japan) were simultaneously added slowly to distilled water kept at 40°C in a water bath. The speed of the dropwise addition was controlled to maintain the pH at 9.0. The initial HAp/Col weight ratio was 80:20. The obtained HAp/Col precipitate was lyophilized and used to prepare porous HAp/Col. Next, 1 g of HAp/Col fibers was homogenized with 6.5 mL of phosphate-buffered saline (PBS) and alkalinized with 50  $\mu$ L of 1M sodium hydroxide solution. This mixture was combined with 1.5 mL of 0.6% collagen in phosphoric acid (pH 2.0). The resultant mixture (pH 7.0) was infused into a mold. To initiate gelation of collagen as a binder, the mold containing the mixture was incubated at 37°C for 2 h. Next, the gelled mixture was frozen at -60°C, allowing the remaining liquid to form ice crystals

within the gel. The spaces occupied by the ice crystals were converted to pores by subsequent lyophilization. These lyophilized porous HAp/Col composites were dehydrothermally treated at 140°C for 12 h in a vacuum to introduce crosslinking of the collagen molecules. Porous HAp/Col was then cut to samples 10 mm  $\times$  10 mm  $\times$  10 mm in size for mechanical testing, 10 mm  $\times$  10 mm  $\times$  5 mm for *in vitro* bioabsorption testing, and 2 mm  $\times$  2 mm  $\times$  3 mm for *in vivo* analyses. The porous HAp/Col constructs were sterilized by gamma-ray irradiation at 16, 25, 35, or 50 kGy or treated with 70% ethanol for 10 min as a control.

### Mechanical Testing

The compressive mechanical properties of porous HAp/Col with a 10 mm  $\times$  10 mm  $\times$  10 mm cubic shape were analyzed using a mechanical examination machine with a load cell capacity of 20 N, ( $N = 5$ ) (EZ test-20N; Shimadzu Corporation, Japan). The specimens were moisturized by soaking in distilled water for 2 min under vacuum to remove the remaining air in the pores, causing them to become elastic and sponge-like. Each specimen was compressed perpendicularly at a cross-head speed of 10 mm/min to achieve a strain of 25% and then was immediately returned to the initial position. This cyclic test was repeated four times. The recovery rate and the compressive elastic modulus at 10 and 20% strain were calculated from the stress-strain curve of the fourth compression. After the compression test, the specimens were prepared for observation by scanning electron microscopy (SEM).

### SEM Examination

After mechanical compressive testing, the specimens were fixed with 2.5% glutaraldehyde, cut at the center, and post-fixed with 0.1% osmium tetroxide. Next, the samples were dehydrated through a graded series of ethanol washes, dried by a critical point dryer, mounted onto aluminum stubs, and sputter-coated with platinum. The samples were observed from the cut plane using a SEM (Hitachi S4200 FESEM, HITACHI, Tokyo, Japan).

### In Vitro Bioabsorption

The *in vitro* bioresorbability of porous HAp/Col was tested using the collagenase digestion model reported by Yunoki et al.<sup>16</sup> Blocks of porous HAp/Col (10 mm  $\times$  10 mm  $\times$  5 mm) with a weight of 70.3  $\pm$  3.0 mg (mean  $\pm$  SD) were immersed in 5 mL PBS containing 375 U of bacterial collagenase (*Clostridium histolyticum*; Wako Pure Chemical Industries) and incubated at 37°C for 1 ( $N = 3$ ) or 2 ( $N = 6$ ) h. The collagenase solution and the sediment of degraded HAp/Col were then removed using a pipette. The remaining HAp/Col blocks were frozen, freeze-dried, and weighed. The remaining weight was normalized against the initial weight by taking the percentage.

### Surgical Procedures

All experimental animals used in these studies were maintained and treated according to the guidelines for the care and use of laboratory animals of the Tokyo Medical and Dental University. After anesthesia by intraperitoneal administration of 7% trichloroacetaldehyde monohydrate solution (0.5 mL per 100 g of body weight) and aseptic preparation, HAp/Col implants were transplanted into bone defects created in the distal ends of the femurs and into pouches in the back muscles of male rats (F344, age 11 weeks, weight 240–250 g). The distal end of the femur shaft was exposed by lateral approach and a 3-mm diameter hole was bored using an electric drill with continuous saline irrigation. The drill hole was then rinsed with saline, and the implant was placed in the hole. At 2, 4, and 8 weeks post-transplantation, the rats were euthanized and the femurs and composites transplanted into the back muscles were excised.

### Quantification of Porous HAp/Col in Back Muscle

Porous HAp/Col implants removed from the dorsal muscle were scanned using a micro-CT scanner (ScanXmate-E090; Comscantecno, Japan) before fixation to exclude the effects of fixation on sample volume. Three-dimensional image data were then reconstructed, and the volume of the porous implant was calculated using the three-dimensional bone analysis software TRI/3D-BON (Ratoc System Engineering, Japan).

### Histological Analysis

After micro-CT analysis, samples were fixed in 4% paraformaldehyde, decalcified in 20% ethylenediaminetetraacetic acid (EDTA), dehydrated using a graded alcohol series, and embedded in paraffin wax. Sections of 5- $\mu$ m thickness were cut and stained with hematoxylin and eosin (HE) or tartrate-resistant acid phosphatase stain (TRAP).

### Statistical Analysis

Overall differences among groups in the mechanical test were determined by two-factor repeated measures analysis of variance (ANOVA), and those in the other experiments were determined by two-factor ANOVA, and differences between individual groups were estimated using the Tukey-Kramer multiple comparison test. Differences were considered statistically significant when the  $p$  value was  $<0.05$ .

## RESULTS

### Compressive Mechanical Test

Swelling of the samples after rehydration was not detected by macroscopic observation. All tested samples behaved elastically, and recovery rates after the fourth compression were greater than 92% in all groups, although there was a

TABLE I. Recovery Rates of Compression Test

Control	16 kGy	25 kGy	35 kGy	50 kGy
94.3%	93.4%	93.5%	93.1%	92.8%

slight irradiation dose-dependent decrease in recovery rate (Table I). Compared to the nonirradiated control, the compressive elastic modulus of all irradiated groups was markedly decreased at strains of both 10 and 20%, with the lowest modulus observed in the 50-kGy group [Figure 1(A)]. SEM images of porous HAp/Col specimens after mechanical testing demonstrated that although slight compressive deformation of the porous structures remained, there was no apparent disruption of the materials in any group, including those treated with 50-kGy irradiation [Figure 1(B)].

### In Vitro Bioresorbability

Figure 2 shows the remaining weight of the HAp/Col composite after enzymatic digestion by collagenase. As the irradiation dose increased, the remaining weight decreased. The reduction ratios of the remaining porous HAp/Col in the 35- and 50-kGy groups were significantly higher than those of the other groups.

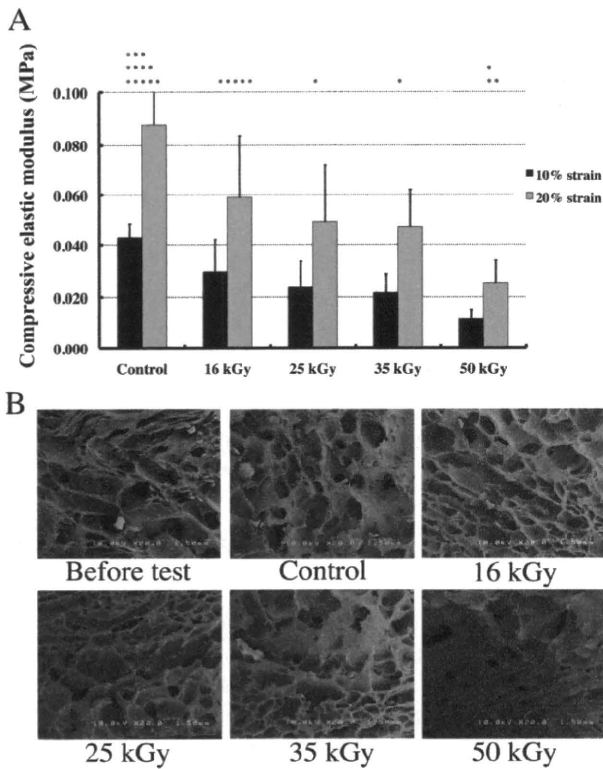
### Quantification of Porous HAp/Col in Back Muscle

Figure 3 shows the volume of residual porous HAp/Col extracted from the back muscle. Control implants were scarcely absorbed until 8 weeks after transplantation. In contrast, all irradiated implants showed dose-dependent decrease in volume, with the 35- and 50-kGy irradiation-treated specimens exhibiting drastic volume decreases 2 weeks postoperatively. The average volume of the 50-kGy group was increased slightly at 4 and 8 weeks, where this may have been due to dispersion of the fragmented implant following tissue invasion.

### Histological Analysis

**Porous HAp/Col at the Extraskelatal Site.** Fragmentation of porous HAp/Col implants and multinucleated macrophage attachment were observed in all irradiated groups at 2 weeks post-transplantation. The 50-kGy-treated implants were the most severely fragmented and reduced, with a large number of multinucleated macrophages attaching to the fragments. In the control group, despite the presence of macrophages, the porous structure of the extracted implants was maintained (Figure 4).

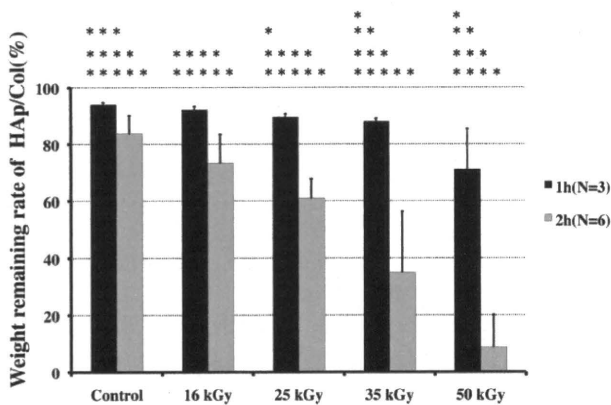
At 4 weeks after implantation, although the extracted implants in the 16-, 25-, and 35-kGy groups were almost completely fragmented and the original porous structures could not be recognized, the volume of the remnants of the 35- and the 50-kGy implants was still lower. Partial fragmentation of the control implant was also observed at 4 weeks postoperatively. At 8 weeks, absorption of the 25-



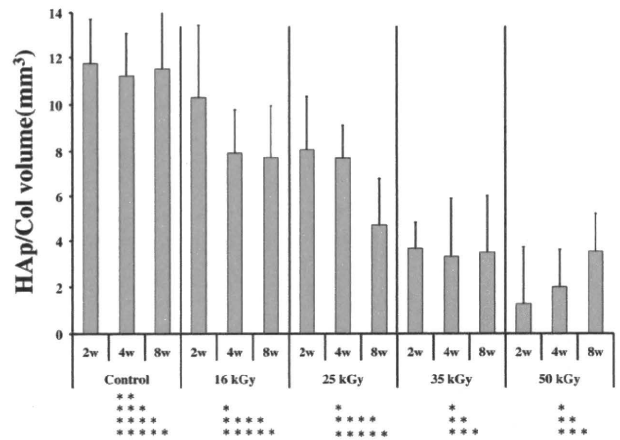
**Figure 1.** (A) Elastic moduli of porous HAp/Col after gamma-ray irradiation at 10 and 20% strain ( $N = 5$ ). \* $p < 0.05$  versus control, \*\* $p < 0.05$  versus 16 kGy, \*\*\* $p < 0.05$  versus 25 kGy, \*\*\*\* $p < 0.05$  versus 35 kGy, \*\*\*\*\* $p < 0.05$  versus 50 kGy. (B) SEM images of the porous structure of HAp/Col before and after compression testing.

kGy implant was advanced when compared with the 16-kGy and control groups. Some control implants even maintained their original porous structure at 8 weeks.

**Porous HAp/Col at the Skeletal Site.** At 2 weeks after implantation, absorption of the irradiated implants was



**Figure 2.** Weight of porous HAp/Col remaining after collagenase digestion. Incubation time of 1 ( $N = 3$ ) or 2 h ( $N = 6$ ). \* $p < 0.05$  versus control, \*\* $p < 0.05$  versus 16 kGy, \*\*\* $p < 0.05$  versus 25 kGy, \*\*\*\* $p < 0.05$  versus 35 kGy, \*\*\*\*\* $p < 0.05$  versus 50 kGy.



**Figure 3.** Residual volume of porous HAp/Col in back muscle quantified by micro-CT analysis. \* $p < 0.05$  versus control, \*\* $p < 0.05$  versus 16 kGy, \*\*\* $p < 0.05$  versus 25 kGy, \*\*\*\* $p < 0.05$  versus 35 kGy, \*\*\*\*\* $p < 0.05$  versus 50 kGy.

dose-dependent, with bioabsorption, fragmentation, and soft tissue invasion from the extraskeletal site observed for 50-kGy implants. Bone formation in the marrow cavity also showed irradiation dose-dependent increases (Figure 5).

At 4 weeks postoperatively, the bone defects of each group were almost completely closed, with no significant differences in the amount of residual implant or bone tissue in the marrow cavity. At 8 weeks, normal bone marrow cavities were restored; implants and surplus bone in the cavities were almost completely absorbed, and small remnants of the implants surrounded by the bone tissue were observed in all groups.

In 2-week implants, TRAP-positive multinucleated cells were attached to both the cavity and extraskeletal sides, and the number of TRAP-positive cells increased as the irradiation dose increased (Figure 6).

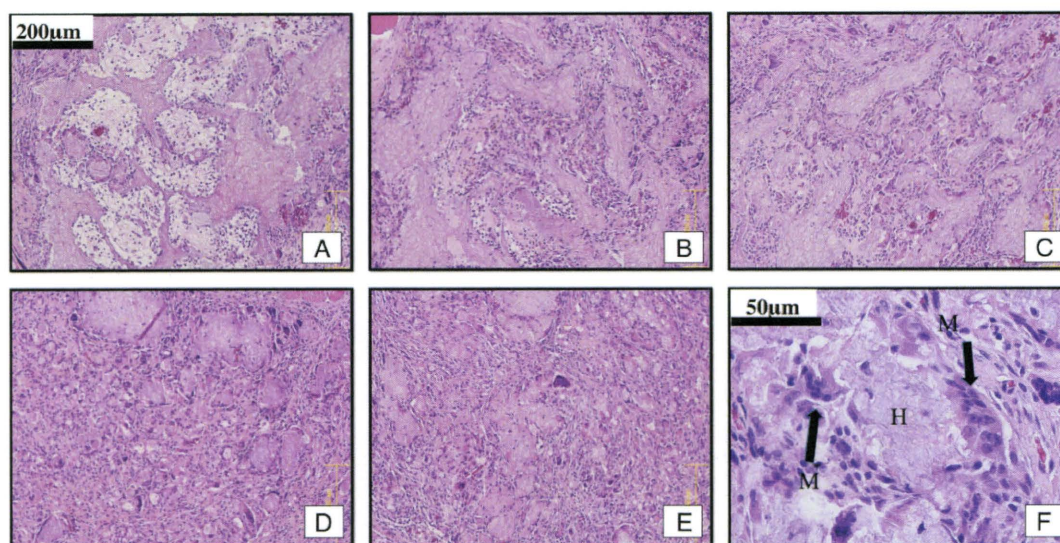
The 4-week implants also exhibited a large number of TRAP-positive cells in every group. The cells were mainly attached to newly formed bone in the 50-kGy gamma-irradiated implants, whereas in the control group, cells mainly adhered to the implant remnant. The number of TRAP-positive cells was markedly decreased at 8 weeks when compared with 2 and 4 weeks postimplantation.

**DISCUSSION**

In this study, gamma-ray irradiation resulted in dose-dependent reduction of elastic modulus and resistance to biological digestion for porous HAp/Col, although bone conductivity at skeletal implantation sites was not impaired.

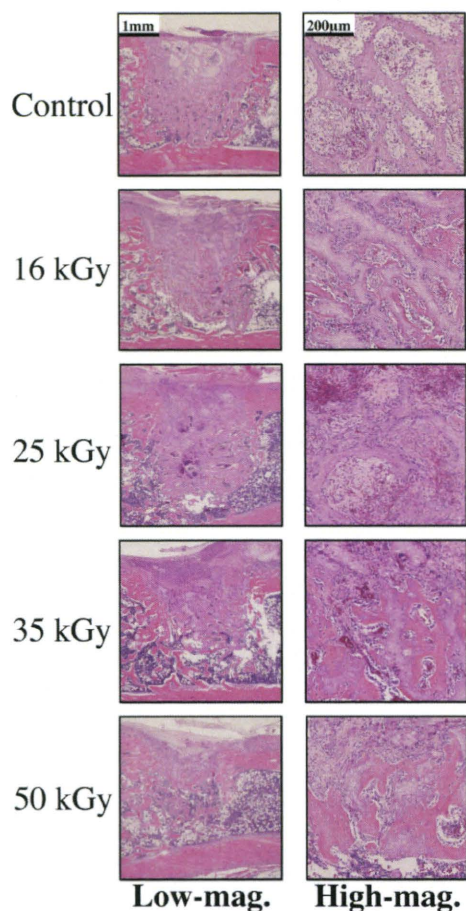
Gamma-ray irradiation from Cobalt 60 sources is a popular sterilization method for medical devices, including implant materials. However, gamma-ray irradiation has also been reported to degrade collagen by splitting the peptide chains, thus adversely affecting the mechanical and biological properties of collagen-containing materials.<sup>13,17</sup> Porous





**Figure 4.** Sections of the HAp/Col implant harvested from back muscle at 2 weeks after implantation (HE staining). (A) Control group (70% ethanol), (B) 16-kGy-irradiated group, (C) 25-kGy-irradiated group, (D) 35-kGy-irradiated group, (E) 50-kGy-irradiated group, (F) high-magnification views of 25-kGy-irradiated group. H, HAp/Col. M, multinucleated macrophage. Implant fragmentation in the 35- and 50-kGy groups was severe. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

## 2 weeks post-implantation

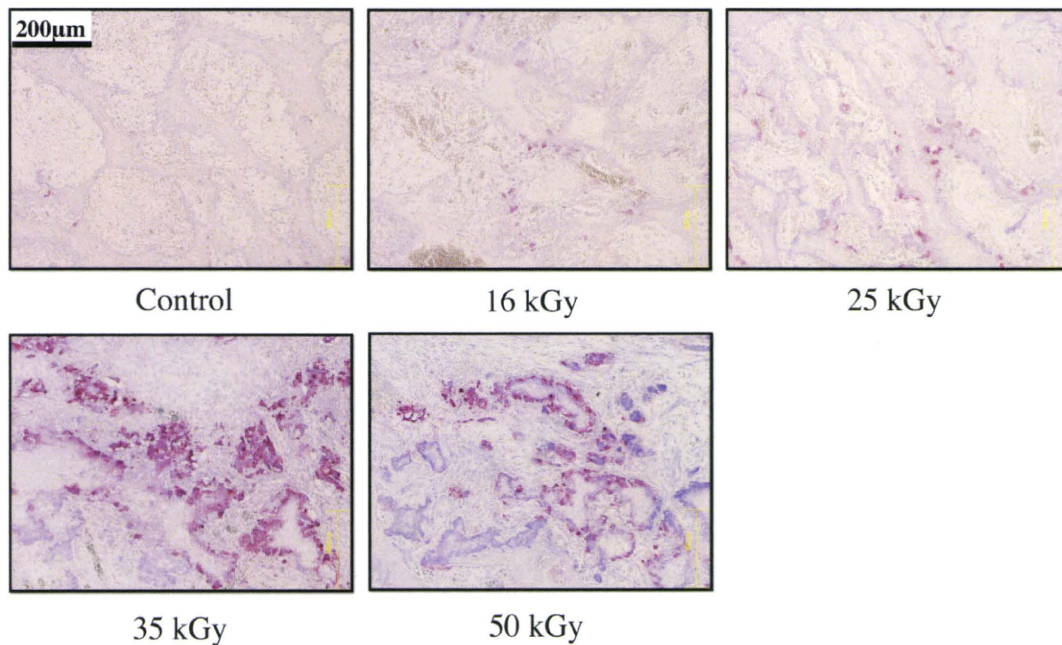


HAp/Col is a bioabsorbable bone substitute with high osteoconductivity and unique mechanical properties including elasticity; these properties permit facile handling during surgery.<sup>9,18,19</sup> Thus, the fragmentation of collagen fibers in HAp/Col by gamma-ray irradiation may significantly affect the ease of use of porous HAp/Col implants.

Porous HAp/Col is elastic and sponge-like and does not have weight-bearing mechanical strength. As a result, its mechanical properties do not contribute directly to its clinical usefulness and, for orthopedic applications, fixation devices must also be used at weight-bearing sites. However, the elasticity of HAp/Col is important for ease of handling and implantation, and therefore, we evaluated the effects of gamma-ray irradiation on elasticity. The elastic moduli of all HAp/Col implants showed irradiation dose-dependent decrease, with the most noticeable decrease observed for the 50-kGy group. Additionally, the 50-kGy-irradiated implants were noticeably fragile during *in vivo* transplantation experiments, although SEM images and the recovery rate after uniaxial compression did not reveal much degradation.

Bone prosthetic materials are commonly used to fill bone defects and accelerate natural bone ingrowth. Ideally, these materials should be absorbed and completely replaced

**Figure 5.** Coronal sections of femurs crossing the center of the implant 2 weeks postoperatively (HE staining). Left column shows lower magnification ( $\times 1.25$ ), right column shows higher magnification ( $\times 10$ ). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



**Figure 6.** TRAP staining of femur sections 2 weeks postoperatively. The number of TRAP-positive cells was irradiation dose-dependent. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

by natural bone in the long term. However, in the clinic, bone defects are not always surrounded by cortical bone or periosteum, and healing may be disrupted by soft tissue invasion of the defect.<sup>20</sup> Bone substitutes implanted in bone defects promote healing by accelerating bone ingrowth due to osteoconductivity and prevention of soft tissue invasion.<sup>21,22</sup> Hence, bone substitutes should resist bioabsorption by macrophages with subsequent soft tissue invasion. Therefore, the effects of gamma-ray irradiation on the bioabsorbability of porous HAp/Col were tested using *in vitro* enzymatic digestion test and *in vivo* implantation models.

*In vitro* tests were performed using collagenase, which is responsible for collagen degradation *in vivo*.<sup>16</sup> *In vitro* digestion of the HAp/Col implant by collagenase was irradiation dose-dependent, with particularly notable degradation after gamma-ray irradiation at 35 or 50 kGy.

In muscular tissue, the porous HAp/Col nonirradiated implant maintained its volume and porous structure until 8 weeks after implantation, whereas in skeletal implantation sites, implants were almost completely absorbed and replaced by bone tissue. Control implants resisted bioabsorption from the extraskelatal site and soft tissue invasion. Intramuscular absorption of irradiated HAp/Col progressed faster than that of the control, especially for 35- and 50-kGy implants, whose volume decreased markedly at early time points. Absorption of the irradiated implants in the marrow cavities also progressed faster than that of the control, although this absorption was

followed by the ingrowth of new bone. Notably, implant absorption and bone ingrowth were synchronized; thus, bone ingrowth of the 50-kGy group was most vigorous at early time points after transplantation. These findings indicate that osteoclast–osteoblast coupling mechanisms might be involved in the osteoconductivity of porous HAp/Col. Although all bone defects treated with irradiated implants eventually healed in the *in vivo* model, concavity and soft tissue invasion were observed on the extraskelatal side of 50-kGy implants due to macrophage adhesion. Thus, if the bone defects had been larger and a porous HAp/Col implant sterilized with 50 kGy or more gamma-ray irradiation had been transplanted, the implant may have been absorbed and the defect may not have healed completely.

In this study, porous HAp/Col was treated with gamma-ray irradiation at 16, 25, 35, and 50 kGy. Irradiation of porous HAp/Col at 16 kGy is the minimum dose for which we could validate sterility (ISO11137-2006, data not shown). Gamma-ray irradiation of porous HAp/Col has few beneficial effects and should thus be applied sparingly. Our results suggest that the proper gamma-ray irradiation dose for sterilization of porous HAp/Col is 16 or 25 kGy.

Porous HAp/Col was jointly developed by the Tokyo Medical and Dental University, the HOYA Corporation, and the National Institute for Materials Science (NIMS). The authors thank Dr. Masanori Kikuchi at NIMS for excellent technical support and advice.

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# 骨のバイオマテリアル—人工骨

Artificial bone substitute

特集

早乙女進一<sup>\*1\*2</sup> 四宮 謙一<sup>\*3</sup> 大川 淳<sup>\*4</sup>  
 SOTOME Shinichi SHINOMIYA Kenichi OKAWA Atsushi

すべての医師のための骨粗鬆症診療ガイド2010 Key words 骨移植 人工骨 組織工学

## 背景

骨は自然治癒能力の高い組織であるが、骨腫瘍や外傷、人工関節の緩みなどで大きな骨欠損が生じた場合や脊椎の固定手術などでは、骨移植が必要となる。移植材料には自家骨、同種骨(他家骨)、人工骨などがある(図1)<sup>1)2)</sup>。自家骨移植は、骨癒合率も高く成績も良好でゴールドスタンダードで

あるが、採取量に限りがあるだけでなく、採取部における合併症の頻度も高く問題となっている。同種骨移植とは、死体骨や人工股関節手術などで摘出した大腿骨頭を移植に用いる方法であるが、国内では同種骨移植に対する抵抗感も強く、普及していないのが現状である。人工骨は、これらの移植材料の問題点を克服するために研究開発が始められた。

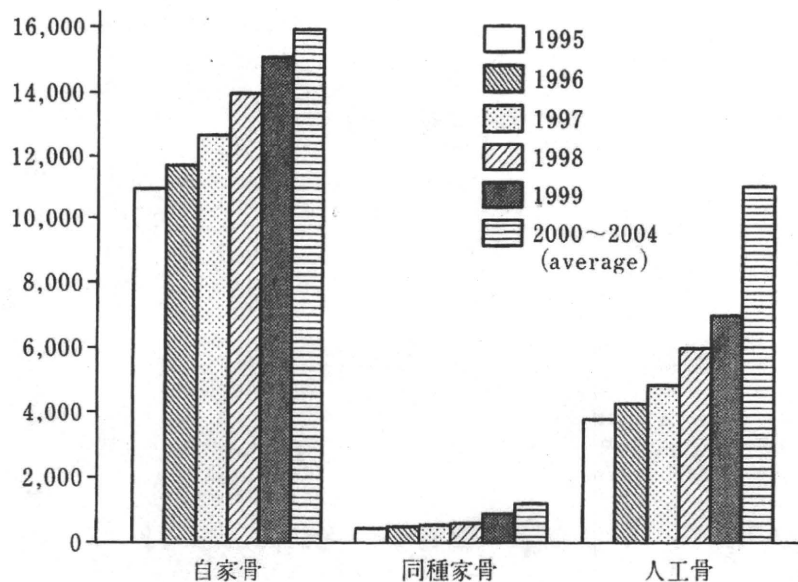


図1 移植骨組織別の推移(文献1, 2より改編)

\*1東京医科歯科大学大学院医歯学総合研究科整形外科先端治療開発学講座 寄附講座准教授 \*2同 整形外科科学分野 \*3教授 \*4准教授