for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br 2005;87:640–5.
23. Erggelet C, Browne JE, Fu F, Mandelbaum BR, Micheli LJ, Mosely JB. Autologous chondrocyte transplantation for treatment of cartilage defects of the knee joint. Clinical results. Zentralbl Chir 2000;125:516–22 (in German).
24. Browne JE, Anderson AF, Arciero R, Mandelbaum B, Moseley JB Jr, Micheli LJ, Fu F, Erggelet C. Clinical outcome of autologous chondrocyte implantation at 5 years in US subjects. Clin Orthop 2005;(436):237–45.
25. Henderson I, Tuy B, Oakes B. Reoperation after autologous chondrocyte implantation. Indications and findings. J Bone Joint Surg Br 2004;86:205–11.
26. Minas T. Autologous chondrocyte implantation for focal chondral defects of the knee. Clin Orthop 2001;(391, suppl):S349–61.
27. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop 2000;(374):212–34.

特集
変形性膝関節症の治療戦略

【装具療法】

変形性膝関節症に対する膝装具療法の最近の知見

Recent observation of treatment for knee osteoarthritis with knee orthosis.

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Key words
●変形性膝関節症(knee osteoarthritis)
●保存療法(conservative treatment)
●機能的膝装具(functional knee brace)

0286-5394/10/¥400/論文/JCOPY

はじめに

近年，日本では急速な高齢化に伴い，運動器疾患の罹患人口は増加の一途を辿っている。とくに膝関節などの荷重関節ではメカニカルストレスにより退行性変性が生じるため，変形性膝関節症(膝OA)が発症，進行しやすい。将来的にも膝OA患者は増加していくことが容易に予測でき，その治療にかかる医療費の増大は社会的な問題であり，早急な解決が必要な課題である。

膝OA治療の主な目的は除痛，身体機能の改善，およびアライメント異常の修正であり，手術療法と保存療法に大別される。一般的に進行した重症例の場合は手術療法が行われるが，圧倒的多数は保存療法の適応となる。保存療法は運動療法，物理療法などを含めた理学療法，薬物療法，そして装具療法がある。

本稿では，装具療法のなかでもとくに膝装具についての膝OA治療における知見を述べる。

膝装具の種類

膝OAでは膝関節の内反変形を呈し，内側関節面への圧縮ストレスが増大することで関節軟骨の摩耗を引き起こし，変形が助長されるという悪循環が生じる。その結果，疼痛や関節運動制限や活動制限が生じ，ADLやQOLの低下につながる。これらに対する膝装具の役割として，

①変形の矯正と予防
②関節の運動制限と固定
③関節運動の補助
④免荷

があげられる[1]。膝装具にはいくつかの種類があり，それぞれに異なる機能と効果をもつ。

軟性装具(図1)

局所的な保温作用と，着用による安心感という心理的作用が主な目的で使用される。安価で

かつ簡易的に装着が可能なため高齢者は好んで着用するが，サポータータイプの場合，膝関節に対する直接的な矯正力はなく，精神的な安定感が疼痛を軽減していることが推測される。過去の報告として関節固有感覚の向上や姿勢制御機能の改善[2),3)]などの報告があるが，これらは膝関節全体を覆うことにより膝関節周囲筋を圧迫，刺激し，固有感覚受容器や身体機能に影響を与えたことが考えられる。

機能的膝装具(brace)(図2)

braceには膝関節を安定化させる作用があり，歩行立脚期における不安定性が制動され，関節の安定性と除痛効果を得ることができる。また筋電図による下肢の筋活動の評価では，膝関節周囲の筋活動が変化することで疼痛が軽減しているという報告もある[4)]。臨床的には中，長期的な治療結果が国内外で報告されており，その効果の持続性が報告されている[5)]。しかし，その機能的なメリットの反面，軟性装具と比較すると高価で，またその重量感，着用感から患者のコンプライアンスが低く，汎用性に欠けるともいえる。しかし，最近では膝OA患者の大半である高齢者向けに開発され，意匠性と実用性を配慮された装具も徐々に出現してきている。

braceの効果

外反矯正のメカニズム

膝OAでは内側関節面への圧縮ストレス増大が病態と密接に関係しており，その指標として三次元動作解析により計測される外部膝関節内反モーメント(膝内反モーメント)が注目されている。braceは外反矯正力(①)を加えることで膝関節にかかるストレスを減少させる(②)が，そのメカニズムは装具の種類によって異なる。たとえば，内側継ぎ手付き装具の場合，内側の支柱の大腿部分と下腿部分による圧迫と，外側のストラップによる(③)膝関節裂隙部分における圧迫によって三点支持機構をなしており，これによって外反矯正力を加える**(図3)**。

図1 支柱付き軟性膝装具

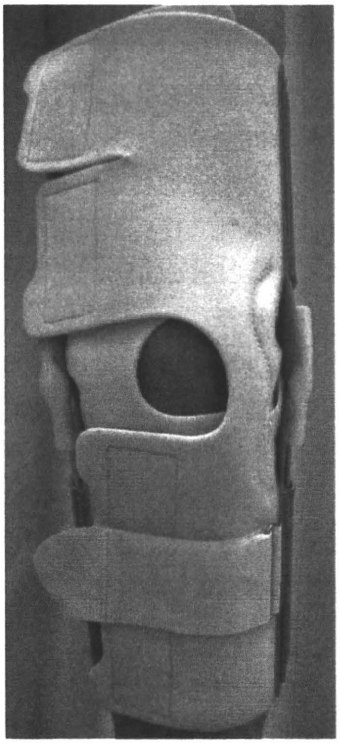
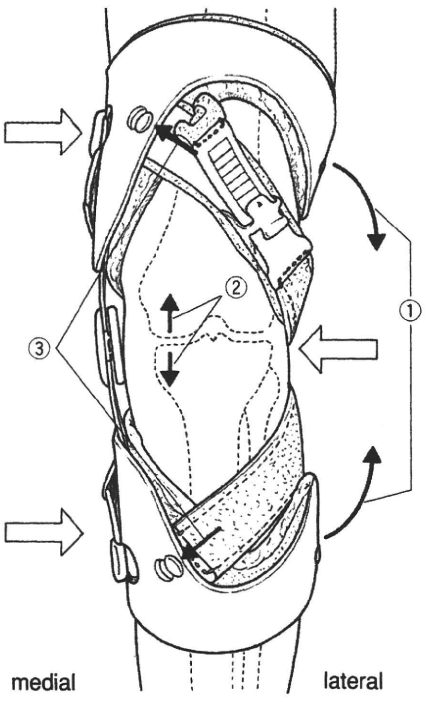


図2 機能的膝装具(brace)

図3 三点支持機構のメカニズム



白矢印：三点支持

バイオメカニクス

過去の研究においてbraceによる膝内反モーメントへの影響に関してはさまざまな報告があり，その多くにおいて膝内反モーメントを減少させることが示されている[5]。また動的な下肢アライメントに対する影響としては，歩行中の踵接地時の関節裂隙の拡大を認めたという報告がある[6]。

著者らは最近開発されたbrace装着前後での歩行への影響に関する調査を行っている**(図4)**。braceを装着した際，装着した下肢の立脚期に骨盤が装具側へ傾斜しており，体幹もやや側屈している。またとくに高齢者の場合はbrace装着状況に適応するため，顔が下を向き，視野が狭まるため転倒のおそれもある。

このようにbraceを装着することで通常とは異なる状況におかれた患者では，環境に適応しようと動作を変化させることがあり，その歩容の変化もさまざまである。もし，この瞬時的な動作の変化が長期にわたって残存する場合は，ほかの筋骨格系に影響を及ぼす可能性があり，単にbraceを処方するだけでなく，理学療法士と連携をとりながら，歩行動作の改善も視野に含めた全身的なアプローチも考慮することが必要である。

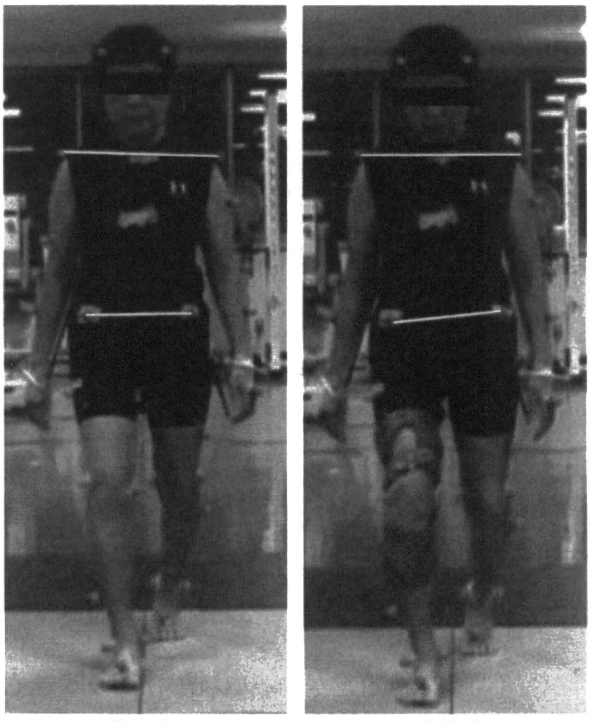
臨床効果

braceを装着することにより，非荷重位では関節裂隙に変化はみられないが，荷重位では関節裂隙の拡大がみられ，立位や歩行時など荷重活動時にbraceの有効性が出現すると予測される**(図5〜7)**。braceによる臨床的評価に関する過去の研究では，12カ月の介入後に大腿脛骨角(FTA)が改善したという報告がある一方で[7]，3カ月間ではX線像上での変化がなかったという報告も存在する[8]。装具の種類にもよるが，長期間のbraceの使用によってアライメント異常に対する効果が出現する可能性が考えられる。

また臨床症状に関する介入の報告も多くなされており，著者らはbrace装着による主観的症状とQOLに対する影響を調査している。

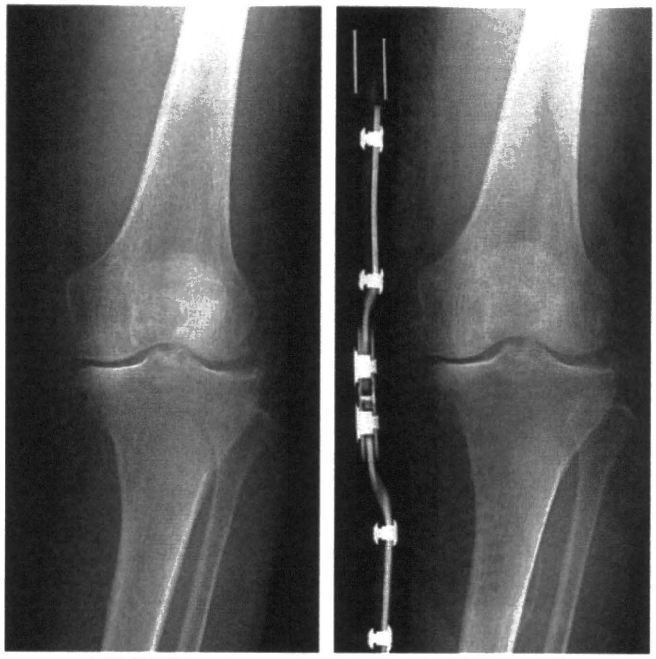
図4 brace装着前後での歩行の変化

装着により骨盤と体幹の傾斜が出現し，視線が下方に向いている。



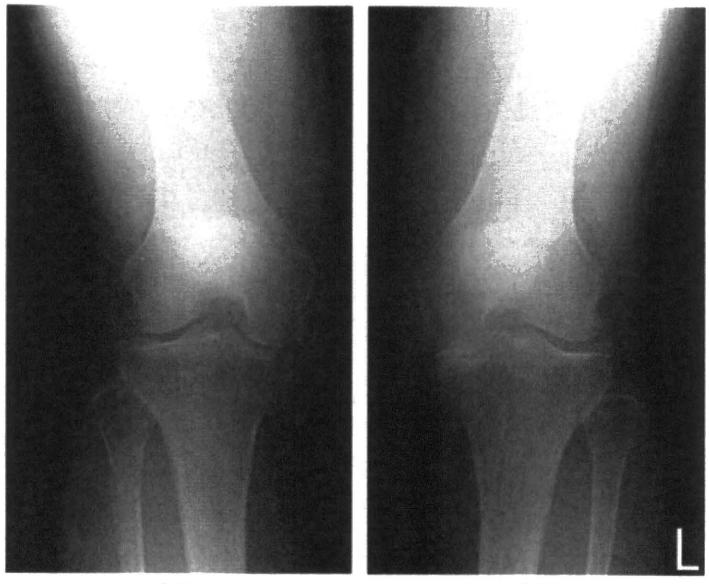
装着前　　装着後

図5 非荷重位での正面像
関節裂隙に違いはみられない。



左足(装具なし)　　左足(装具あり)

図6 Rosenberg view (装具なし)



右足　　左足

対象群(n=32)とbrace群(n=16)を設定し，6カ月経過した症例(各n=11，n=8)について3カ月後と6カ月後に評価を行ったところ，身体機能に関してbrace装着による効果が認められた(F<1,17>=6.418，p=0.021)。その後の検定では3カ月後と6カ月後ではbrace群が対象群よりも有意に身体機能スコアが高かった(**図8**，各p<0.05)。これらのことから，短期的ではあるがbraceによる身体機能の維持効果が期待できる。

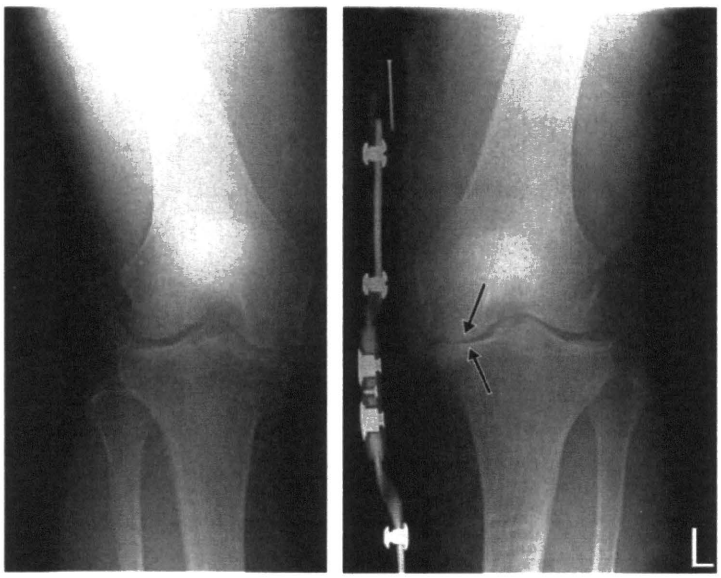
膝装具療法の問題点

使用感の問題と副作用

brace装着の際には装具がずれるなどのコン

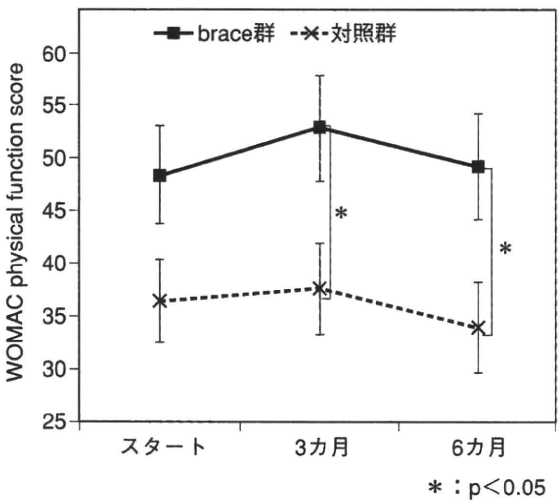
図7 Rosenberg view（装具あり）

荷重位では装具装着により図6より関節裂隙が拡大しているのがわかる（矢印）。



右足　　左足

図8 brace着用による身体機能への影響



プライアンスを低下させる諸問題がある[9]。また長期の使用で，皮膚刺激，機械的な問題，装具の不適合などが生じることもある[10]。さらに最も重要な問題として，まれに肺塞栓による呼吸困難を呈する[10]。

このためbraceによる治療を行う際は，既往歴や潜在的な心血管疾患のリスクなどを確認して慎重に処方を判断する必要がある。また患者にコンプライアンスを維持してもらうために，使用法の適切な指導と身体的な変化に対応するために適宜braceを調整する必要がある。

身体への影響

膝装具の8週間に及ぶ使用の影響として，筋力に変化はなかったものの，内側広筋の著明な萎縮，姿勢制御の反応劣化，関節固有感覚における誤認角度の増大が認められたとの報告がある[11]。この研究から，使用期間がさらに長期に及ぶ場合，筋力低下や運動制御機能の低下による転倒リスクが懸念される。これらを未然に防ぐために疼痛のない範囲で運動習慣を維持するように指導

し，必要に応じて薬物療法や理学療法を併用して行っていくことが重要である。

おわりに

保存療法では適切に疼痛や機能障害がコントロールできない場合，活動量の低下に合併して筋力低下を引き起こし，さらに活動量が減少するという悪循環が生じる可能性がある。膝装具療法は保存療法の1つとして身体活動向上の手助けとなるツールではあるが，それを処方するのみでは，必ずしも膝OAの治療としては成立しないと考える。装具の機能を最大限に発揮させるためには，義肢装具士や理学療法士と検討しながら，装具の種類の検討，装着による姿勢や歩行を始めとした動作への影響を観察し，適切な指導を行うことが必要である。膝装具療法に加え各種保存療法を組み合わせることで膝OAの病態，症状の進行や腰部や足・股関節などのほかの身体部位の関節疾患の発生を防ぐことが可能となり，そこで初めて膝OA患者の病態進行，症状の緩和が可能になると考える。

文献

1）元田英一：膝装具に必要な力学的特性. 日本義肢装具学会誌, 24(1)：12-14, 2008.
2）McNair PJ, Stanley SN, Strauss GR：Knee bracing：effects of proprioception. Arch Phys Med Rehabil, 77(3)：287-289, 1996.
3）Chuang SH, Huang MH, Chen TW, et al：Effect of knee sleeve on static and dynamic balance in patients with knee osteoarthritis. Kaohsiung J Med Sci, 23(8)：405-411, 2007.
4）Ramsey DK, Briem K, Axe MJ, et al：A mechanical theory for the effectiveness of bracing for medial compartment osteoarthritis of the knee. Bone Joint Surg Am, 9(11)：2398-2407, 2007.
5）Beaudreuil J, Bendaya S, Faucher M, et al：Clinical practice guidelines for rest orthosis, knee sleeves, and unloading knee braces in knee osteoarthritis. Joint Bone Spine, 76(6)：629-636, 2009.
6）Komistek RD, Dennis DA, Northcut EJ, et al：An *in vivo* analysis of the effectiveness of the osteoarthritic knee brace during heel-strike of gait. J Arthroplasty, 14(6)：738-742, 1999.
7）Matsuno H, Kadowaki KM, Tsuji H：Generation II knee bracing for severe medial compartment osteoarthritis of the knee. Arch Phys Med Rehabil, 78(7)：745-759, 1997.
8）Finger S, Paulos LE：Clinical and biomechanical evaluation of the unloading brace. J Knee Surg, 15(3)：155-159, 2002.
9）Liu K, Lao L, Asami T, et al：Clinical care of osteoarthritic knee with knee orthoses. Fukuoka Igaku Zasshi, 89(10)：298-302, 1998.
10）Giori NJ：Load-shifting brace treatment for osteoarthritis of the knee：a minimum 2 1/2-year follow-up study. J Rehabil Res Dev, 41(2)：187-194, 2004.
11）三輪　恵ほか：膝装具装着が下肢機能に及ぼす影響. 日本義肢装具学会誌, 7：117-118, 1992.

In vitro cartilage formation using TGF-β-immobilized magnetic beads and mesenchymal stem cell-magnetic bead complexes under magnetic field conditions

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Received 25 January 2007; revised 27 November 2007; accepted 19 March 2008
Published online 26 January 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.32365

Abstract: We evaluated the efficacy of transforming growth factor (TGF)-β-immobilized magnetic beads for chondrogenesis *in vitro* using a mesenchymal stem cell (MSC) delivery system and an external magnetic force (EMF). MSCs isolated from the bone marrow of Sprague Dawley rats were mixed with carboxyl group-combined magnetic beads (Ferri Sphere 100C®) coated with anti-rat CD44 mouse monoclonal antibodies. TGF-β3 (10 and 1 ng/mL) was attached magnetically to such other Ferri Sphere 100C® beads via an amide bond formed between a primary amino group on the TGF-β3 and the carboxyl groups on the surface of the beads. MSC-magnetic bead complexes were centrifuged to form a pellet and cultured in chondrogenic differentiation medium (CDM) supplemented with either 10 or 1 ng/mL TGF-β-immobilized magnetic beads (10 or 1 ng/mL TGF-β-immobilized magnetic bead groups) or in CDM supplemented with 1 or 10 ng/mL TGF-β (1 or 10 ng/mL TGF-β group). TGF-β-immobilized magnetic beads were gathered effectively under an EMF. Chondrogenesis was achieved from the MSC-magnetic bead complexes in the presence of 1 ng/mL TGF-β-immobilized magnetic beads. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 92A: 196–204, 2010

Key words: transforming growth factor (TGF)-β; magnetic beads; magnetic force; mesenchymal stem cell; chondrogenic differentiation

INTRODUCTION

In recent years, autologous chondrocyte implantation or autologous osteochondral mosaicplasty have been widely performed to treat limited chondral and osteochondral lesions because articular cartilage has a limited potential to repair itself, and the cartilage defects treated by conventional surgical methods do not lead to hyaline cartilage regeneration.[1–5] However, the disadvantages of these procedures are that they involve undergoing an arthrotomy to harvest and transplant cartilage,[1] and they are limited by a lack of suitable donor sites from which large cartilage samples can be harvested.[5]

A treatment strategy that avoids undergoing an arthrotomy would ideally involve combining a group of multipotential cells and a controlled concentration of cytokines at a localized area with a single intra-articular injection. We originally developed a technique for cell-based cartilage repair in which cells were coupled with magnetic beads in association with an external magnetic force.[6] The underlying concept of this technique involves injection of cells coupled with magnetic beads into a joint and the use of an external magnetic force to position the transplanted cells in the desired location.[1,6] We isolated mesenchymal stem cells (MSCs) from bone marrow to use as our cell source and developed two types of MSC-magnetic bead complexes using anti-CD44 antibodies and a synthetic cell adhesion peptide (arginine-glycine-aspartic acid-serine, RGDS peptide).[6] Anti-CD44 antibody-bead complexes are useful for the identification of MSCs because the CD44 antigen is popularly regarded as a positive phenotypic maker of MSCs.[7] The RGDS peptide-bead complexes are useful for clinical applications as the RGDS peptide is a biodegradable material. We have also demonstrated that MSC-magnetic bead complexes could proliferate and differentiate into chondrocytes when supplemented with chondro-

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Contract grant sponsor: Japan Ministry of Education, Culture, Sports, Science and Technology; contract grant number: 16209045

genic differentiation factors,[6] as first described by Johnstone et al.[8] We have shown that MSC-magnetic bead complexes that have been localized to a specific area by an external magnetic force could generate a chondrogenic matrix when cultured in a monolayer.[6] However, we have not examined whether MSC-magnetic bead complexes still possess chondrogenic potential under three-dimensional culture conditions against gravity and under the influence of an external magnetic force.

To induce differentiation of MSCs, cultivation under appropriate culture conditions and stimulation with several bioactive factors, including transforming growth factor-β (TGF-β), bone morphogenic protein (BMP), fibroblast growth factor (FGF), and insulin-like growth factor (IGF-I) are required.[7–11] In particular, TGF-β is essential to the *in vitro* chondrogenic differentiation of MSCs.[8] However, in terms of the clinical application of TGF-β, several investigators have shown that free injection of TGF-β into the joint cavity precipitates severe adverse effects such as osteophyte formation and inflammatory joint disease.[12,13] That is to say, because the injected TGF-β does not directly target and attach to the lesion, it affects the lesion and also extends into the normal intra-articular structure. Therefore, to assist this intra-articular cell transplantation without scaffolds and to solve the problems associated with injecting free TGF-β, we developed TGF-β-immobilized magnetic beads, which localized the TGF-β and enhanced our unique MSC delivery system.

On the basis of this information, this study had two main objectives. Firstly, we wanted to assess whether chondrogenesis using MSCs as a cell source could be induced under conditions against gravity and under the influence of an external magnetic force. Secondly, we hoped to evaluate a new cytokine delivery system involving TGF-β-immobilized magnetic beads and an external magnetic force. This included examining whether the localization of TGF-β-immobilized magnetic beads could lower the concentration of TGF-β necessary for chondrogenesis to occur from the MSC cell source.

MATERIALS AND METHODS

Animal experiments were performed in accordance with the Guide for Animal Experimentation, Hiroshima University, and the Committee of Research Facilities for Laboratory Animal Sciences, Graduate School of Biomedical Sciences, Hiroshima University.

Isolation and expansion of MSCs

A modification of Kotobuki's culture method,[9] as described previously,[6] was used for the isolation and *in vitro* expansion of MSCs. Briefly, the bone marrow of Sprague Dawley rats (12-weeks-old) was aspirated from the tibial marrow cavities with 24G injection needles and mixed in a culture medium consisting of high-glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO) and penicillin-streptomycin-fungizone (Bio-Whittaker, ML). The cells, including buffy coat and red blood cells, were seeded onto 100-mm culture dishes (Falcon, BD Bioscience, Franklin Lakes, NJ) in culture medium, and incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The medium remained unchanged for the first 7 days, and was subsequently changed every 2–3 days. After 14–21 days, the cells had proliferated and reached confluence. The cells were then harvested by using 0.25% trypsin and 0.02% EDTA, and then rinsed twice with culture medium. To expand the MSCs, 2–3 × 10^5 of the harvested cells were seeded onto 100-mm culture dishes. On reaching confluence again, the cells were reseeded under the same conditions.

Carbodiimide-mediated immobilization of CD44 antibody to the magnetic beads by amide bond formation

Firstly, to examine the expression of the cell surface antigen CD44 in rat MSCs expanded in a monolayer culture, immunohistochemical staining was performed as described previously.[6] Then, according to Yanada's method,[6] we coated uniform, mono-sized magnetic beads composed of styrene-acryl polymers with a thin film of magnetic ferrite (diameter: 310 nm; density: 1.8 g/cm^3; the amount of magnetization: 27 emu/g; carboxyl groups introduced on the surface: 0.01–0.04 mol/mg; Ferri Sphere 100 C®, Nippon Paint, Tokyo, Japan). The coupling procedure involved the formation of an amide bond between a primary amino group of the CD44 antibody (Chemicon International, Temecula, CA) and the carboxyl groups on the surface of the magnetic beads, mediated by carbodiimide activation (Fig. 1). Because the intermediate product of the reaction between the carboxylic acid and the carbodiimide is very labile and hydrolyzed quickly, a less labile intermediate (NHS: *N*-hydroxy succinimide) was used. Briefly, after 3 mg of magnetic beads were washed twice with 500 μL 0.01*N* NaOH for 10 min with thorough mixing, they were washed three times with deionized water in the same manner to remove excess liquid. Then, 50 μL of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) solution (50 mg/mL in 25 m*M* MES (2-[*N*-morpholino]ethane sulfonic acid), pH 5) and 50 μL NHS solution (50 mg/mL in 25 m*M* MES, pH 5) were added, mixed well, and incubated with slow tilt rotation at room temperature for 30 min. After incubation, the tube containing the magnetic beads was placed on a magnet for 4 min and the supernatant was removed. The beads were washed twice with 25 m*M* MES (pH 5). Twenty micrograms of CD44 antibody dissolved in 25 m*M* MES (pH 5) were added to the activated beads, making a total volume of 500 μL. The mixture was vortexed and then incubated for 3 h at 25°C with slow tilt rotation. After incubation, the

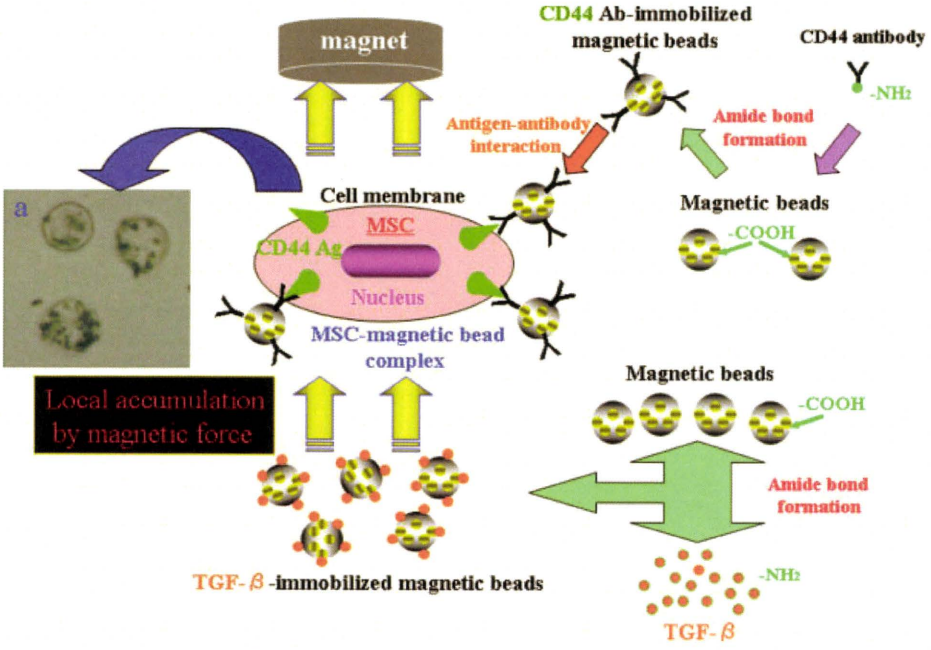

Figure 1. Schema with CD44 antibody-immobilized magnetic bead-conjugated MSC (MSC-magnetic bead complex) and TGF-β-immobilized magnetic beads. Light microscopic view (magnification ×400). Some massive bead conglomerates are attached to the surface of MSCs (a). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tubes were placed on a magnet for 4 min, and the supernatant was removed. Ethanolamine (0.05*M*) in PBS(-) (pH 8) was added and the magnetic beads were incubated for 1 h at room temperature with slow tilt rotation to quench nonreacted groups. Finally, the beads were washed four times with 0.5% BSA in PBS(-) and suspended in 0.5% BSA in PBS(-) at a concentration of 3 mg beads/mL. Thus, CD44 antibody-immobilized magnetic beads composed of magnetic beads and CD44 antibodies were prepared (Fig. 1).

Complexing CD44 antibody-immobilized magnetic beads to MSCs

Forty-five microliters of CD44 antibody-immobilized magnetic beads (225 μL beads) and 1×10^6 MSCs were mixed in 355 μL of 0.5% BSA in PBS(-) with slow tilting and rotation for 1 h at 4°C or at 37°C. The tube was then placed on a magnet for 4 min to collect the complexes. The assembled MSC-CD44 antibody-bead complexes were washed four times with 0.5% BSA in PBS(-) and resuspended in culture medium at 5×10^6 cells/mL (Fig. 1).

Carbodiimide-mediated immobilization of TGF-β3 to the magnetic beads by amide bond formation

To label TGF-β3 (Sigma-Aldrich, St. Louis, MO) magnetically, we used such other Ferri Sphere 100C® beads. The coupling procedure involved the formation of an amide bond between a primary amino group of TGF-β3 and the carboxyl groups on the surface of the magnetic beads, mediated by carbodiimide activation. After the magnetic beads were activated as described above, 50-μL of 1000 ng/mL TGF-β3 dissolved in 25 m*M* MES buffer (pH 5) was added to the activated beads, making a total volume of 500 μL. The mixture was vortexed and then incubated for 3 h at 25°C with a slow tilt rotation. After incubation, the tube was placed on a magnet for 4 min and the supernatant was removed. Ethanolamine (0.05*M*) in PBS(-) (pH 8) was added, and the magnetic beads were incubated for 1 h at room temperature with a slow tilt rotation to quench the nonreacted groups. Finally, the beads were washed four times with 0.5% BSA in PBS(-) and resuspended in 0.5% BSA in PBS(-) at a concentration of 1 mg beads/mL. The concentration of TGF-β3 was 100 ng/mg beads (Fig. 1).

Chondrogenesis of MSC-magnetic bead complexes using TGF-β-immobilized magnetic beads under an external magnetic force

To evaluate the effect of an external magnetic force on the chondrogenic potential of the MSC-magnetic bead complexes, a modification of Johnstone's pellet culture system was performed.[8] Approximately 2×10^5 MSCs, existing as complexes, were resuspended in chondrogenic differentiation medium (CDM) lacking TGF-β3. The CDM consisted of high-glucose DMEM supplemented with 10^{-8} M dexamethasone (Sigma), 50 μg/mL ascorbic acid-2-phosphate (Sigma), 40 μg/mL L-proline (Nacalai Tesque, Kyoto, Japan), ITS-A supplement (Invitrogen, 10 μg/mL insulin, 6.7 ng/mL sodium selenite, 5.5 μg/mL transferrin, 110 μg/mL sodium pyruvate), and 1.25 mg/mL BSA (Sigma). The MSC-magnetic bead complexes and non-

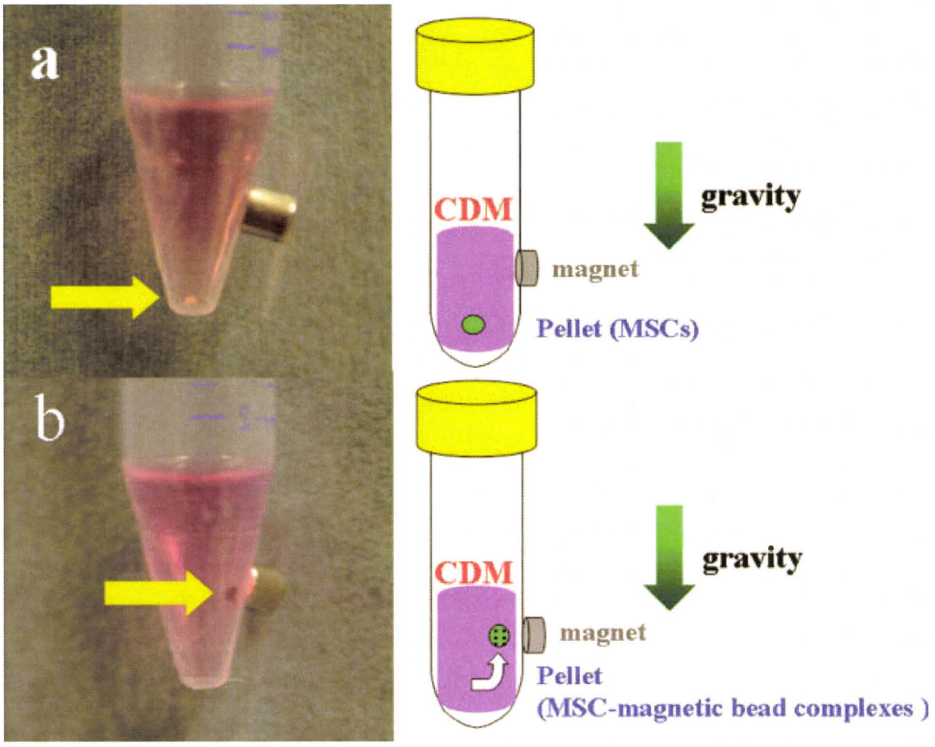

Figure 2. Pellet culture system in which a neodymium magnet was set on the lateral side of the polypropylene tube. Approximately 2 × 10^5 MSC-magnetic bead complexes (b) or MSCs alone (a) were centrifuged to form a pellet. Although the pellet of the MSCs alone remained on the bottom of the tube (a), the pellets of the MSC-magnetic bead complexes could be moved up against gravity by an external magnetic force (b). The pellets of the MSC-magnetic bead complexes (b) were cultured in CDM containing either 100 or 10 μL/mL TGF-β-immobilized magnetic beads (TGF-β concentration: 10 or 1 ng/mL, respectively) or 1 or 10 ng/mL TGF-β protein, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

labeled rat MSCs (MSCs alone) were centrifuged to form a pellet and a neodymium magnet (diameter: 5 mm; height: 5 mm; magnetic flux density: 0.43 Tesla (T))[6] was positioned on the lateral side of a 15-mL polypropylene tube [Fig. 2(a,b)]. The pellet of the MSCs alone remained on the bottom of the tube [Fig. 2(a)].

Next, to evaluate the effect of TGF-β-immobilized magnetic beads on the chondrogenic potential of the pelleted

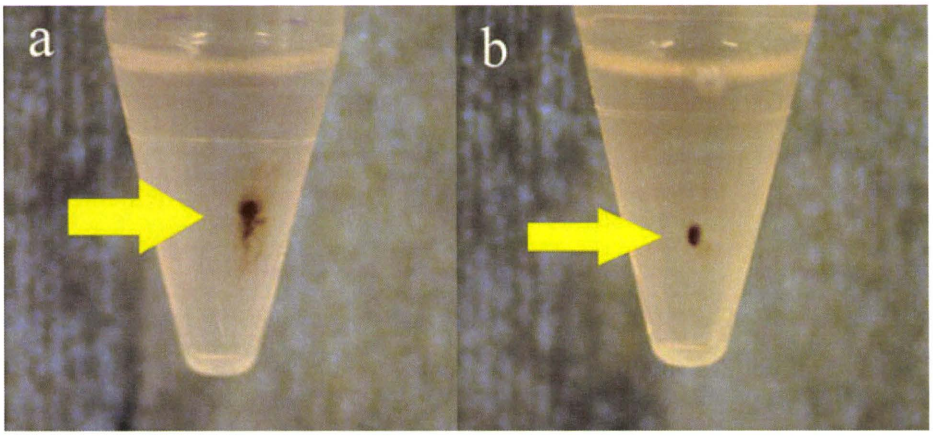

Figure 3. Macroscopic findings of the MSC-magnetic bead complexes pelleted after being cultured for 21 days. The pellet was transferred into PBS(-) under the influence of an external magnetic force, and these photographs were taken. Photograph (a) indicates the pellet cultured in the CDM lacking TGF-β, but supplemented with 10 μL/mL TGF-β-immobilized magnetic beads (1 ng/mL TGF-β) localized under an external magnetic force whenever the medium is changed. Photograph (b) indicates the pellet cultured in the CDM containing 1 ng/mL TGF-β. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Each Pellet Culture System of the Four Groups

	10 ng/mL TGF-β-immobilized magnetic bead group	1 ng/mL_TGF-β-immobilized magnetic bead group	1 ng/mL_TGF-β group	10 ng/mL TGF-β group
Cultured Cells	MSC-magnetic bead complexes	MSC-magnetic bead complexes	MSCs-magnetic bead complexes	MSC-magnetic bead complexes
TGF-β (ng/mL)	0	0	1	10
TGF-β-immobilized magnetic bead (ng/mL)	10	1	0	0

The basic CDM consisted of high-glucose DMEM supplemented with 10^{-8} M dexamethasone, 50 μg/mL ascorbic acid-2-phosphate, 40 μg/mL L-proline, ITS-A supplement (10 μg/mL insulin, 6.7 ng/mL sodium selenite, 5.5 μg/mL transferrin, 110 μg/mL sodium pyruvate), and 1.25 mg/mL BSA, and was applied under an external magnetic force.

MSC-magnetic bead complexes, TGF-β was labeled magnetically (10 ng/mL and 1 ng/mL TGF-β-immobilized magnetic bead groups) using the method described above (Fig. 1). The pelleted MSC-magnetic bead complexes were cultured at 37°C with 5% CO_2 and 95% air in 1 mL of CDM supplemented with either 100 μL/mL or 10 μL/mL TGF-β-immobilized magnetic beads (TGF-β concentration: 10 ng/mL or 1 ng/mL [Fig. 3(a)], respectively) and localized under an external magnetic force [Fig. 2(b)]. We have demonstrated previously that MSC-magnetic bead complexes had the ability to differentiate into the chondrogenic lineage in the presence of 10 ng/mL TGF-β.[6] To assess the influence of TGF-β concentrations lower than 10 ng/mL, the pellet from the MSC-magnetic bead complexes was cultured in CDM supplemented with 1 ng/mL TGF-β under an external magnetic force (1 ng/mL TGF-β group) [Fig. 3(b)]. As the positive control group, the pellet of the MSC-magnetic bead complexes was cultured for 21 days in CDM supplemented with 10 ng/mL TGF-β (10 ng/mL TGF-β group) and localized under an external magnetic force [Fig. 2(b)]. Table I is a list to clarify all the test groups. The medium was changed every 3 days and either 100 μL/mL or 10 μL/mL TGF-β-immobilized magnetic beads (TGF-β concentration: 10 ng/mL or 1 ng/mL, respectively) were added at the same time. In the 1 ng/mL TGF-β group and the 10 ng/mL TGF-β group, 1 ng/mL or 10 ng/mL TGF-β in solution was respectively added. After 14 and 21 days in culture, the chondrogenic ability of the four different groups was evaluated (Table I).

RNA preparation and reverse transcription-polymerase chain reaction analysis

Total RNA was prepared from the pellets of MSC-magnetic bead complexes using the RNeasy Micro kit (Qiagen, Tokyo, Japan). Prepared RNA was converted to cDNA using the Superscript™ First-Strand Synthesis System for reverse transcription-polymerase chain reaction (RT-PCR) (Invitrogen) according to the manufacturer's protocol. PCR was performed in a Minicycler (PTC-150, Bio-Rad, Hercules, CA). PCR amplification conditions for rat aggrecan, type II collagen, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: 94°C for 2 min followed by 35 cycles of 94°C for 15 s, 56°C (or for GAPDH: 58°C) for 30 s, and 68°C for 1 min. The reaction products were resolved by electrophoresis on a 2% agarose gel and visualized with ethidium bromide under UV illumination. The aggrecan and type II collagen primers have been described previously,[14] and the GAPDH primers were designed specifically for this study. The primer sequences used are as follows: aggrecan (forward): 5′-TAGAGAA GAAGAGGGGTTAGG-3′; aggrecan (reverse): 5′-AGCAG TAGGAGCCAGGGTTAT-3′; type II collagen (forward): 5′-GAAGCACATCTGGTTTGGAG-3′; type II collagen (reverse): 5′-TTGGGGTTGAGGGTTTTACA-3′; GAPDH (forward): 5′-GCCAAAAGGGTCATCATCTC-3′; GAPDH (reverse): 5′-GCCTGCTTCACCACCTTCTT-3′.

Histological evaluation

Pelleted MSC-magnetic bead complexes that had been cultured for 21 days were fixed with 4% paraformaldehyde and embedded in paraffin. After deparaffinization, sections (5 μm) were stained with toluidine blue and safranin O solution.

RESULTS

Assembly and proliferation of MSC-magnetic bead complexes

As described previously,[6] immunohistochemical staining for CD44 antigens revealed that CD44 antigens are expressed in ~90% of expanded MSCs up to at least passage four. We decided to use MSCs that had been expanded up to passage four for assembling MSC-magnetic bead complexes. Next, CD44 antibody-immobilized magnetic beads were combined with the expanded MSCs (Fig. 1). Electron and light microscopy demonstrated that some massive bead conglomerates had attached to the surface of the labeled MSCs (Fig. 1). In contrast, large bead conglomerates had not attached to the nonlabeled rat MSCs. We showed that MSCs could be combined with small magnetic beads via rat CD44 antibodies.

As described previously,[6] the MSC-magnetic bead complexes did not proliferate during the first 3 days of culture although the complexes remained attached to the bottom of the well. However, microscopic

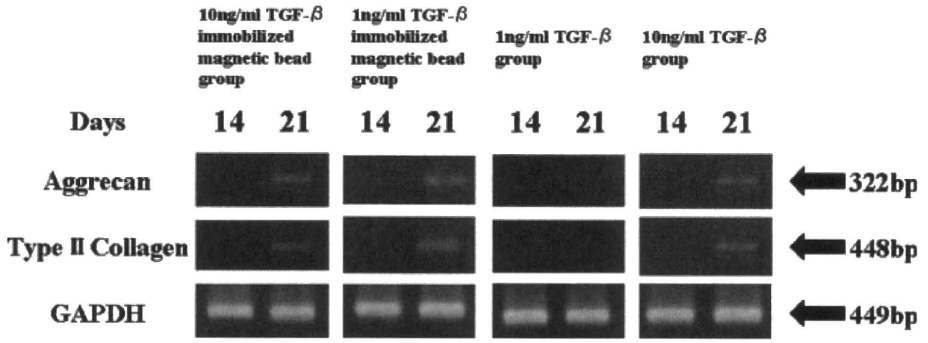

Figure 4. RT-PCR analysis of the 10 ng/mL and 1 ng/mL TGF-β-immobilized magnetic bead groups, 1 ng/mL and 10 ng/mL TGF-β groups. The expression of aggrecan and type II collagen mRNA could be detected in both the 10 ng/mL and 1 ng/mL TGF-β-immobilized magnetic bead groups after being cultured for 21 days, but not after being cultured for 14 days. The data shown are typical of four independent experiments (n = 6).

observation revealed that the cells proliferated after the CD44 antibody-immobilized magnetic beads separated from the surface of MSCs in the well. After 7 days of culture, cell proliferation of MSC-magnetic bead complexes was ~50% that of normal rat MSCs. The assembled MSC-magnetic bead complexes were able to proliferate after separating from the mediator-immobilized magnetic beads, although the proliferation of the complexes was slower during the early period of culture than that of nonlabeled rat MSCs.[6]

Macroscopic findings from the three-dimensional culture system of pellets influenced by an external magnetic force

All of the pellets in each group were attached to the wall of the tube by an external magnet after 21 days in culture [Fig. 3(a,b)]. Moreover, in the 10 ng/mL and 1 ng/mL TGF-β-immobilized magnetic bead groups [Fig. 3(a)], the TGF-β-immobilized magnetic beads gathered effectively under the influence of the external magnet.

Chondrogenesis of MSC-magnetic bead complexes in the three-dimensional culture system under the influence of an external magnetic force

RT-PCR analysis of the pellet cultures demonstrated that aggrecan and type II collagen mRNA expression could not be detected in the 1 ng/mL TGF-β group after either 14 or 21 days in culture (Fig. 4). On the other hand, mRNA expression of these two markers was not detected in the 10 ng/mL or 1 ng/mL TGF-β-immobilized magnetic bead groups or the 10 ng/mL TGF-β group after 14 days in culture (Fig. 4). However, after 21 days in culture, aggrecan and type II collagen mRNA expression could be detected (Fig. 4). The same RT-PCR analysis results were obtained in all six pellets (n = 6) from each group.

In addition, after 21 days in culture, MSC-CD44 antibody-bead complex-derived-MSCs were partially surrounded by a chondrogenic matrix in the 10 ng/mL and 1 ng/mL TGF-β-immobilized magnetic bead groups, as well as the 10 ng/mL TGF-β group (Fig. 5). This matrix stained metachromatically with toluidine blue and safranin O, albeit sparsely (Fig. 5). The complexes in the 1 ng/mL TGF-β-immobilized magnetic bead group had higher chondrogenic potential than that of 1 ng/mL TGF-β group based on these histological findings. The complexes in the 10 ng/mL TGF-β-immobilized magnetic bead group also had a higher chondrogenic potential than the complexes in the 10 ng/mL TGF-β group (Fig. 5). This pattern of histological findings was consistent in four of six pellets (n = 6) in each group.

DISCUSSION

This study demonstrated that MSC-magnetic bead complexes could differentiate along the chondrogenic lineage in the presence of 1 ng/mL TGF-β. This concentration of TGF-β is 10% lower than the 10 ng/mL generally used, and was made possible by using TGF-β-immobilized magnetic beads.

The chondrogenic differentiation of MSCs *in vitro* requires the addition of certain bioactive factors, such as TGF-β and dexamethasone. Several investigators have speculated that TGF-β initially increases the proliferation of cells with chondrogenic potential to the point where a critical number is reached and differentiation can then occur.[7,8,10,11] Johnstone et al.[8] reported that lowering the TGF-β concentration decreased chondrogenesis in the aggregates of rabbit MSCs, and that the optimal TGF-β concentration was 10 ng/mL. In addition, Bosnakovski et al.[15]

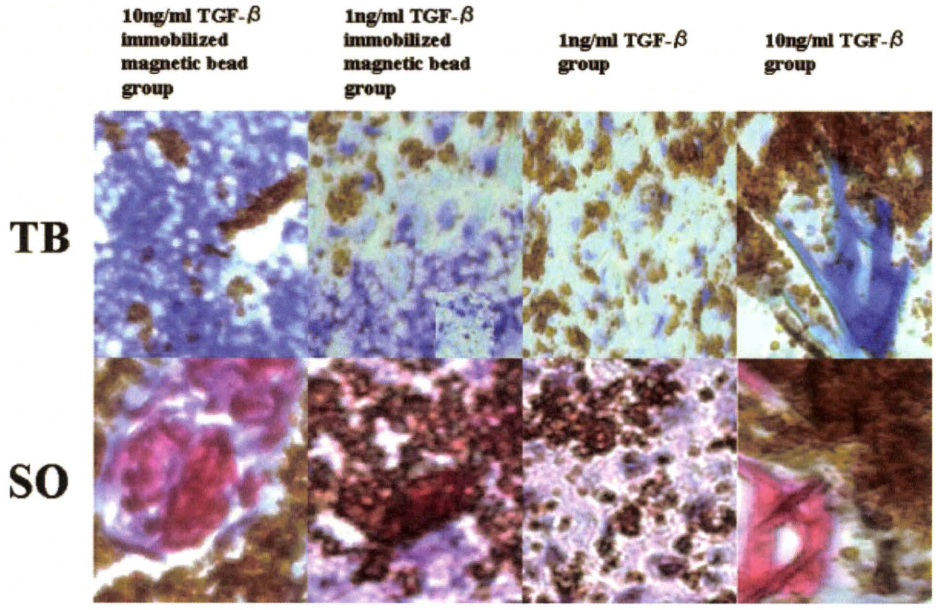

Figure 5. Histological results using light microscopy. Representative histology of cartilage pellets stained with Toluidine blue or safranin O under high (×200) magnification: the 10 ng/mL TGF-β-immobilized magnetic bead group; the 1 ng/mL TGF-β-immobilized magnetic bead group; the 1 ng/mL TGF-β group; and the 10 ng/mL TGF-β group. The histological results shown are typical of four independent experiments (n = 6).

reported that chondrogenic differentiation of bovine MSCs in monolayer culture appeared to be dose-dependent and time-dependent in relation to TGF-β. The optimal TGF-β concentration was >5 ng/mL, with higher concentrations able to promote chondrogenic differentiation of bovine MSCs better than lower concentrations. Several investigators[7,10,11] have reported that chondrogenic differentiation of human MSCs can be promoted by supplementing the cell cultures with 10 ng/mL TGF-β. Similarly, 10 ng/mL TGF-β was often added to the CDM when culturing rat MSCs *in vitro*.[16] Thus, regardless of these interspecies differences, TGF-β promotes chondrogenesis of MSCs *in vitro*, and the minimal and optimal TGF-β concentration required is 10 ng/mL in a number of species. In addition, the concentration of TGF-β in the medium correlates with the chondrogenic potential of the MSCs. However, there are some complications regarding the clinical application of TGF-β. Several investigators have demonstrated that injection of free TGF-β into the joint cavity precipitates severe adverse effects such as osteophyte formation, cartilage destruction, and inflammatory joint disease.[12,13,17] Therefore, to inject TGF-β into the joint at the lowest concentration possible for effective chondrogenic differentiation, we devised a method that allowed the accumulation of TGF-β at the site of interest only by immobilizing TGF-β to Ferri Sphere 100 C® magnetic beads (Fig. 1). That is, by using TGF-β-immobilized magnetic beads and an external magnetic force, we enabled TGF-β to act at the local site more effectively. Although we have already shown that MSC-magnetic bead complexes can differentiate along the chondrogenic lineage in both monolayer and three-dimensional cultures grown in CDM supplemented with 10 ng/mL TGF-β,[6] in this study, we established new culture conditions for chondrogenesis comprising of TGF-β-immobilized magnetic beads and an external magnetic force.

In this study model, we have developed a new pellet culture system in which a magnet was applied to the lateral wall of a tube to properly evaluate the effect of TGF-β-immobilized magnetic beads under an external magnetic force (Fig. 2). In the conventional pellet culture system described by Johnstone et al.,[8] the pellet is usually found on the bottom of the tube. Without an external magnetic force, TGF-β-immobilized magnetic beads should also gravitate to the bottom of the tube under the influence of gravity. Under these conditions, it is difficult to use the TGF-β-immobilized magnetic beads to their full capacity and the effects of the beads cannot be evaluated precisely. TGF-β-immobilized magnetic beads should be applied against gravity. Therefore, to prevent the pellet from falling to the bottom of the tube because of gravity, we made a pellet composed of MSC-magnetic bead complexes (Fig. 2) and showed that the pellet of MSC-magnetic bead complexes could be held up off the bottom of the tube under an external magnetic force for 21 consecutive days in

culture (Fig. 3). In addition, we also demonstrated that the pellet of MSC-magnetic bead complexes could differentiate along the chondrogenic lineage even in a three-dimensional culture system against gravity and under the influence of an external magnetic force (Figs. 4 and 5).

Recently, we investigated the efficacy of a magnetic drug delivery system (DDS). We demonstrated successfully that the combined treatment of a topical injection of magnetic liposomes containing magnetite (Fe_3O_4; mean diameter: 10 nm) and cytokines, such as recombinant human bone morphogenetic protein-2 (rhBMP-2) and TGF-β1, together with a magnet implanted at the target defect site is effective for bone formation in a segmental bone defect rat model and for promotion of chondrogenesis in the osteochondral defect rat model.[18,19] These studies led to the use of an external magnetic force, which is less invasive and more useful than the surgical implantation of a magnet. We modified this magnetic DDS and demonstrated that this magnetic stem cell delivery system could direct MSC-magnetic bead complexes to the desired location. We had previously performed a pilot study demonstrating the clinical application of this system, in which a cartilage defect in the bilateral femoral condyle of a rabbit was placed between two magnetic poles after an intra-articular injection of MSC-magnetic bead complexes.[1] In this model, the accumulation of the MSC-magnetic bead complexes in the defects after 60 min was markedly higher in the group exposed to an external magnetic force than the group that was not exposed to a magnetic force.[1] In contrast, in another *in vitro* experimental model, we had found that the chondrogenic potential of the complexes was slightly reduced compared to that of normal rat MSCs.[6] On the basis of these previous results, we tried to control the concentration of TGF-β at the local site under an external magnetic force by using TGF-β-immobilized magnetic beads to form a denser cartilage matrix. Consequently, we found that as long as the CDM was supplemented with 10 μL/mL TGF-β-immobilized magnetic beads (TGF-β concentration: 1 ng/mL) and these TGF-β-immobilized magnetic beads were localized under an external magnetic force, the MSC-magnetic bead complexes could differentiate along the chondrogenic lineage even in three-dimensional culture systems in CDM lacking TGF-β.

In this study, Ferri Sphere 100C® magnetic beads were used. As this model has potential for clinical application, but several investigators have shown that micro-sized magnetic beads are phagocytosed by some cells such as active dendritic cells[20] or CD8-positive lymphocytes,[21] we speculated that mediator-immobilized magnetic beads may be taken up by cells such as macrophages once released from the cell surface[6] or from an external magnetic force.

The mechanism underlying the action of the TGF-β-immobilized magnetic beads remains unknown. The TGF-β superfamily contains multifunctional growth factors that are responsible for many cellular processes such as differentiation, proliferation, and apoptosis.[22,23] However, the effects of TGF-β during chondrogenesis are still unclear. Chondrocyte differentiation is regulated by the conflicting effects of TGF-β. TGF-β promotes the differentiation of embryonic chick limb cartilage.[24] MSC-derived primary chondrogenesis needs TGF-β signals.[7] TGF-β activates the TGF-β type I receptor by forming a ligand-receptor complex with the type II receptor. Several TGF-β responsive pathways, such as the Smad2/3 and mitogen-activated protein kinase (MAPK) pathways, have been identified as key signaling processes and are regulated by the activation of the TGF-β receptor.[25–27] We speculate that the TGF-β-immobilized magnetic beads linked to the MSCs might stimulate the Smad2/3 signaling pathway and therefore produce Smad 2 and Smad 3, which are phosphorylated and translocated into nuclei along with nonlabeled TGF-β.[28] Studies that focus on the function of TGF-β-regulated Smads (Smad2/3) during chondrogenesis from MSCs are required to further investigate the mechanism underlying the action of the TGF-β-immobilized magnetic beads.

In conclusion, we have demonstrated that our novel TGF-β-immobilized magnetic beads could lower the concentration of TGF-β necessary for chondrogenesis of MSC-magnetic bead complexes. Our cell and growth factor delivery systems, which can be influenced by an external magnetic force *in vitro*, has the potential to support minimally invasive cartilage repair such as intra-articular cell transplantation without scaffolds. Our results suggest that the use of TGF-β-immobilized magnetic beads under an external magnetic field can enhance chondrogenesis of MSC and decrease the incidence of side effects caused by injection of TGF-β directly into the joint. However, further studies will be necessary to evaluate the effectiveness of our new stem cell and growth factor delivery system for cartilage repair *in vivo*.

A part of this study won the Best Poster Presentation at the 20th Annual Research Meeting of the Japanese Orthopedic Association, October 20-21, 2005.

References

1. Ochi M, Adachi N, Nobuto H, Yanada S, Ito Y, Agung M. Articular cartilage repair using tissue engineering technique—Novel approach with minimally invasive procedure. Artif Organs 2004;28:28–32.

2. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889–895.
3. Ochi M, Uchio Y, Tobita M, Kuriwaka M. Current concepts in tissue engineering technique for repair of cartilage defect. Artif Organs 2001;25:172–179.
4. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J. Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br 2002;84:571–578.
5. Matsusue Y, Yamamuro Y, Hama H. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 1993;9:318–321.
6. Yanada S, Ochi M, Adachi N, Nobuto H, Agung M, Kawamata S. Effects of CD44 antibody- or RGDS peptide-immobilized magnetic beads on cell proliferation and chondrogenesis of mesenchymal stem cells. J Biomed Mater Res A 2006;77: 773–784.
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.
8. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998;238:265–272.
9. Kotobuki N, Hirose M, Takakura Y, Ohgushi H. Cultured autologous human cells for hard tissue regeneration: Preparation and characterization of mesenchymal stem cells from bone marrow. Artif Organs 2004;28:33–39.
10. Sekiya I, Larson BL, Vuoristo JT, Reger RL, Prockop DJ. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. Cell Tissue Res 2005;320:269–276.
11. Shirasawa S, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: Optimal condition and comparison with bone marrow-derived cells. J Cell Biochem 2006; 97:84–97.
12. Elford PR, Graeber M, Ohtsu H, Aeberhard M, Legendre B, Wishart WL, Mackenzie AR. Induction of swelling, synovial hyperplasia and cartilage proteoglycan loss upon intra-articular injection of transforming growth factor β2 in the rabbit. Cytokine 1992;4:232–238.
13. Bakker AC, van de Loo FAJ, van Beuningen HM, Sime P, van Lent PLEM, van der Kraan PM, Richards CD, van den Berg WB. Overexpression of active TGF-β1 in the murine knee joint: Evidence for synovial-layer-dependent chondro-osteophyte formation. Osteoarthritis Cartilage 2001;9:128–136.
14. Hanada K, Solchaga LA, Caplan AI, Hering TM, Goldberg VM, Yoo JU, Johnstone B. BMP-2 induction and TGF-β1 modulation of rat periosteal cell chondrogenesis. J Cell Biochem 2001;81:284–294.
15. Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Isolation and multilineage differentiation of bovine bone marrow mesenchymal stem cells. Cell Tissue Res 2005;319:243–253.
16. Dennis JE, Caplan AI. Porous ceramic vehicles for rat-marrow-derived (*Rattus norvegicus*) osteogenic cell delivery: Effects of pre-treatment with fibronectin or laminin. J Oral Implantol 1993;19:106–115.
17. Hunziker EB. Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. Osteoarthritis Cartilage 2001;9:22–32.
18. Matsuo T, Sugita T, Kubo T, Yasunaga Y, Ochi M, Murakami T. Injectable magnetic liposomes as a novel carrier of recombinant human BMP-2 for bone formation in a rat bone-defect model. J Biomed Mater Res A 2003;66:747–754.
19. Tanaka H, Sugita T, Yasunaga Y, Shimose S, Deie M, Kubo T, Murakami T, Ochi M. Efficiency of magnetic liposomal transforming growth factor-β1 in the repair of articular cartilage defects in a rabbit model. J Biomed Mater Res A 2005;73:255–263.
20. Lutjens P, Siekmeier R, Holzel V, Bedorf M, Hanfland P, Potzsch B. Uptake of magnetic beads by dendritic cells, a major obstacle for the use of beads in biological assays. Acta Haematol 2004;112:172–176.
21. Burkhardt O, Merker HJ. Phagocytosis of immunobeads by CD8 positive lymphocytes during magnetic cell sorting. Ann Anat 2002;184:55–60.
22. Heldin CH, Miyazono K, ten Dijke P. TGF-β signaling from cell membrane to nucleus through SMAD proteins. Nature 1997;390:465–471.
23. Shi Y, Massague J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 2003;113:685–700.
24. Kulyk WM, Rodgers BJ, Greer K, Kosher RA. Promotion of embryonic chick limb cartilage differentiation by transforming growth factor-β. Dev Biol 1989;135:424–430.
25. Liu F. Receptor-regulated Smads in TGF-β signaling. Front Biosci 2003;8:1280–1303.
26. Hanafusa H, Ninomiya-Tsuji J, Masuyama N, Nishita M, Fujisawa J, Shibuya H, Matsumoto K, Nishida E. Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-β-induced gene expression. J Biol Chem 1999;274:27161–27167.
27. Sano Y, Harada J, Tashiro S, Gotoh-Mandeville R, Maekawa T, Ishii S. ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-β signaling. J Biol Chem 1999;274:8949–8957.
28. Furumatsu T, Tsuda M, Taniguchi N, Tajima Y, Asahara H. Smad3 induces chondrogenesis through the activation of Sox9 via CREB-binding protein/p300 recruitment. J Biol Chem 2005;280:8343–8350.

Arch Orthop Trauma Surg (2010) 130:231–236
DOI 10.1007/s00402-009-0998-2

TRAUMA SURGERY

Repair of a large osteochondral defect in the knee joint using autologous and artificial bone graft combined with motion preserving distraction arthroplasty: a case report

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Received: 10 January 2009 / Published online: 5 November 2009

Abstract The biological reconstruction of a large osteochondral defect in the weight-bearing area of the knee joint has long been a challenge to orthopedic surgeons. We present a case of a large posttraumatic defect in the weight-bearing area of knee joint treated with a novel distraction arthroplasty device after reconstruction of the joint surface using combined autologous and artificial bone graft.

Keywords Large osteochondral defect repair · Knee · Distraction arthroplasty

Introduction

Large osteochondral defects are associated with mechanical instability and are accepted indications for surgical intervention to prevent development of degenerative joint disease.

Ideally, a large osteochondral defect should be repaired with a graft that can provide mechanical stability and allow early postoperative function under physiologic loading conditions [1].

Osteochondral defects in the weight-bearing area are difficult to treat effectively using biological methods [2, 3].

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The fragile repair tissue induced from the bone marrow is thought to be damaged by overloading. Furthermore, it is well known that joint motion promotes repair of osteochondral defects in the joint [5, 6]. Therefore, to minimize damage to the repaired tissue, long-term unloading with continuous passive motion was applied [1].

Distraction arthroplasty is a technique that has been used mostly at the elbow, hip, and ankle joints to preserve joint space and decrease the weight-bearing load. It delays the need for arthrodesis or joint replacement surgery [7–10].

The senior author [11] developed a new articulated arthroplasty device for the knee joint (Meira, Nagoya, Japan). In addition to the merits of previous devices, which include preservation of the joint surface and protection against overload on the regenerating fibrocartilage during weight bearing, this new articulated device permits smooth exercising of the joint during fixation [11].

We hypothesize that articulated joint distraction may be a useful treatment for osteochondral defects in the weight-bearing area. So we present a case of large osteochondral defect in the knee joint treated with a novel distraction arthroplasty (DA) device after reconstruction of the joint surface using combined autologous and artificial bone graft.

Case report

An 18-year-old female patient presented to us with her chief complaints: right knee pain with a limited range of motion, instability and deformity of the knee especially on weight bearing.

In March 2003, she was injured by traffic accident and resulting an open fracture of the right knee joint. Her plain X-rays revealed also a remarkable bone defect in the right

medial tibial condyle due to a markedly depressed fracture of the right medial tibial plateau (Schatzker classification type IV B, AO/ASIF type B2 pure depression) with a corresponding bony defect of the right medial femoral condyle due to an impacted compression fracture. Initial MRI demonstrated only medial meniscus tear and medial collateral ligament (MCL) injury. There were no associated neurovascular injuries. At this time, debridement and open reduction of the dislocation fracture, and bracing were carried out.

In mid-October, she was referred to our hospital for the purpose of the treatment of the large bone defect in the right knee joint as revealed in plain X-ray (Fig. 1a, b) as well as CT (Fig. 2).

On presentation; the wound on the anteromedial aspect of the knee was completely healed with no evidence of infection. There was limited range of motion of the affected knee from 0 to 50. Marked varus malalignment of the right lower limb could be attributable to the marked bony defect on the medial side of the knee, meanwhile there was no clinical, or MRI evidence of lateral collateral ligament (LCL), anterior cruciate ligament (ACL) or posterior cruciate ligament (PCL) injuries.

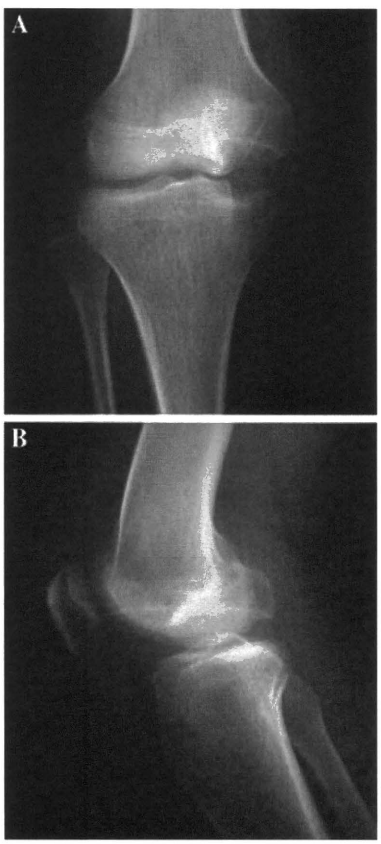

Fig. 1 Preoperative plain X-ray **a** AP view, **b** lateral view; revealing bony defect of the right medial femoral and tibial condyles

Surgical procedure

On exploration there was a large bony defect of the right medial tibial and femoral condyles, and irreparable complex injury of the medial meniscus.

The first step

The first step was reconstruction of the original shape of the medial femoral condyle using a combination of autologous iliac crest bone graft fixed by Herbert screws and artificial bone grafts shaped to the configuration of the remaining defect and consisting of hydroxyapatite ceramic with an interconnected porous structure (IP-CHA) (NEOBONE_, Toshiba Ceramics Co., Tokyo, Japan). Mesenchymal stem Cells and growth factors can readily penetrate the IP-CHA center to provide good osteoconduction in the early phase of the graft healing. This artificial bone was fixed by absorbable pins [poly-L-lactide (PLLA) pins of diameter 2 mm (Neofix; Gunze, Kyoto, Japan)] [12].

Lastly, reconstruction of medial tibial plateau using the reformed bone retrieved from a free bone fragment, supplemented by iliac crest cortico-cancellous bone graft fixed with cancellous screws (Figs. 3, 4).

The second objective

The second objective was stimulating bone adhesion and cartilage regeneration by preserving the joint space and reducing the load, meanwhile preserving the joint motion and function. This could be accomplished through external fixation using a novel articulated distraction arthroplasty (DA) device that decreases the load to bone grafted area, meanwhile allowing early start of joint motion to get the advantage of the stimulating effect of continuous passive motion on cartilage regeneration (Fig. 5a, b).

Partial medial meniscectomy was performed due to the associated irreparable complex damage, while the associated MCL injury was managed conservatively.

Postoperatively continuous passive motion exercise commenced the day after surgery and was continued for approximately 2 weeks, partial weight bearing was encouraged 6 weeks after surgery, This articulated distraction devise was removed 3 months postoperative, full weight bearing was then encouraged. During the period of external fixation, the patient was encouraged to do range of motion (ROM) exercises at first passively by physiotherapist and then active ROM exercises as tolerated by the patient. The screws were removed 6 months postoperatively. Follow-up of the patient later on demonstrated satisfactory arthroscopic (Fig. 6a, b), as well as radiological results taken as late as 4.5 years postoperatively (Figs. 7, 8, 9).

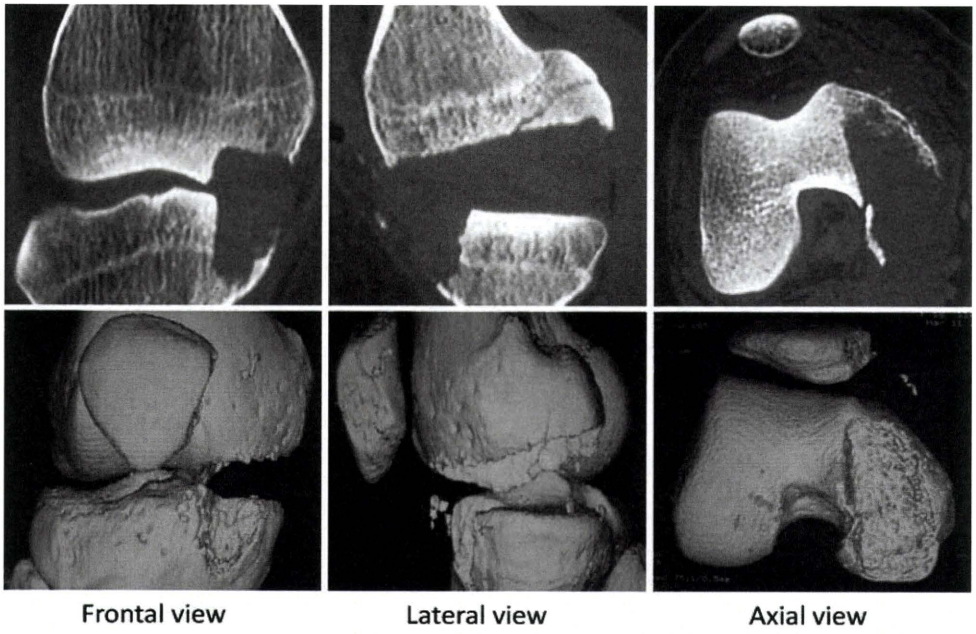

Fig. 2 Preoperative CT with three dimensional reconstruction showing a large osteochondral defect of the right medial femoral and tibial condyles

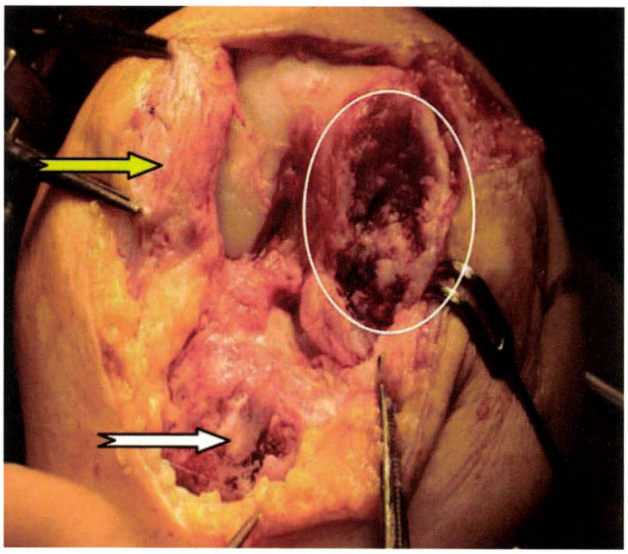

Fig. 3 Shows the bone defect in the medial femoral condyle (within the *white circle*), bone defect of the right medial tibial plateau (*white arrow*), patella (*yellow arrow*)

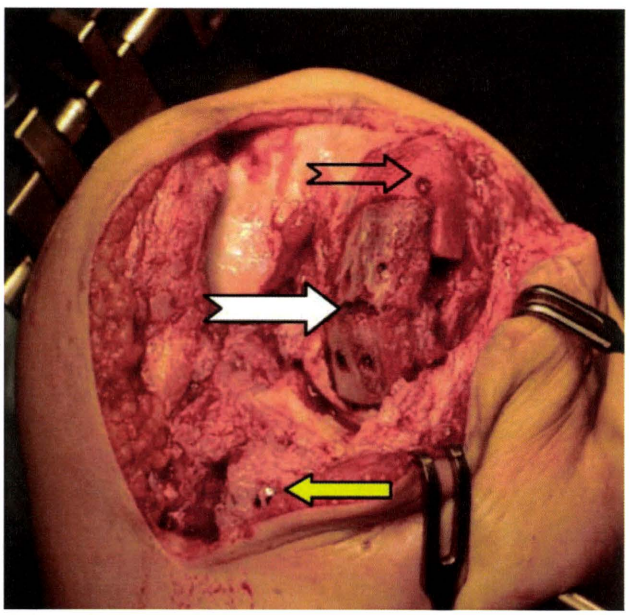

Fig. 4 Artificial bone (NEO BONE) graft fixed by absorbable pins (*transparent arrow*). Autologous iliac bone graft fixed by Herbert screw (*white arrow*). Reconstruction of the tibial condyle by the reformed bone from free bone fragment fixed by cancellous screw (*yellow arrow*)

The patient was completely satisfied having a painless stable knee joint with a pain-free range of motion of 0–150° (Fig. 10).

Discussion

The biological reconstruction of a large osteochondral defect in the weight-bearing area of the knee joint has been a challenge to orthopedic surgeons. This comprises reconstruction of the bony portion and restoration of the original shape of the reconstructed condyles, and the restoration of the hyaline articular cartilage to preserve the joint function and prevent or halt the likely progression towards osteoarthritis. The fragile repair tissue induced from the bone marrow is thought to be damaged by overloading; moreover, a defect of the cartilage in the weight-bearing area eventually progresses to osteoarthritis by causing friction and overload to the opposing articular surface. [13–16].

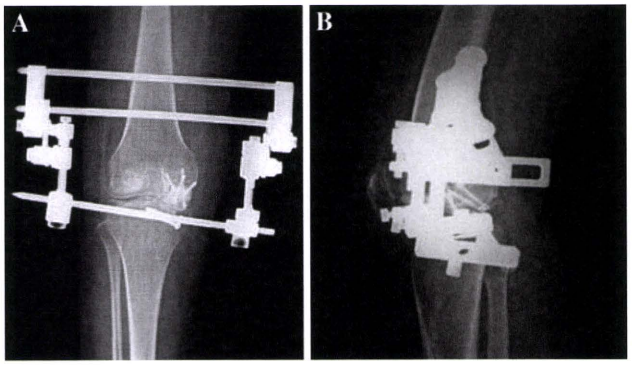

Fig. 5 Postoperative plain X-ray **a** frontal, and **b** lateral views

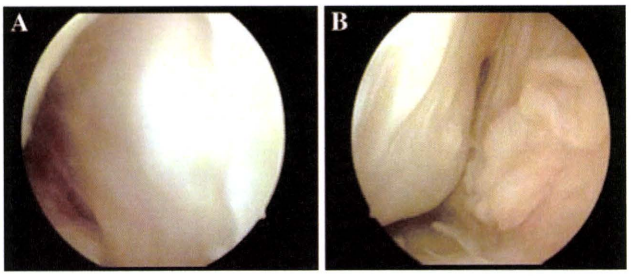

Fig. 6 **a**, **b** Postoperative arthroscopic findings showing resurfacing of the reconstructed bony surface. **a** Represents the reconstructed central weight bearing area of the medial femoral condyle (MFC), while **b** represents the adjacent area of the MFC, as well as the medial gutter as illustrated in MRI Fig. 7

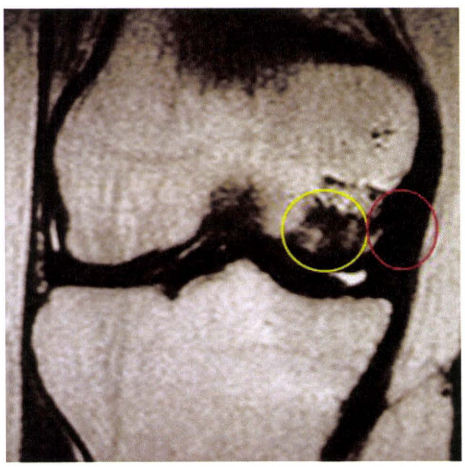

Fig. 7 Two and a half years postoperative coronal MRI showing the reconstructed bony defects, the cartilage layer showing mild residual irregularities [the *yellow circle* represents the arthroscopic view (**a**), while the *red circle* represents the arthroscopic view (**b**)]

The effective role of distraction arthroplasty in cartilage repair was demonstrated in a lot of studies; whereby joint distraction widens the joint space and decreases of the load to regenerated fibrocartilage. [11, 14, 17–19]

It is also well known that joint motion promotes repair of osteochondral defects in the joint [20–22]. Moreover, Kajiwara et al. [4] demonstrated that articulated joint

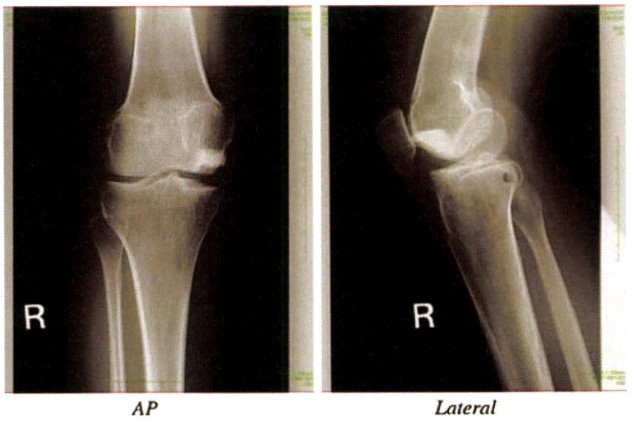

Fig. 8 Shows the latest follow-up X-rays taken about 4.5 years postoperative

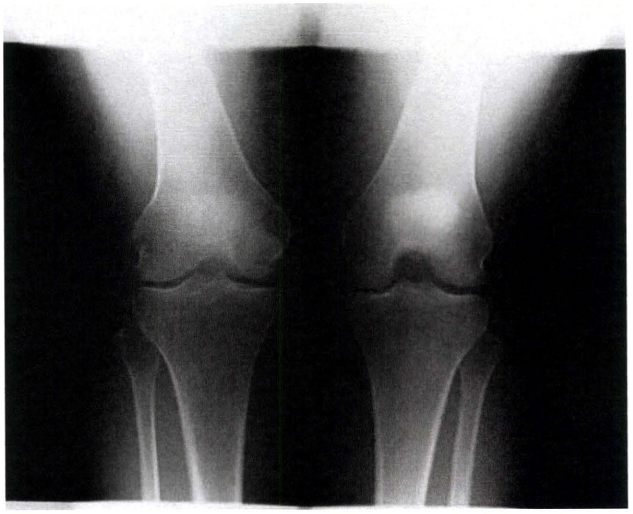

Fig. 9 AP weight-bearing X-ray of both knees taken in the latest follow-up about 4.5 years postoperative showing reconstruction of the bony defects with preservation of the joint space

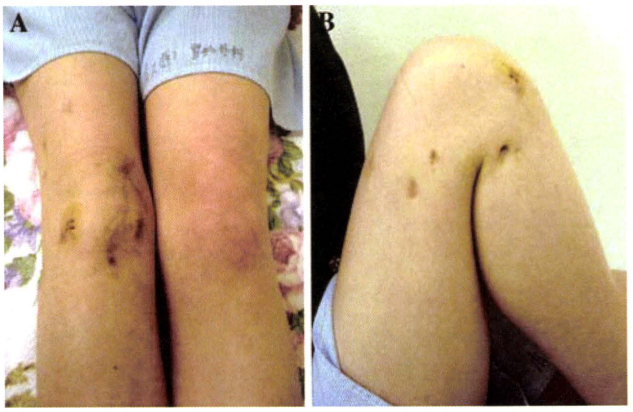

Fig. 10 Shows the postoperative range of movement from 0° (**a**) to 140° flexion (**b**)