induction (8 weeks after MSC transplantation). Two weeks after induction it was 83±3%, 85±1% at 6 weeks, 88±5% at 10 weeks, 90±4% at 18 weeks and 91±2% at 26 weeks ($P<0.05$) (Fig. 1).

MRI T2-weighted signal intensities in the sham-treated and MSC transplantation groups decreased significantly shortly after the induction of IVD degeneration, with only the MSC transplantation group restored after MSC transplantation. For the sham-operated groups figures were 72±6% 2 weeks after induction, 64±8% 6 weeks after induction, 62±8% at 10 weeks, 56±5% at 18 weeks and 60±4% at 26 weeks. For the MSC transplantation group the figures were 70±6% at 2 weeks, 80±8% at 6 weeks after induction (4 weeks after transplantation), 82±4% at 10 weeks, 84±6% at 18 weeks and 81±2% at 26 weeks ($P<0.05$) (Fig. 2).

From macroscopic evaluations, discs from NC group showed an intact NP without narrowing of the disc space, whereas discs from sham-operated rabbits showed NP with connective tissue invasion accompanied by apparent disc space narrowing. The MSC-transplanted group discs demonstrated reappearance of the NP with restoration of disc space narrowing (Fig. 3).

Histological analysis also proved significant regenerative effects of the procedure. NC group discs show oval-shaped nucleus with no collapse of the inner annular structure. Sham-operated discs show collapse

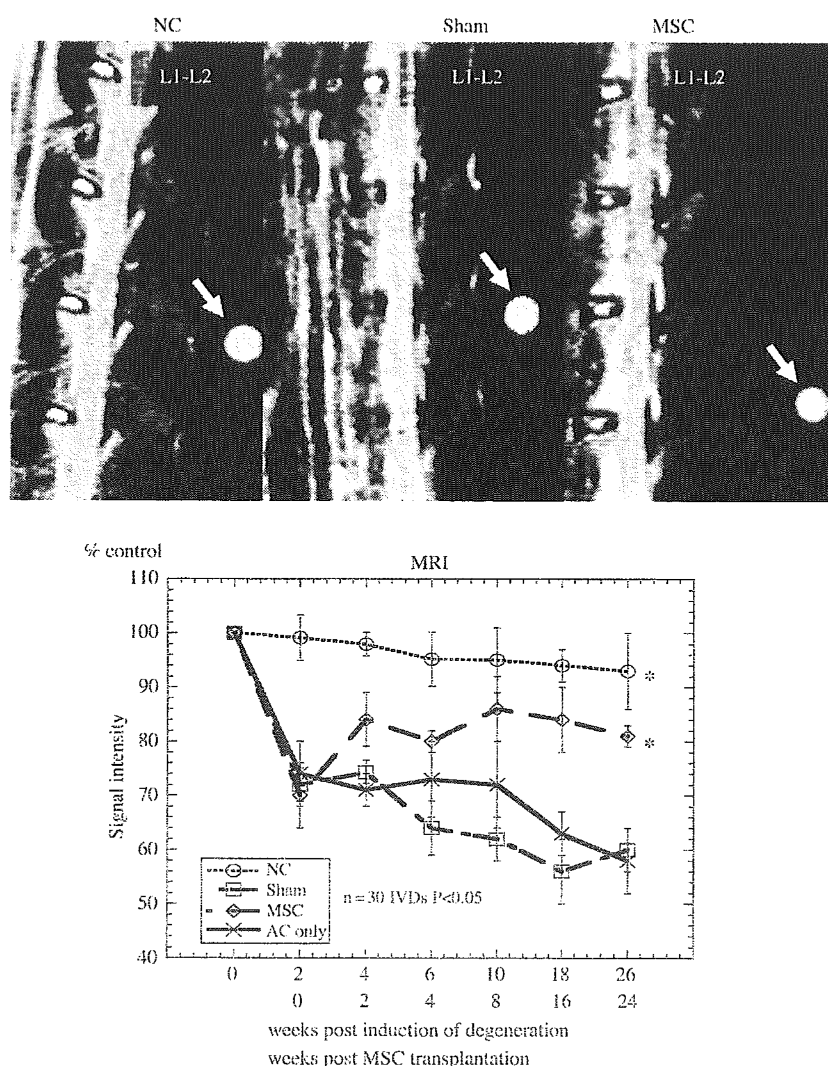


Fig. 2. MRI image of normal control, sham and MSC-transplanted animals taken 26 weeks post-induction of degeneration (24 weeks after MSC transplantation in MSC-transplanted animals). Images of sham and MSC-transplanted animals are among the best model obtained by means of degeneration and restoration. Significant recovery of T2-weighted signal intensity is seen in L2–L3, L3–L4 and L4–L5 discs of MSC-transplanted discs compared to very low signal intensity in sham. The arrows indicate the garlic oil supplement capsules used for standardizing control. Data for animals with atelocollagen (AC) injected, but without cells, are also shown for comparison.

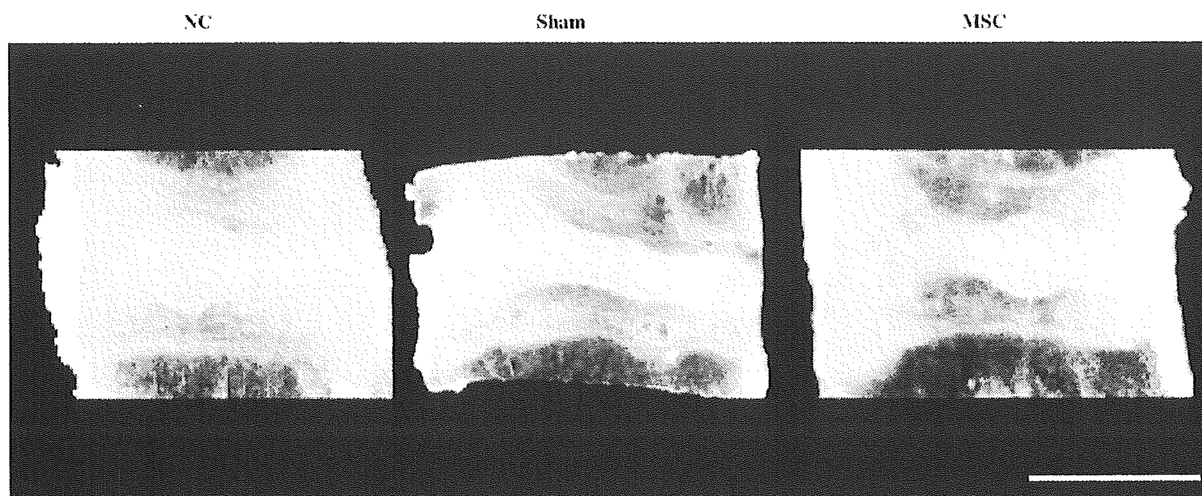


Fig. 3. Macroscopic view of normal control, sham-operated and MSC-transplanted discs harvested at period equivalent to 24 weeks after MSC transplantation in the MSC transplantation group. Note that depletion of nucleus and disc height narrowing is evident in sham group disc, but not so apparent in MSC-transplanted group disc. Bar = 5 mm.

of the inner annulus morphology from 4 weeks (6 weeks after induction of degeneration). Fibrosis in the nucleus due to cell invasion from the surrounding region is completed at 24 weeks. MSC group discs showed relatively preserved inner annulus structure with minimal fibrosis in the nucleus region (Fig. 4a). Disc degeneration grading results showed that MSC transplantation group discs were graded as 1–2 at the most, compared with 4–5 in the sham-operated group. NC group discs maintained grade 0 throughout the study (Fig. 4b and c). Close observation of the sections demonstrated that cells composing the NP in the discs of the MSC transplantation group expressed two distinct cell types: spindle-shaped cells with mildly dense matrix and large oval-shaped cells with occasional vacuoles. Vertebrae from the sham-operated rabbits showed replacement of the disc with fibrosis and dense extra cellular matrix.

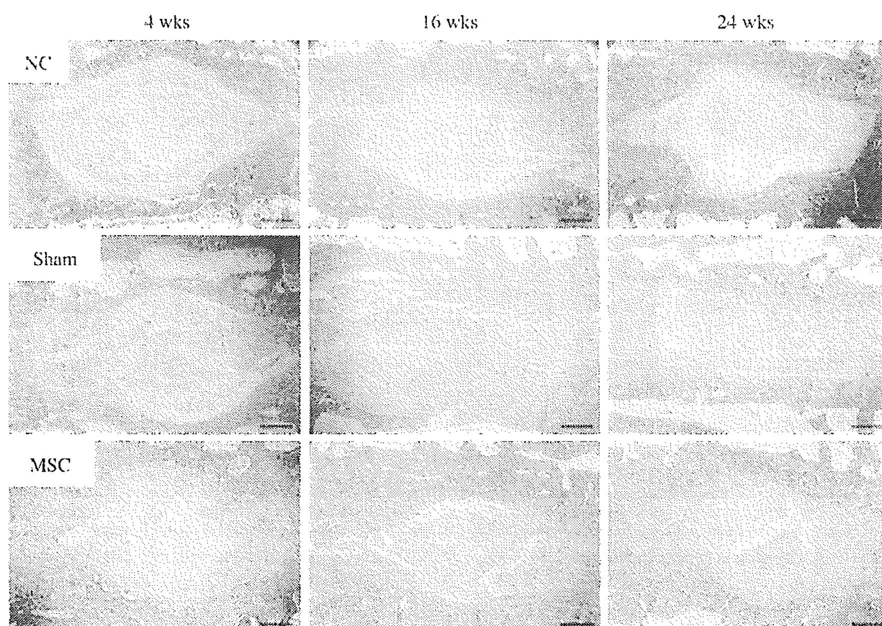
No apparent depositions of lipid or calcium were noted in the nucleus of MSC-transplanted discs showing absence of adipogenesis and osteogenesis. Safranin-O staining and immunohistochemistry results revealed staining patterns similar to normal discs in the MSC transplantation group discs, whereas the sham-operated group discs stained poorly (Fig. 5a and b). This was further confirmed at the mRNA level. RT-PCR analysis of the discs harvested 24 weeks after transplantation showed significant upregulation of both aggrecan and versican gene expression. Type II collagen genes were also restored ($P<0.05$, Fig. 6).

X-gal staining of MSC-transplanted discs 2 weeks after transplantation demonstrated that $27\pm8\%$ of the cells seen in NP were positively stained. The mean percentage of X-gal positive cells increased significantly up to $72\pm12\%$ by 8 weeks after transplantation, thus proving survival and proliferation ($P<0.05$, Fig. 7). X-gal positive cells gradually decreased in sections harvested 8 weeks after transplantation, because adenovirus-mediated *LacZ* gene transfer is transient.

4. Discussion

The multi-lineage differentiation potential and highly viable nature of stem cells has provided many potential techniques to treat various diseases [37]. Clinically, MSC transplantation has become one of the treatment options for full-thickness articular cartilage defects, osteogenesis imperfecta and myocardial infarction, while applications to other diseases are being developed [23–27]. The advantages of MSC transplantation over other cell transplantation therapies is because MSCs are easy to harvest, isolate and grow, with minimum involvement of in vitro techniques [19]. Moreover, MSCs are considered suitable not only for autologous but also allogeneic transplantations, as they lack the expression of HLA class II antigens [20]. Consequently, MSCs serve as a practical cell source widely applicable in clinical settings. Despite such an interest and the growing number of research data, studies directed toward regeneration of the IVD have only just begun.

An atelocollagen gel solution was used here for the cell delivery scaffold because it allows embedded cells to grow in a three-dimensional environment, which is very suitable for disc cells and chondrocytes in vitro [8,31]. Our pilot study on the feasibility of using this scaffold resulted in successful cell delivery [34]. Atelocollagens are clinically safe collagen solutions with reduced



Histological grading of disc degeneration seen from inner annulus structure

Grade 0: normal structure

Grade 1: mildly serpentine appearance of the AF with rupture

Grade 2: moderately serpentine appearance of the AF with rupture

Grade 3: severely serpentine appearance of the AF with mildly reversed contour

Grade 4: severely reversed contour

Grade 5: indistinct

(b) *Nishimura and Mochida 1998*

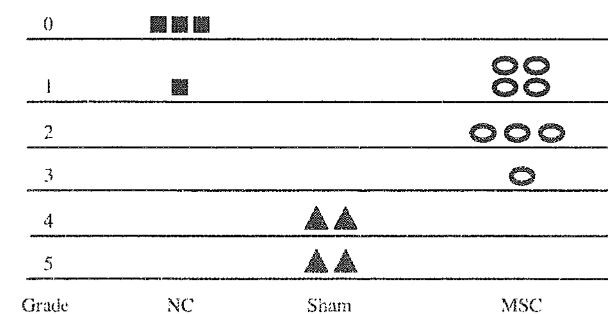


Fig. 4. (a) Histological changes seen over the observed time course after MSC transplantation in MSC-transplanted group discs. Normal control (NC) group discs show oval-shaped nucleus with no collapse of the inner annular structure. Sham-operated discs show collapse of the inner annulus morphology from 4 weeks (6 weeks after induction of degeneration). Fibrotic change in the nucleus due to cell invasion from the surrounding region is completed at 24 weeks. MSC group discs showed relatively preserved inner annulus structure with minimal fibrosis in the nucleus region. Bar = 200 μm. (b) A histological grading system for disc degeneration by Nishimura and Mochida that focuses on morphological change in the inner annulus cells. (c) Diagram showing result of histological grading evaluated at 24 weeks after MSC transplantation among three groups (NC and sham: $n = 4$; MSC: $n = 8$).

immunogenicity. The antigenic region of the collagen molecule—the telopeptide region—is removed by pepsin digestion and differential salt precipitation during purification. The type II atelocollagen used in our study was prepared from bovine cartilage by pepsin digestion and differential salt precipitation in acidic solution. This

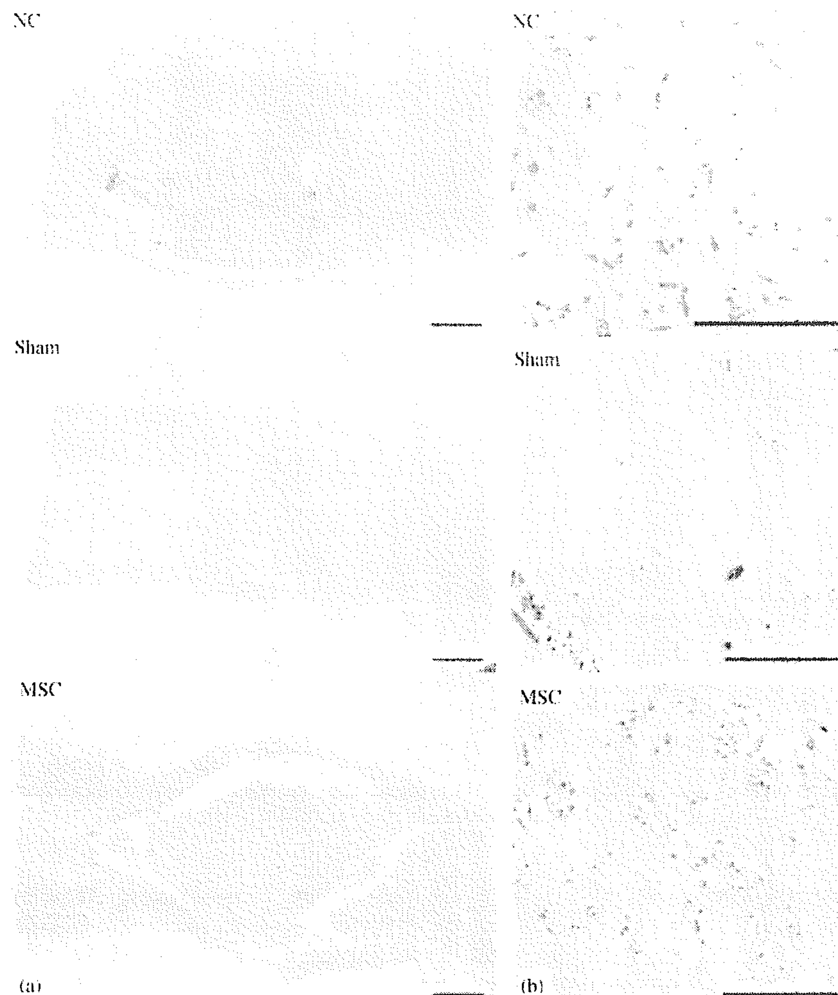


Fig. 5. (a) Safranin-O staining and (b) immunohistochemistry of anti-proteoglycan antibody show strong staining in normal control (NC) and MSC-transplanted discs (MSC). Sham-operated discs demonstrate insufficient staining. Cells in the nucleus region of MSC group showing round-shaped cells similar to NC. Bar = 200 μm.

solution is in liquid form when cooled to a temperature of about 4 °C but gelatinizes when heated to about 37 °C. It holds its three-dimensional structure for about 2 weeks, but eventually decompose to an aqueous solution [38]. Preliminary experiments showed that when atelocollagen scaffold alone was injected into the degeneration-induced discs, it did not show any regenerative effects (Figs. 1 and 2). We have also studied the regenerative effects of hyaluronate sodium solution and type I collagen gel solution injections, but neither of these were effective. Thus, the cellular component is essential to achieve structural and functional regeneration by injections.

Degenerated IVDs were significantly regenerated after MSC transplantation. Restoration of disc height and T2-weighted signal intensity on MRI are two major parameters for evaluating disc degeneration in clinical settings. In the normal aging process, a decrease of disc height occurs with a decrease in water content associated with a reduction in proteoglycans in the nucleus [4,5]. A high signal intensity of T2-weighted images in MRI is often used indirectly to evaluate water content in the IVD [39]. Based on these parameters, regeneration of the disc was achieved successfully with water and proteoglycan restoration. Regaining a high content of glycosaminoglycans as shown by Safranin-O staining and immunohistochemistry for keratan sulfate supports this. Regeneration was also confirmed at the gene expression level using semi-quantitative RT-PCR. However, type II collagen mRNA expression did not show any significant difference between the groups 24 weeks after induction. This is consistent with the report by Anderson et al. [40], who showed that type II collagen mRNA levels tend to increase in the first week but

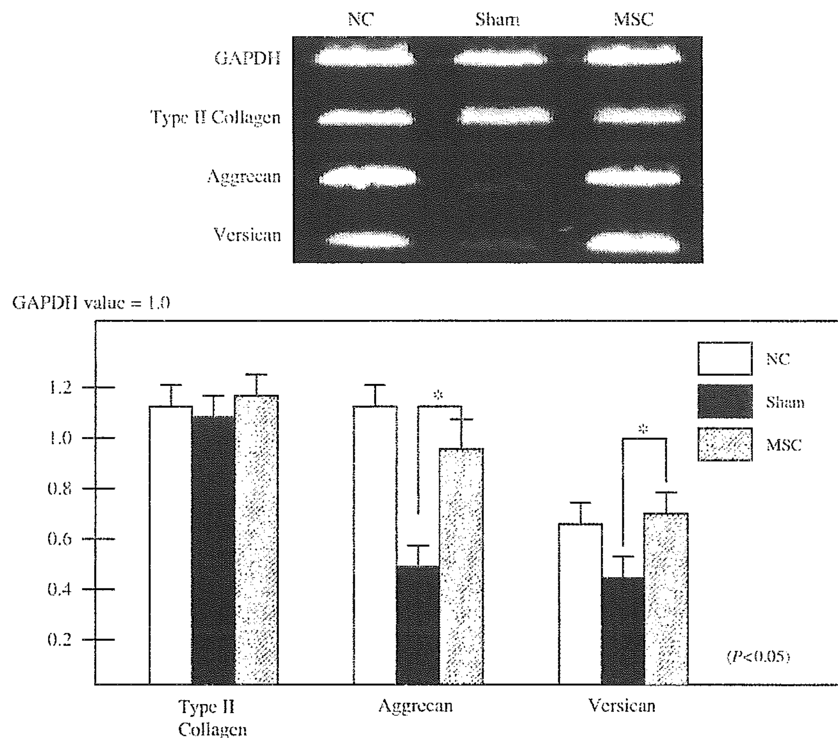


Fig. 6. RT-PCR ($n = 8$, mean data of two discs each from NC and sham-operated rabbits; four discs from MSC-transplanted discs at 24 weeks post-transplantation). Aggrecan and versican mRNA expressions recovered significantly after MSC transplantation.

eventually do not greatly differ against NCs using a rabbit disc degeneration model.

The micro-environment of the nucleus may have directed the transplanted MSCs to differentiate toward an NP cell phenotype, or at least, we have shown in the current study that the procedure enhanced chondrogenic activity in the degenerated discs. The absence of adipogenesis and osteogenesis was also a strong indicator of transplanted site-dependent differentiation.

Histological evaluation using Nishimura's grading system resulted in significant preservation of annular structure after MSC transplantation in MSC group discs compared to sham group discs. This confirmed that reconstitution of annular structure in the disc, an important effect of this procedure that we reported in the previous pilot study, was evident even after 24 weeks. We suspect that the transplanted MSCs functioned as the nucleus preventing invasion of inner AF cells and thus maintaining annular structure without flattening of the disc.

This study focused on the effectiveness of the technique, and we can only hypothesize on the mechanism by which degeneration was prevented. It is possible that the transplanted MSCs differentiated into cells expressing a chondrocyte-like phenotype with restoration of proteoglycan synthesis, as shown by the increased production of glycosaminoglycans and keratan sulfate proteoglycans. Whether the transplanted MSCs expressed a phenotype specific for NP cells or just chondrocyte-like phenotype is not yet clear. In addition, it will be important to compare the effectiveness of transplanting immature stem cells, as in this study, with transplantation of more differentiated progenitor cells of the disc. This is difficult to clarify from the present approach, as the *LacZ* reporter gene expression only lasts for about 8 weeks and there is no specific marker for NP cells. It is also possible that the MSCs served to support or promote regeneration in residual NP cells. MSCs are known for their potential to enhance the viability of neighboring cells [41], and we have used MSCs to improve the viability of NP cells using coculture [42,43]. It is possible that both transformation and helper cell effects were at work.

To clarify the mechanisms completely, further studies in vitro and in vivo are essential. Since our first pilot study, similar studies had been conducted using rat tail IVD model and in rabbits, confirming survival, proliferation and increase in proteoglycan content of the MSC-transplanted disc [44,45]. Establishing a way to induce disc cells from MSCs in vitro is a major challenge as there are currently no specific markers for NP or AF cells [8,33,46]. Moreover, the cellular composition of NPs differs among species and with age, which makes this task more complex [30,47,48]. A more detailed

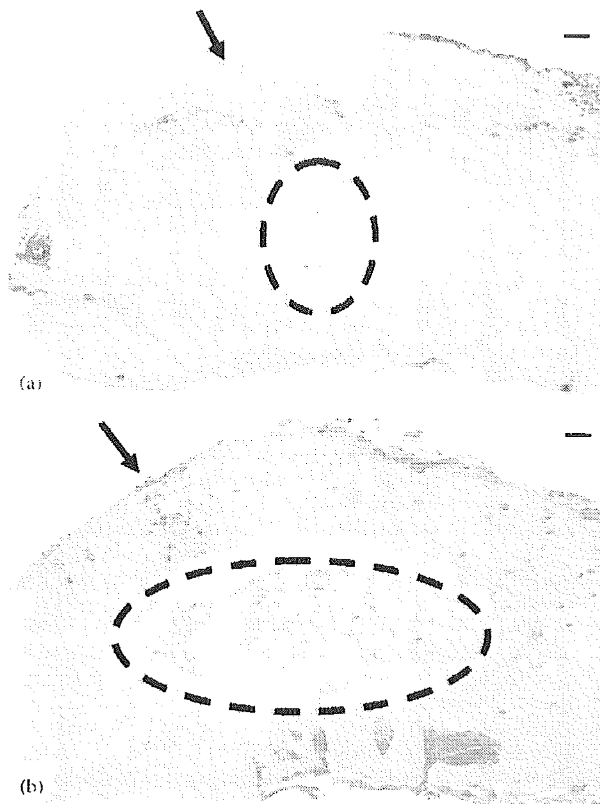


Fig. 7. (a) Frozen section of an MSC-transplanted group disc at week two. X-gal positive cells are primarily seen inside the circled area of the NP. (b) The X-gal stained area has expanded 24 weeks after transplantation. The arrow indicates the site of injection. Bar = 200 μm.

analysis of the NP cells, especially in humans, is thus essential. Furthermore, the regenerative effects of this procedure must also be thoroughly studied using larger animal models or bipedal animals with biomechanical evaluations if we are to consider applying this approach to humans. Although there are obstacles to overcome, results of our study provide an insight that clinical application of MSCs in the treatment of disc degeneration, via transplantation of undifferentiated autologous fresh bone marrow MSCs with the minimal involvement of in vitro techniques, is an attractive proposition both technically and ethically.

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References

[1] Deyo RA, Weinstein JN. Low back pain. N Engl J Med 2001;344:363–70.
[2] Frymoyer JW, Cats-Baril WL. An overview of the incidence and costs of low back pain. Orthop Clin North Am 1991;22:263–71.
[3] Nishida K, Kang JD, Gilbertson LG, Moon SH, Suh JK, Vogt MT, et al. 1999 Volvo Award in basic science: modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenovirus-mediated transfer of the human transforming growth factor beta1 encoding gene. Spine 1999;24:2419–25.
[4] Kraemer J. Natural course and prognosis of intervertebral disc diseases. Spine 1995;20:635–9.
[5] Antoniou J, Steffen T, Nelson F, Winterbottom N, Hollander AP, Poole RA, et al. The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration. J Clin Invest 1996;98(4):996–1003.
[6] Buckwalter JA. Aging and degeneration of the human intervertebral disc. Spine 1995;20:1307–14.
[7] Lee CK. Accelerated degeneration of the segment adjacent to a lumbar fusion. Spine 1988;13:375–7.
[8] Gruber HE, Hanley EN. Recent advances in disc cell biology. Spine 2003;28:186–93.
[9] Nishida K, Kang JD, Suh JK, Robbins PD, Evans CH, Gilbertson LG. Adenovirus-mediated gene transfer to nucleus pulposus cells: implications for the treatment of intervertebral disc degeneration. Spine 1998;23:2437–43.
[10] Paul R, Haydon RC, Cheng H, Ishikawa A, Nenadovich N, Jiang W, et al. Potential use of sox9 gene therapy for intervertebral degenerative disc disease. Spine 2003;28:755–63.
[11] Takegami K, Thonar EJ, An HS, Kamada H, Masuda K. Osteogenic protein-1 enhances matrix replenishment by intervertebral disc cells previously exposed to interleukin-1. Spine 2002;27:1318–25.
[12] Nishimura K, Mochida J. Percutaneous reinsertion of the nucleus pulposus: an experimental study. Spine 1998;23:1531–638.
[13] Okuma M, Mochida J, Nishimura K, Sakabe K, Seiki K. Reinsertion of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an in vitro and in vivo experimental study. J Orthop Res 2000;18:988–97.
[14] Nomura T, Mochida J, Okuma M, Nishimura K, Sakabe K. Nucleus pulposus allograft retards intervertebral disc degeneration. Clin Orthop 2001;389:94–101.
[15] Gruber HE, Johnson TL, Leslie K, Ingram JA, Martin D, Hoelscher G, et al. Autologous intervertebral disc cell implantation: a model using *Psammomys obesus*, the sand rat. Spine 2002;27:1626–33.
[16] Ganey T, Libera J, Moos V, Alasevic O, Fritsch KG, Meisel HJ, et al. Disc chondrocyte transplantation in a canine model: a treatment for degenerated or damaged intervertebral disc. Spine 2003;28:2609–20.
[17] Okano H. Stem cell biology of the central nervous system. J Neurosci Res 2002;15:698–707.
[18] Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641–50.
[19] Prockop DJ. Marrow stromal cells for nonhematopoietic tissues. Science 1997;276:71–4.
[20] Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875–84.

[21] Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. Bone 1992;13:81–8.

[22] Gerson SL. Mesenchymal stem cells: no longer second class marrow citizens. Nat Med 1999;5:262–4.

[23] Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309–13.

[24] Liechty KW, MacKenzie TC, Shaaban AF, Radu A, Moseley AM, Deans R, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 2000;6:1282–6.

[25] Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002;105:93–8.

[26] Caplan AI, Elyaderani M, Mochizuki Y, Wakitani S, Goldberg VM. Principles of cartilage repair and regeneration. Clin Orthop Rel Res 1997;342:254–69.

[27] Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Jt Surg 1994;76-A:579–92.

[28] Im GI, Kim DY, Shin JH, Hyun CW, Cho WH. Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow. J Bone Jt Surg 2001;83-B:289–94.

[29] Quintavalla J, Uziel-Fusi S, Yin J, Boehnlein E, Pastor G, Blancuzzi V, et al. Fluorescently labeled mesenchymal stem cells (MSCs) maintain multilineage potential and can be detected following implantation into articular cartilage defects. Biomaterials 2002;23:109–19.

[30] Horner HA, Roberts S, Bielby RC, Menage J, Evans H, Urban JP. Cells from different regions of the intervertebral disc. Spine 2002;27:1018–28.

[31] Gruber HE, Hanley Jr EN. Human disc cells in monolayer vs 3D culture: cell shape, division and matrix formation. BMC Musculoskeletal Disorders 2000;5(1):1.

[32] Gruber HE, Stasky AA, Hanley Jr EN. Characterization and phenotypic stability of human disc cells in vitro. Matrix Biol 1997;16:285–8.

[33] Risbud MV, Albert TJ, Guttapalli A, et al. Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy. Spine 2004;29:2627–32.

[34] Sakai D, Mochida J, Yamamoto Y, Nomura T, Okuma M, Nishimura K, et al. Transplantation of mesenchymal stem cells embedded in atelocollgen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. Biomaterials 2003;24: 3531–41.

[35] Kanegae Y, Makimura M, Saito I. A simple and efficient method for purification of infectious recombinant adenovirus. Jpn J Med Sci Biol 1995;47:157–66.

[36] Lu DS, Shono Y, Oda I, Abumi K, Kaneda K. Effects of chondroitinase ABC and chymopapain on spinal motion segment biomechanics an in vivo biomechanical, radiologic, and histologic canine study. Spine 1997;22:1828–34.

[37] Preston SL, Alison MR, Forbes SJ, Direkze NC, Poulsom R, Wright NA. The new stem cell biology: something for everyone. Mol Pathol 2003;56:86–96.

[38] Lynn AK, Yannas IV, Bonfield W. Antigenicity and immunogenicity of collagen. J Biomed Mater Res 2004;71B(2):343–54.

[39] Luoma K, Vehmas T, Riihimaki H, Raininko R. Disc height and signal intensity of the nucleus pulposus on magnetic resonance imaging as indicators of lumbar disc degeneration. Spine 2001;26:680–6.

[40] Anderson DG, Izzo MW, Hall DJ, Vaccaro AR, Hilibrand A, Arnold W, et al. Comparative gene expression profiling of normal and degenerative discs: analysis of a rabbit annular laceration model. Spine 2002;27:1291–6.

[41] Kawada H, Ando K, Tsuji T, Shimakura Y, Nakamura Y, Chargui J, et al. Rapid expansion of human umbilical cord hematopoietic projenitors using a novel culture system. Exp Hematol 1999;27:904–15.

[42] Yamamoto Y, Mochida J, Sakai D, Nomura T. Reinsertion of nucleus pulposus cells activated by mesenchymal stem cells using coculture method decelerated intervertebral disc degeneration. Spine J 2003;3:101S.

[43] Yamamoto Y, Mochida J, Sakai D, Nakai T, Nishimura K, Kawada H, et al. Upregulation of the viability of nucleus pulposus cells by bone marrow-derived stromal cells: significance of direct cell-to-cell contact in coculture system. Spine 2004;29:1508–14.

[44] Crevensten G, Walsh AJ, Ananthakrishnan D, Page P, Wahba GM, Lotz JC, et al. Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng 2004;32:430–4.

[45] Zhang YG, Guo X, Xu P, Kang LL, Li J. Bone mesenchymal stem cells transplanted into rabbit intervertebral discs can increase proteoglycans. Clin Orthop 2005;430:219–26.

[46] Roughley PJ. Point of view. Spine 2001;26:1752.

[47] Horner HA, Urban JPG. 2000 Volvo Award in basic science: effect of nutrient supply on the viability of cells from the nucleus pulposus of the intervertebral disc. Spine 2001;26: 2543–9.

[48] Aguiar DJ, Johnson SL, Oegema TR. Notochordal cells interact with nucleus pulposus cells: regulation of proteoglycan synthesis. Exp Cell Res 1999;246:129–37.

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分担研究報告書

高齢者の腰痛症に係るより効果的かつ効率的な診断、治療、介護及びリハビリテーション等の確立に関する研究

研究課題名：骨粗鬆症性脊椎圧迫骨折に対する新たな低侵襲治療法の開発

分担研究者：武政龍一　高知大学医学部生体機能感染制御学講座運動機能学教室 講師

研究要旨：椎体形成術モデルを用いた実験により、CPC 硬化体の圧縮強度が注入時の諸条件により大きく変化することが示唆された。高粉/液比での使用、血液混入の抑制、骨腔の最深部から CPC を注入充填することなどにより、良好な結果が得られることが明らかになった。

A. 研究目的

本邦独自に開発された Calcium Phosphate Cement（以下 CPC)は、粉剤と溶解液との練和により、注入操作によるセメント充填を可能とするペースト状人工骨であり、非発熱性の水和反応にてハイドロキシアパタイトに組成を変えながら自己硬化し、3 日で約 80MPa の圧縮強度に至る生体活性セメントである。我々は、骨粗鬆症性椎体骨折の進行性椎体圧潰や、偽関節などの骨癒合不全に対し、低侵襲性に行えるCPC椎体形成術を開発し、早期の除痛や椎体変形の矯正に成果を挙げてきた。これまで93例107椎体に対し正中を6cm切開する開創術式にて CPC の椎体内注入術を行い、その安全性と有用性を報告してきたが、現行法の問題点として、CPC 硬化体の圧縮強度が手術手技に大きく依存し、不適切な手技では CPC の fragmentation が生じて処置椎体の再圧潰を来たすなどの強度不足が問題となる場合があること、現行法では約 6cmの正中皮膚切開と傍脊柱筋の剥離を必要としており、欧米で行われている PMMA の経皮的注入法に比べると、侵襲性という点においては劣っていることが挙げられる。今回、実際の椎体形成術の状況を想定した実験モデルを用いて、CPC の最大圧縮強度を得るための術中条件を模索した。また更なる低侵襲術式への改良を目指して新術式の開発を行った。

B. 研究方法

練和後の経過時間、粉液比、血液混入の有無、および CPC 注入充填方法の違いが CPC 硬化体の圧縮強度に与える影響を実験的に調べた。直径 16mm 高さ 32mm の容器を椎体に見立て、それにヒト静脈血を 3cc 入れ、臨床で使用するセメントガン注入システムで CPC を容器に注入充填して円柱状の硬化体を得る椎体形成術モデルを作成した。このモデルにて、2種類の粉液比(高粉/液比：粉剤18g/液剤4.5ml、低粉/液比：粉剤18g/液剤5.7ml）で、血液貯留容器あるいは空の容器内へ、それぞれCPCの排出ノズル先端部を底面付近に設置して注入充填する方法およびノズル先を天井面付近に設置して上部から注入充填する方法にて CPC の練成体を作成した。それらを擬似体液中に,3,12時間、1,3,7日間浸漬して硬化体を作成し、インストロン 1125 力学試験機で圧縮最大強度を求めた。

両側椎弓根上に小切開を加え、直径 18mmX 線透過性円筒状レトラクターにて傍脊柱筋内に portal を作成し、新しく作成した手術器材を用いて portal を介した椎体内操作を行う Biportal transpedicular vertebroplasty を開発した。Biportal 法で行った 5 例と、従来の正中 6cm切開で行ってきた直近 13 例（以下開創群）におい

て、術後早期の背部痛の推移、術中出血量、手術時間、変形矯正効果、合併症発現頻度について比較した。

C. 研究結果

経過時間に関してはどの練成体も約 72 時間で最大圧縮強度近くまで硬化していた。圧縮強度を高く維持する条件は、高い粉/液比での使用、血液の混じらない状況下における容器の底面からの注入法であった。最終の 7 日経過時の圧縮強度は、上記条件で注入すれば 69.5MPa の圧縮強度を表わしたのに対し、低粉/液比、血液貯留容器内に天井部から注入したものの圧縮強度は 34.0MPa であり、その強度は半分以下に低下していた。

Biportal 群において腰背部痛の VisualAnalog Scale は、術前平均 83mm から最終調査時 7mm へと著明に改善した。術後 14 日目までの腰背部痛 VAS の推移を比較すると、術翌日から 5 日目までの期間は Biportal 群が開創群よりも有意に VAS 値が低く、7 日目以降は有意差を認めなかった。手術時間は、まだ不慣れな Biportal 群で平均 109 分と長かったが、術中出血量は、Biportal 群で 23ml と有意に少なかった。Biportal 郡の椎体楔状変形率は、術前 18%の楔状変形が、調査時 73%へと著明に改善しており、椎体内への CPC 充填は開創術式に劣らず良好であった。合併症は創癒合不全で再縫合を 2 例に要し、隣接椎体に骨折を 1 例に認めたが、CPC の椎体外漏出や感染などの合併症は無かった。

D. 考察

椎体形成術モデルを用いた実験により、CPC 硬化体の圧縮強度が注入時の諸条件により大きく変化することが示唆された。すなわち椎体形成術で CPC 硬化体圧縮強度を高く維持する方法として、高粉/液比での使用、低血圧コントロールで骨髄出血を抑制し、骨腔内の貯留液を吸引排出するなどの、出来るだけ血液の混じらない状況を確保した上で、注入ノズル先端を骨腔の最深部に設置して腔の底面から CPC を注入充填することの重要性が明らかとなった。Biportal 法による CPC 椎体形成術は、現在まで 7 例に行い、いずれも従来の開創術式に劣らない椎体変形矯正効果が得られ、より早期の除痛と低侵襲性が達成されていた。漏出などの合併症もなく、背筋の健常性が温存される点で、従来法よりも低侵襲性に優れた術式となるものと思われる。まだ手術器材の改良や症例数を増やして長期にわたり経過観察を続ける必要性はあるものの、従来の開創術式の有効性と安全性を踏襲したまま、より低侵襲性に行える術式として有望と思われた。

E. 結論

椎体形成術モデルを用いた実験により、CPC 硬化体の圧縮強度が注入時の諸条件により大きく変化することが示唆された。高粉/液比での使用、血液混入抑制、骨腔の最深部から CPC を注入充填することなどにより、良好な結果が得られることが明らかになった。

F. 健康危険情報

問題なし。

G. 研究発表

1. 論文発表：

1) 武政龍一、山本博司、谷俊一、北岡謙一：生体活性リン酸カルシウム骨ペーストの椎体内注入補填による骨粗鬆症性椎体骨折修復術. 日整会誌 7:237-242, 2004

2) 武政龍一、谷俊一、北岡謙一、他 2 名：骨粗鬆症性椎体偽関節による遅発性脊髄・神経麻痺に対するリン酸カルシウム骨セメントを応用した脊椎再建術. 中国四国整形外科学会誌 16: 115-121, 2004

3) 武政龍一、溝渕弘夫、谷俊一, 他 1 名：リン酸カルシウム骨セメントによる骨欠損部の注入療法—主として骨粗鬆症性椎体骨折についてー. 関節外科 23:91-98, 2004

4) 武政龍一：リン酸カルシウム骨セメントを使用した骨粗鬆症性椎体骨折の治療. 整形外科最小侵襲ジャーナル 33: 21-28, 2004

5) 武政龍一、谷俊一、北岡謙一、喜安克仁、山本博司：神経症状を有する骨粗鬆症性椎体圧潰に対するリン酸カルシウム骨セメントを用いた後方侵入前後同時再建術. 中部日本整形外科災害外科雑誌 47: 673-674, 2004

6) 武政龍一、溝渕弘夫、谷俊一、山本博司：リン酸カルシウム骨ペースト椎体内注入術の長期経過. 第 4 回バイオアクティブペースト研究会論文記録集：86-90, 2004

7) 喜安克仁、武政龍一、溝渕弘夫、谷俊一、山本博司：リン酸カルシウム骨セメント椎体内注入術を施行した 3 年以上経過例の検討. 中四整会誌 16:277-282, 2004

8) 武政龍一、溝渕弘夫、喜安克仁,他 2 名：生体材料—この 10 年の進歩.我々の使用経験からみたリン酸カルシウム骨セメントの評価. 骨・関節・靱帯 17:1185-1194, 2004

9) 武政龍一：脊椎脊髄外科基本手技 Q&A:リン酸カルシウム骨セメントを使用した椎体形成術について. 脊椎脊髄 17: 1164-1167, 2004.

10) 武政龍一：座談会「脊椎外科における人工骨、人工椎間板」. THE SPINE perspective 4：1-6, 2004

11) Ryuichi Takemasa, Hiroshi Yamamoto, Toshikazu Tani, Kenichi Kitaoka: Calcium phosphate cement kyphoplasty with instrumentation for thoracolumbar ischemic vertebral collapse with spinal cord compression. The Spine Journal 5 : 79-80s, 2005

12) 武政龍一、谷俊一、溝渕弘夫、喜安克仁、山本博司：骨粗鬆症性椎体骨折に対するバイオアクティブペースト椎体内注入術の臨床成績 第 5 回バイオアクティブペースト研究会論文記録集：78-84, 2005

13) 武政龍一、谷俊一、喜安克仁、山本博司：高齢者骨粗鬆症性椎体骨折偽関節に対するリン酸カルシウム骨セメントを用いた椎体形成術 骨・関節・靱帯 18:425-434, 2005

14) Ryuichi Takemasa, Hiroshi Yamamoto, Toshikazu Tani, Kenichi Kitaoka: Calcium phosphate cement vertebroplasty with pedicle screw fixation for osteoporotic thoracolumbar vertebral collapse with spinal cord compression. European Spine Journal 14 : 57s, 2005

15) 武政龍一：骨粗鬆症性椎体骨折偽関節に対するリン酸カルシウム骨セメントを用いた椎体形成術 The Spine Perspective4:10-14, 2005

16) 武政龍一：リン酸カルシウム骨セメント（CPC）を用いた脊椎椎体骨折の外科治療 Journal of Clinical Rehabilitation14:1021-1025, 2005

17) 武政龍一、谷俊一、喜安克仁、北岡健一、山本博司：骨粗鬆症性椎体骨折および偽関節に対するリン酸カルシウム骨セメントを用いた椎体形成術 中部日本整形外科災害外科雑誌 48: 869-870, 2005

2. 学会発表：

1) Takemasa R, Yamamoto H, Tani T, Mizobuchi H, Kitaoka K：Kyphoplasty using bioactive calcium phosphate cement for osteoporotic vertebral fractures. 2004 annual meeting of American Academy of Orthopaedic Surgeons in San Francisco, 2004

2) Takemasa R, Tani T, Kitaoka K, Kiyasu K, Yamamoto H : Kyphoplasty using calcium phosphate cement combined with neural decompression and instrumentation for thoracolumbar ischemic vertebral collapse with neurological compromise. The 31st annual meeting of International Society for the Study of Lumbar Spine. In Porto, Portugal, 2004

3) Takemasa R, Tani T, Kiyasu K, Yamamoto H : The clinical application of calcium phosphate cement to vertebroplasty for repairing osteoporotic vertebral fractures and the pseudarthrosis. 12th International Meeting on Advanced Spine Techniques. In Banff, Canada, 2005

4) Takemasa R, Kiyasu K, Tani T, Kitaoka K, Yamamoto H : Calcium phosphate cement vertebroplsty with pedicle screw for osteoporotic thoracolumbar vertebral collapse with spinal cord compression. EuroSpine 2005. In Barcerona, Spain, 2005

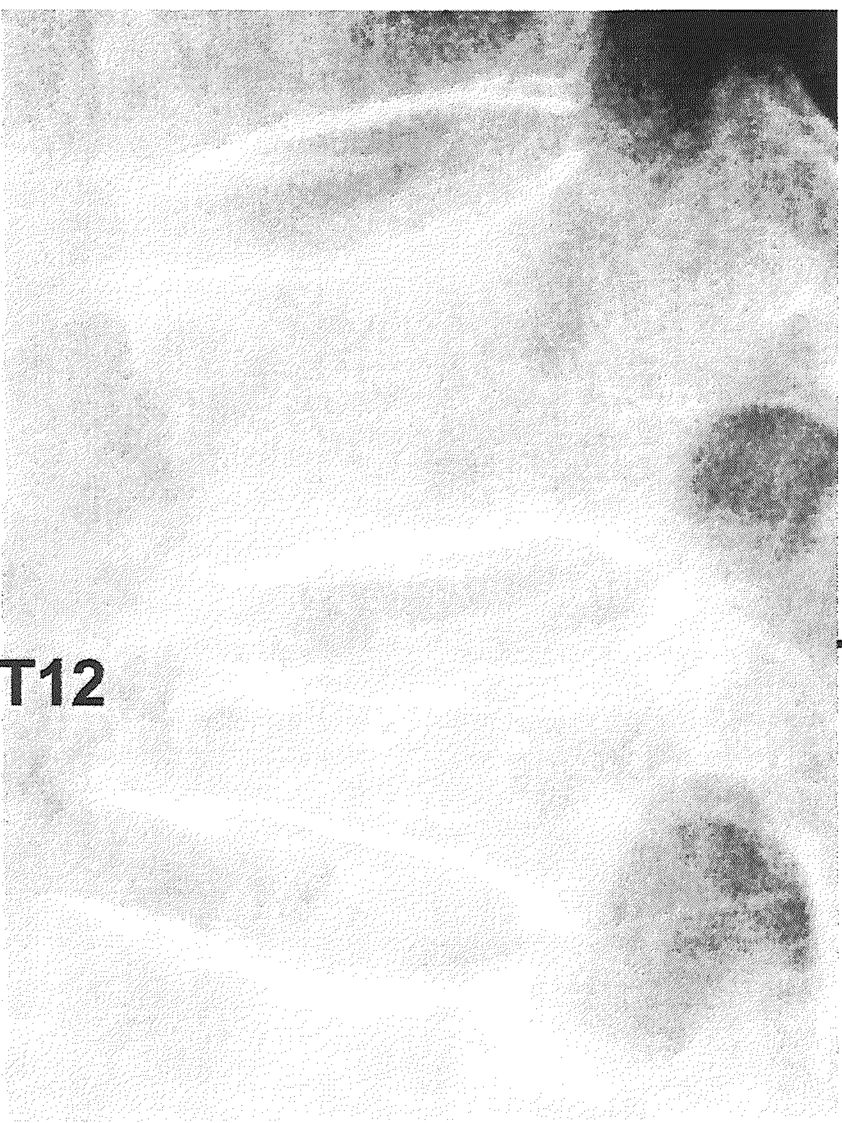
5) Takemasa R, Yamamoto H, Tani T, Kitaoka K :Calcium phosphate cement kyphoplasty with instrumentation for thoracolumbar ischemic vertebral collapse with spinal cord compression.

20th North American Spine Society, Philadelphia, USA, 2005

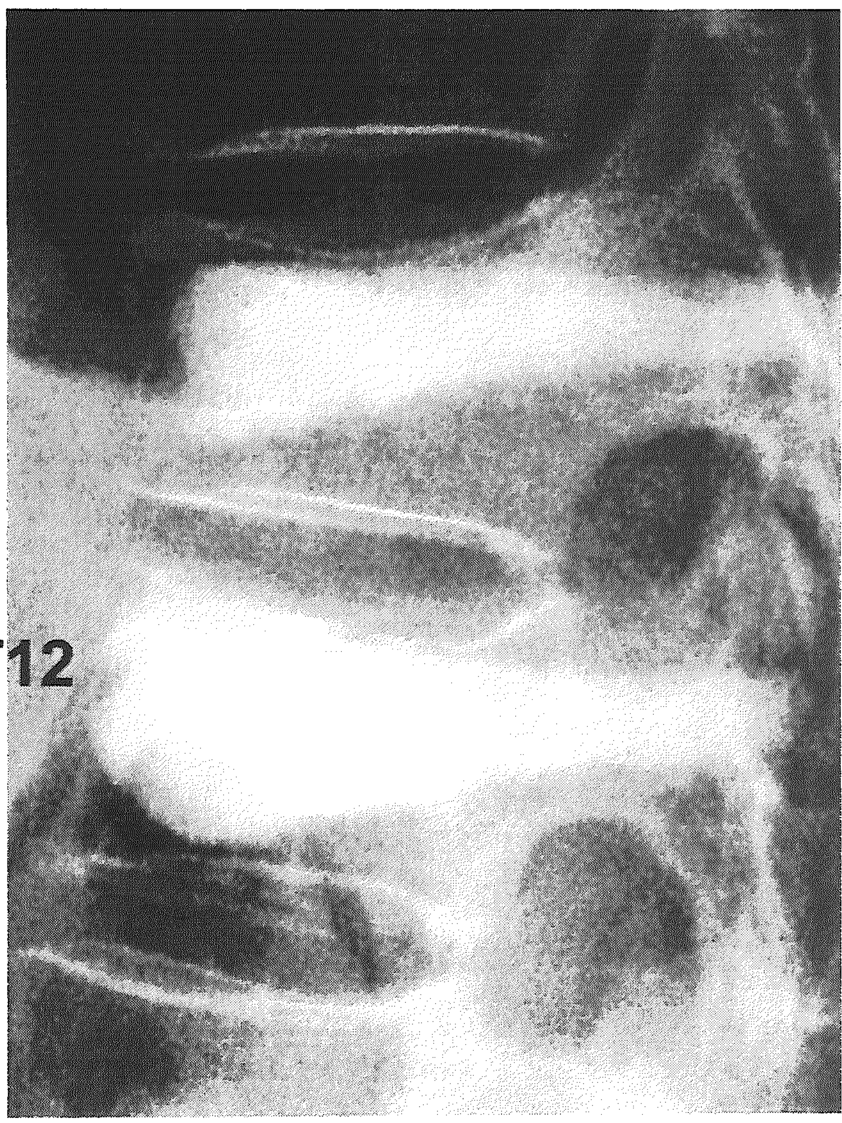
6) 武政龍一：骨粗鬆症性椎体骨折に対するリン酸カルシウム骨セメント椎体内注入術. 第108回西日本整形・災害外科学会特別講演 （日本整形外科学会教育研修講演）2004
7) 武政龍一：高齢者脊椎圧迫骨折の治療上の問題点, 第 78 回日本整形外科学会学術集会. 教育研修講演. 横浜 2005

H. 知的財産権の出願・登録状況
予定していない。

開創式リン酸カルシウムセメント(CPC)椎体内注入術：93例107椎体



Preop



Postop

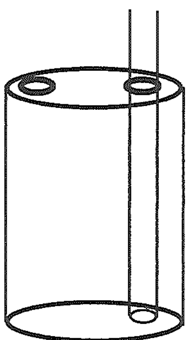
椎体形成術モデル試験

■粉液比　　粉/液＝18g/4.5ml：高粉/液比

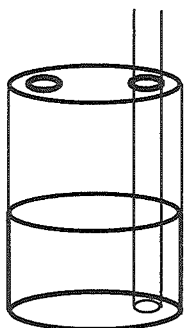
粉/液＝18g/5.7ml：低粉/液比

■血液の有無と注入充填方法

血液なし

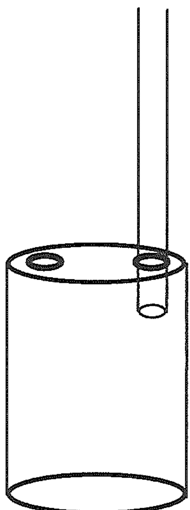


血液あり

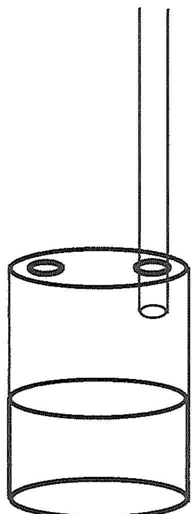


底面から注入

血液なし



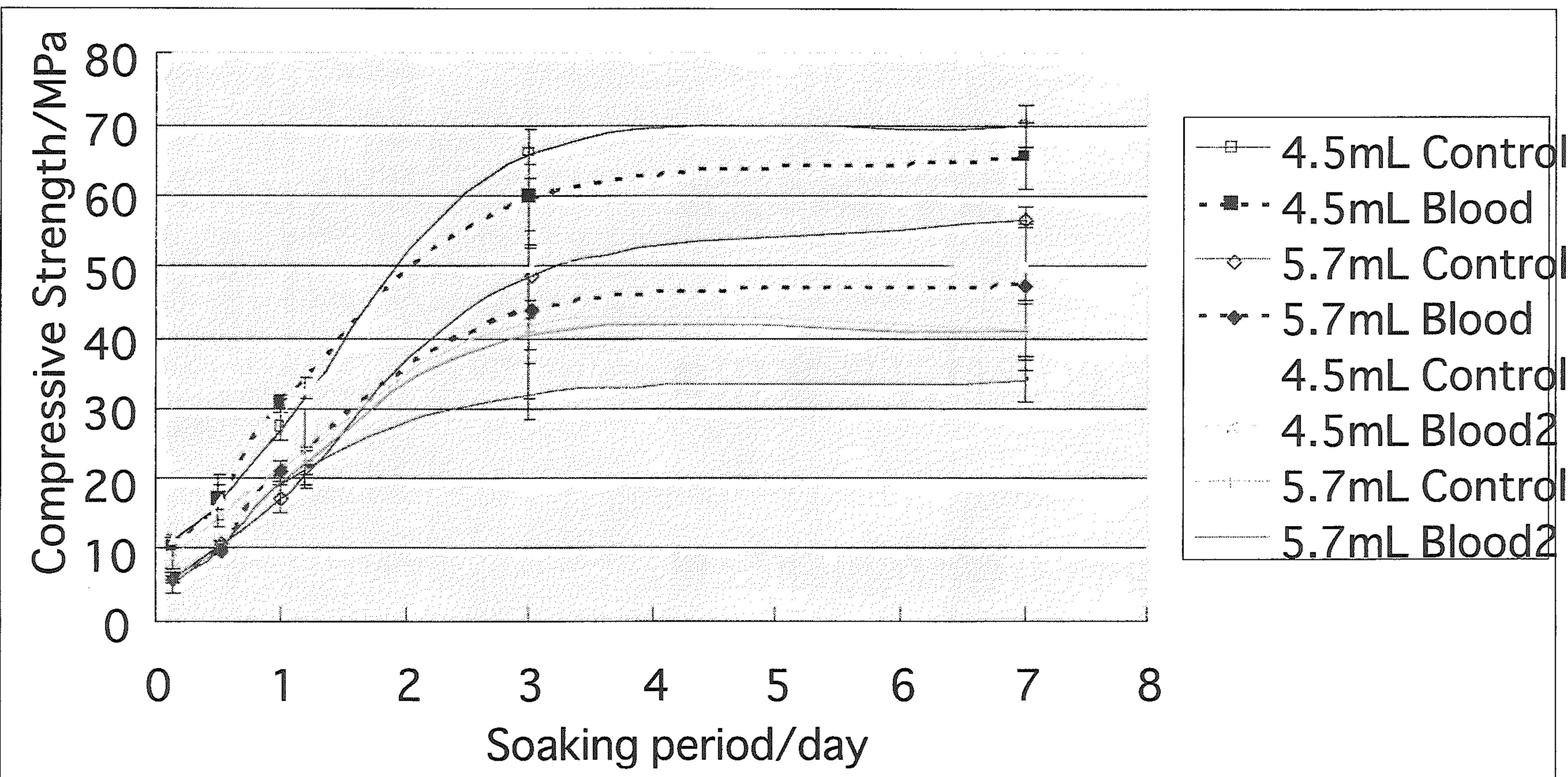
血液あり



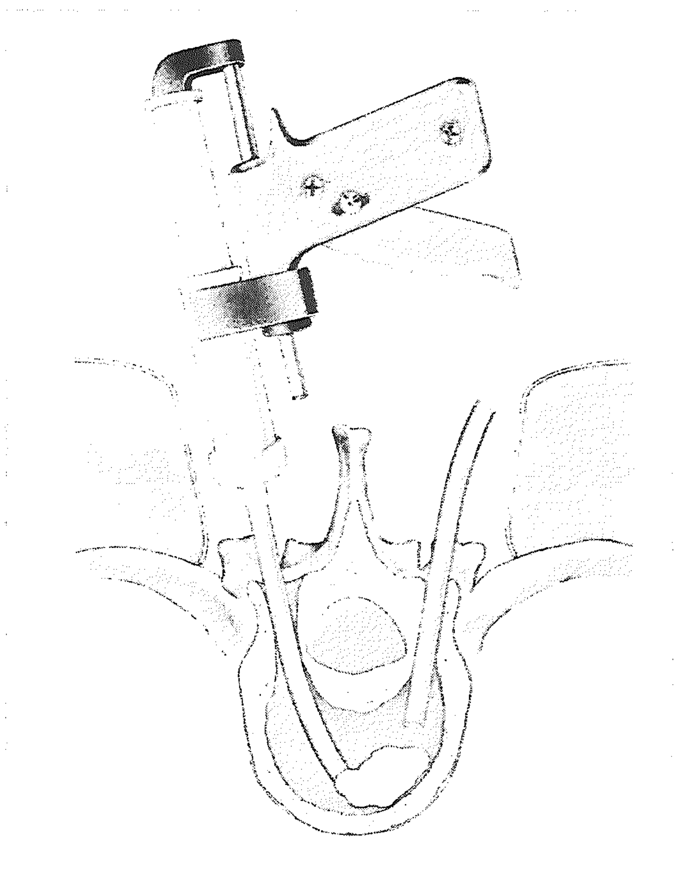
入孔部（天井）から注入

■経過時間　CPC練成体を37℃擬似体液中に3, 12時間, 1,3, 7 日間浸漬

圧縮強度試験結果



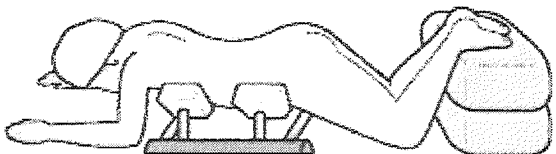
CPC硬化体の圧縮強度を高く維持するためには



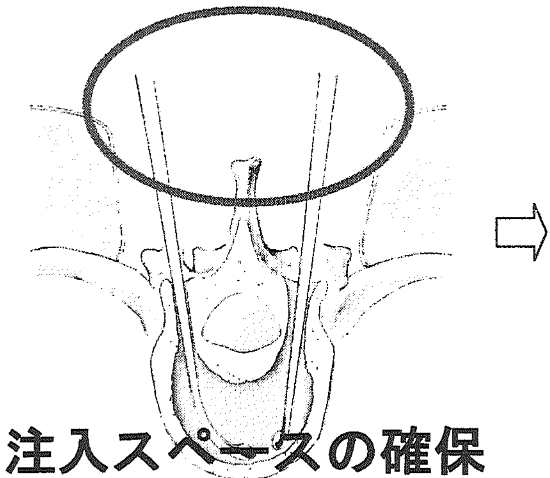
- ■72時間の硬化時間を確保
- ■出来るだけ血液の混入を避ける
- ■高い粉液比
- ■骨腔最深部から注入充填する

開創式術式によるCPC椎体内充填法

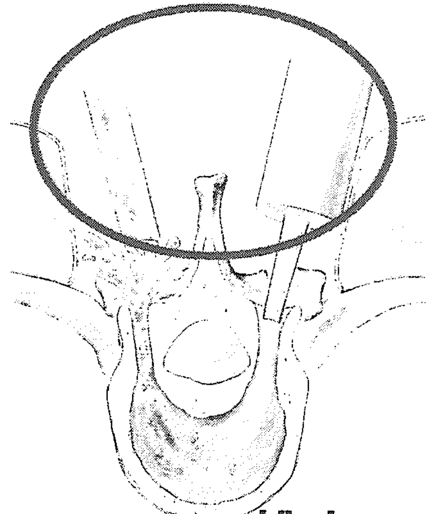
骨伝導能の有効利用
安全性



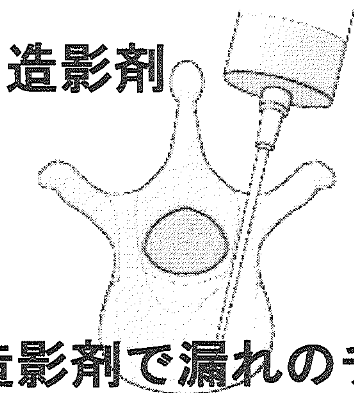
椎体変形の整復



注入スペースの確保

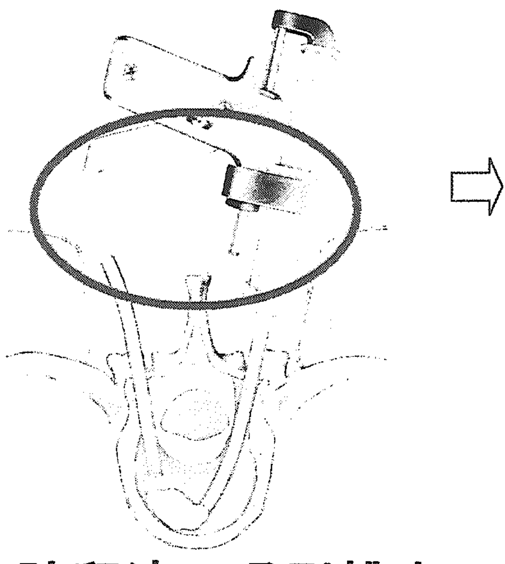


Debrisの排出

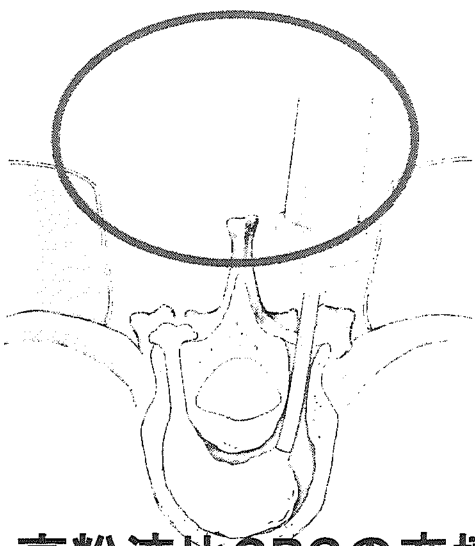


造影剤で漏れのチェック

低血圧コントロール



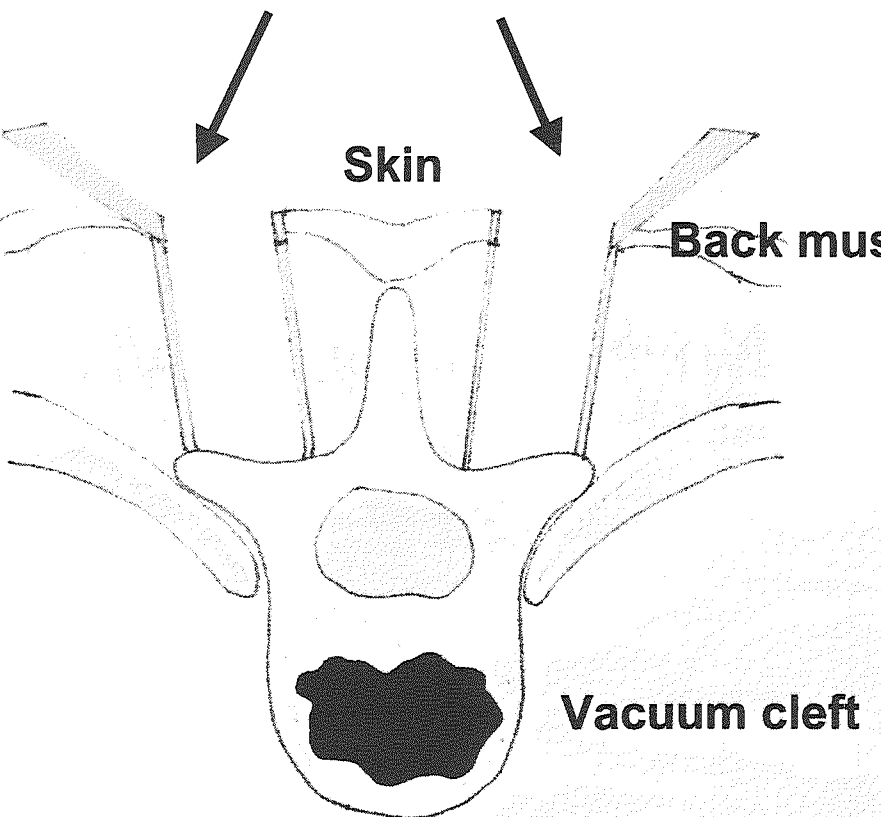
貯留液の吸引排出



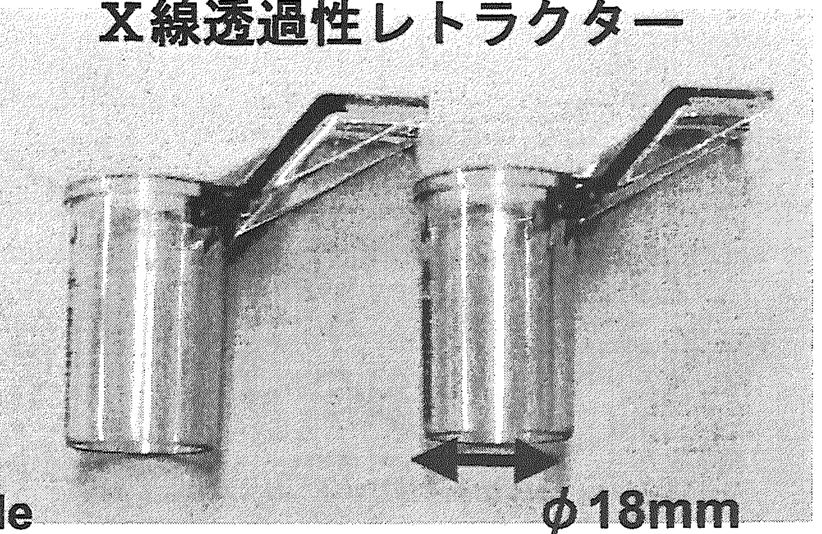
高粉液比CPCの充填

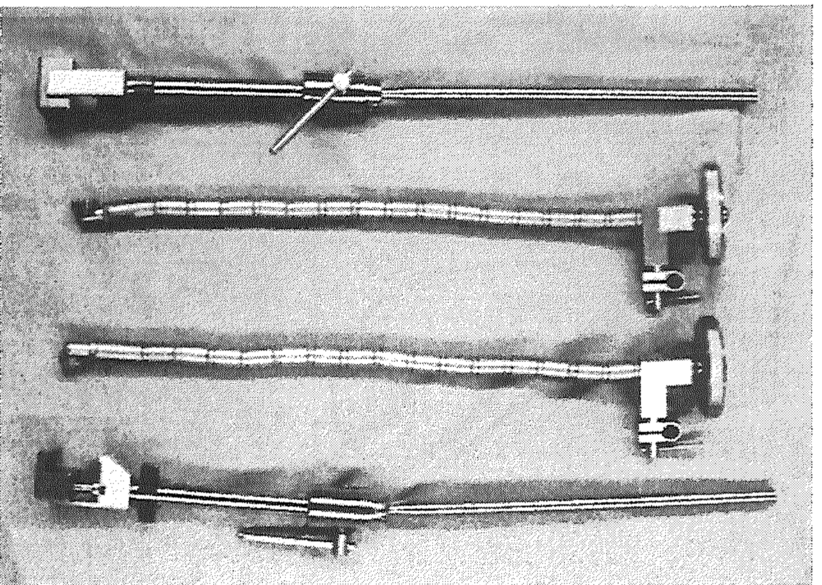
小切開術式（Biportal Transpedicular Vertebroplasty）の開発

椎弓根までのworking spaceを確保



X線透過性レトラクター

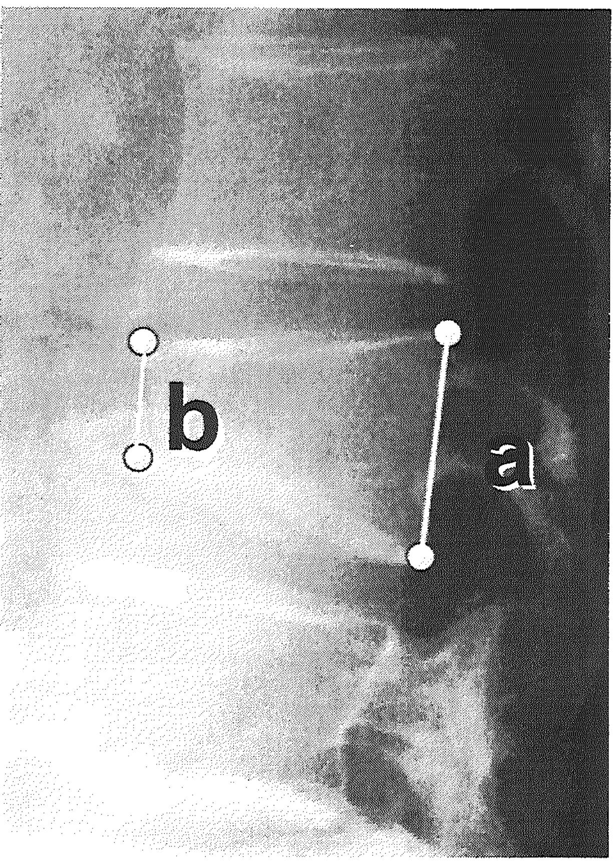


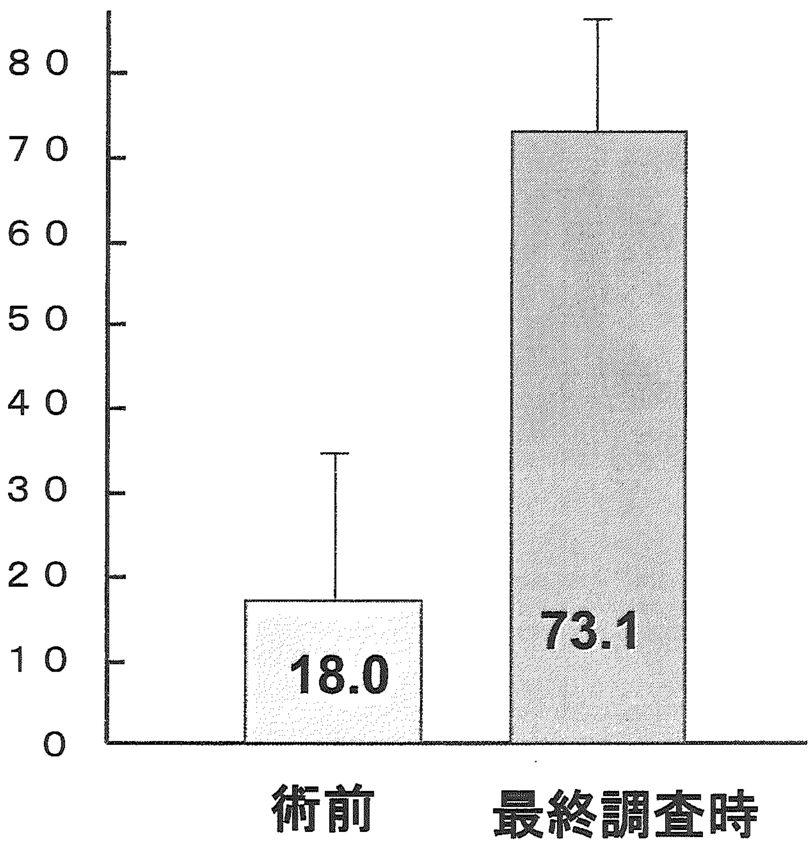


レトラクター保持装置

小切開術式：椎体楔状率

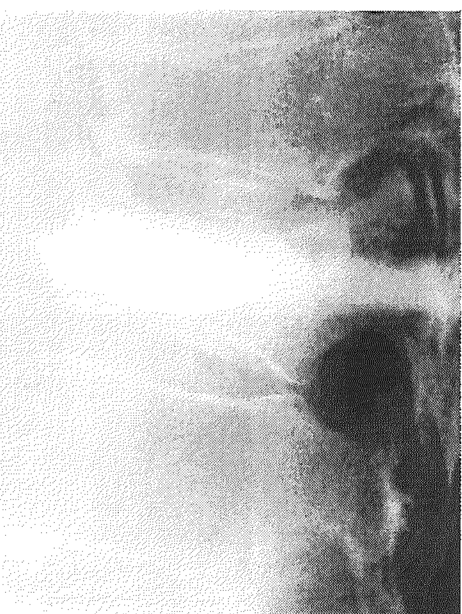
楔状率＝b / a ×100 (%)



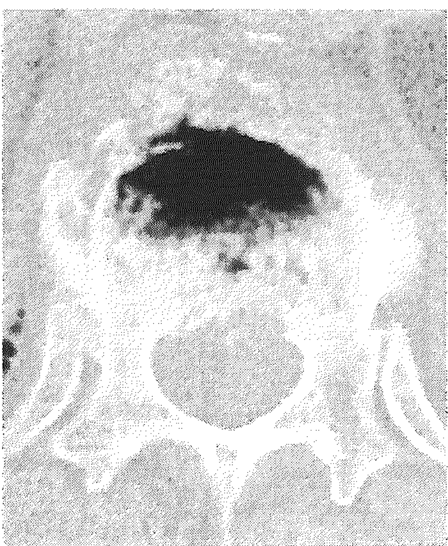


症例1　76歳女性　T12椎体偽関節

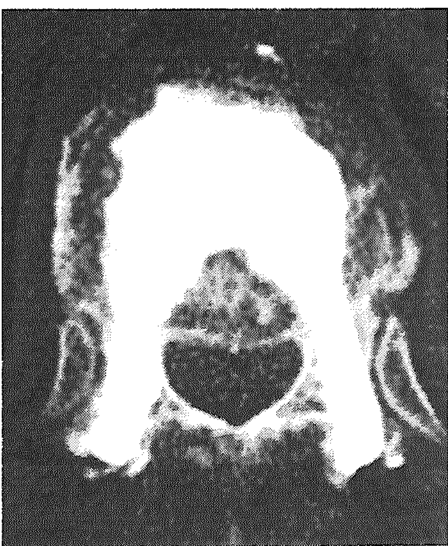
術前



術後

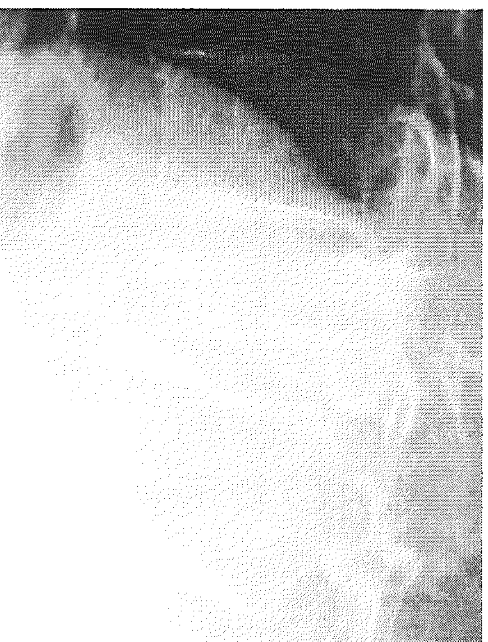


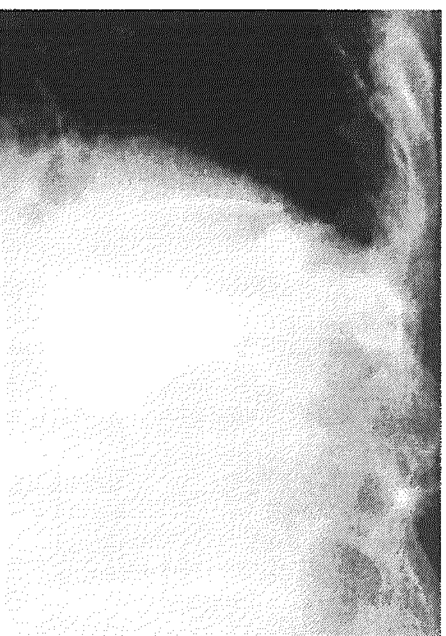
術前CT

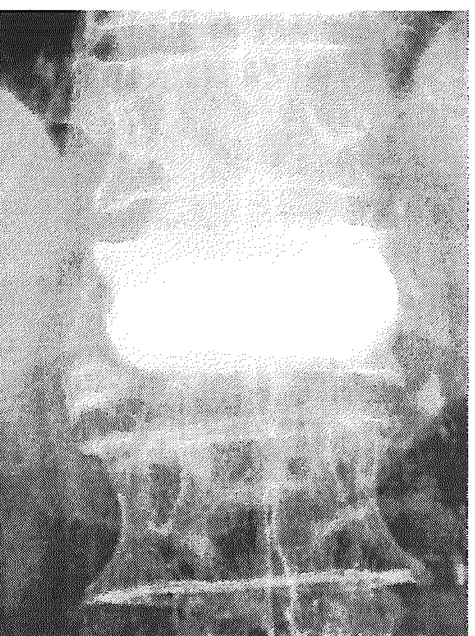


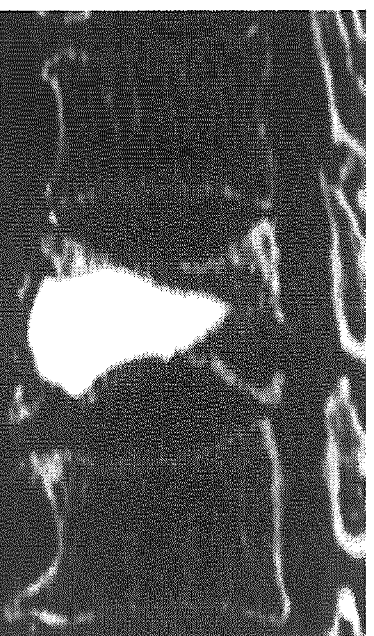
術後CT

症例2　82歳女性　T12椎体偽関節


術前座位


術後1週立位側面


正面


CT再構築