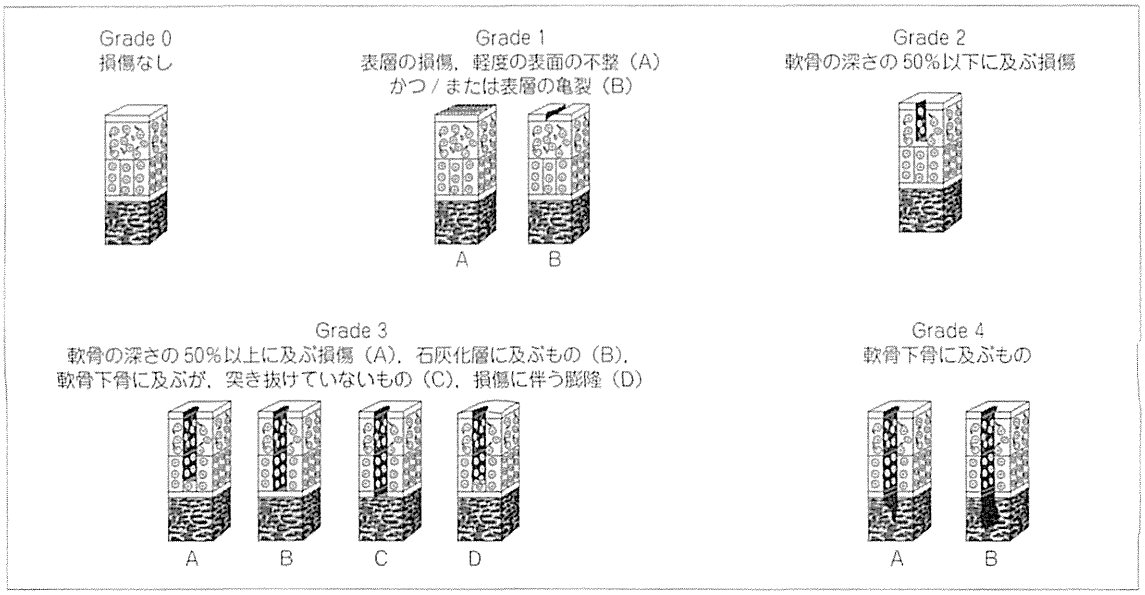

図1 ◆ICRS分類
軟骨損傷の深度により，1～4までのGradeに分類する．
（文献10より引用）

り，Grade 1～3損傷では自己修復にはなかなか至らない[13,14]．

関節軟骨損傷に対する治療

関節軟骨損傷の治療は長年困難であるとされてきた．しかし最近の知見から，受傷早期ではエビデンスを伴ういくつかの治療が確立されつつある．観血的治療，保存的療法の双方の進歩により，以前では放置されていた軟骨損傷でも，軟骨の修復，再生に対していくつかの治療の選択肢を提示できるようになってきている．

1．保存療法

a．無治療

関節軟骨損傷が変形性関節炎に至るメカニズムについて多くの研究がなされているが，特にアスリートにおいては，いまだに軟骨損傷の自然経過についての前向き臨床研究のデータは限られている．Messnerら[15]は荷重域に軟骨欠損を認めた若い28人のアスリートについて，未治療での診断から14年後の膝の機能は比較的保たれており，43%には関節症変化を認めなかったと報告している．

b．内服治療

各種サプリメント(コンドロイチン，グルコサミン，アボカド大豆不鹸化物など)を用いた治療については，変形性関節症(OA)を対象としたものが多いが，大規模試験が多数行われている．コンドロイチン，アボカド大豆不鹸化物については，軟骨再生効果や，疼痛・機能改善効果が示されている[16～19]一方で，グルコサミンについては有効性の一致をみていない[20]．

c．関節内注射

ステロイドの関節内注射は関節炎症症状の急速な緩和目的に用いられ，短期での症状改善は認めるものの，全身，局所の副作用も多い[21,22]．また組織修復能，代謝回転を低下させるため慎重な投与が望まれる．

ヒアルロン酸も軟骨損傷膝に対して短期的に用いられ，OAにかかわるサイトカインと酵素の遺伝子発現を抑えることによる軟骨保護作用と抗炎症作用の2つの作用を有する[23]．ステロイドの関節内注射にみられるような副作用もほとんどないが，約8%の患者に可逆性の偽炎症反応が起こるとされている[24]．

d. パルス電磁場療法

パルス電磁場により，軟骨細胞の増殖・プロテオグリカンの産生の促進，軟骨変性の予防，滑液中の炎症誘発サイトカイン産生と放出の抑制が報告されている．関節鏡による治療後に用いると，副作用なく，炎症を抑え，回復までの時間が短縮できることが報告されており[25]，種々の治療と組み合わせることによる相乗効果が期待できる．

2. 手術療法

患者の要求，症状，軟骨欠損サイズ・深さ，付随する事象(関節のアライメント，靱帯・半月損傷の有無，骨挫傷の有無など)を踏まえ総合的に判断し，術式を選択する必要がある．合併損傷がある場合は，軟骨損傷に対する治療の前もしくは同時に治療を行うべきである．

a. デブリドマン手術

関節鏡下の損傷軟骨のデブリドマン，超音波による焼灼は疼痛を改善するが，硝子軟骨での再生は得られず，また軟骨表面の張力を担う軟骨浅層のコーゲン線維を除去するため，軟骨の機能が損なわれる可能性がある[26]．したがって，これらは小さなフラップ状の軟骨損傷において第一選択となる．

b. 骨髄刺激法

侵襲が少なく，リハビリテーション期間が短いことからアスリートの軟骨損傷の治療として選択される機会も多い．線維軟骨様組織の形成がみられ，時に機能と疼痛の改善を認めることもあるが[27]，時間経過とともに変性の進行を認める[28]．スポーツ選手においては50%しか受傷前のレベルに復帰できなかったとする報告があり，40歳以下，軟骨損傷のサイズが2cm^{2}以下，術前に症状があった期間が12ヵ月以下など，症例を慎重に選べば良好な成績を得られる可能性が報告されている[29]．

c. 自家骨軟骨移植(osteochondral autograft transplantation：OATS)

非荷重部から採取した骨軟骨柱を欠損部に移植することにより，硝子軟骨様の軟骨再生が可能である．ドナーサイトの傷害が問題とされるが，長期に及ぶ合併症の発生率は高くない[30]．アスリートに対する効果としては，複数の前向き研究において，knee function scores，ICRS score，MRI評価でそれぞれ95%，84%と94%でgoodからexcellentという良好な結果が報告されている[31~33]．競技復帰率は61～93%で，6～9ヵ月の間に可能であった．術前の長い有症期間と30歳以上という年齢が，スポーツへの復帰率の低下に関係していた．海外ではアスリートに対する同種骨軟骨移植も行われており，良好な臨床成績が報告されている[34]．

d. 自家培養軟骨細胞移植(autologous chondrocyte implantation：ACI)

Petersonらによって最初に行われた方法で[35]，非荷重部の関節軟骨より分離した軟骨細胞を培養により増やし，骨膜にてパッチした軟骨欠損部に移植するというものである．複数の研究者による軟骨全層欠損の患者に対する移植でも，硝子軟骨様の軟骨再生が得られ，長期間のフォローアップでも関節機能が保たれていたと報告されている[36,37]．しかし，原法では2回の手術を要し，骨膜採取部位および骨膜パッチの変性，培養中に軟骨細胞が脱分化するという問題があった．その問題を解決するため，細胞の足場，スキャフォールドを用いた第2，3世代ACIが開発された．これにより，移植軟骨細胞の状態が安定し，手術手技も簡略化され，現在では関節鏡下に手術が行われている．第2，3世代ACIは若いアスリートで欠損サイズが大きな表層欠損に良い適応であると思われる．加えて膝蓋大腿関節においても良好な結果が得られている[38]．術前の有症状期間が12ヵ月以下，年齢25歳以下であることが予後に重要で，付随するアライメントの矯正や，関節の安定化といった手術歴はアスリートの競技復帰には影響がなかったと報告されている[39]．他方，軟骨欠損が4cm^{2}以上，骨髄刺激法の既往があると成績が悪いとの報告があり[26]，軟骨下骨の状態は軟骨組織の修復に重要であることが示唆されている．

培養中に軟骨細胞が脱分化してしまうことの対策としては，*in vivo*で軟骨形成を促す遺伝子マーカーのプロファイルの発現と細胞の表現型で特徴づけられるサブグループを移植する試みがなされており，骨髄刺激法との比較で，構造的，組

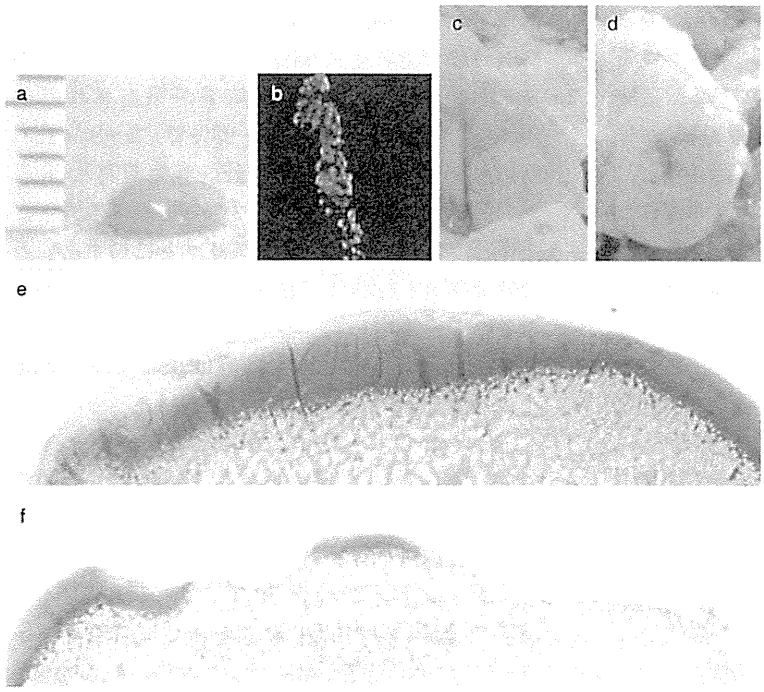

図2 ➔TECを用いた家畜ブタ膝軟骨修復実験
a　TECのマクロ像，b　TECの免疫染色(fibronectin)，c，e　大腿骨の軟骨単独欠損にTECを移植後6ヵ月，d，f　軟骨欠損未治療群
(文献46より引用一部改変)

織的に優れた軟骨再生が得られ，臨床成績も良好であったとの報告がある[40]．

また，体外での軟骨細胞の培養を要さない方法も検討されている．非荷重部より採取した健常軟骨を粉砕し，そのままスキャフォールドに包埋し移植する方法では，細断した軟骨片から軟骨細胞がスキャフォールド内に再分布し，6ヵ月で硝子軟骨様の再生組織が得られたとの報告がある[41]．この場合手術は1回で行うことが可能である．

e．間葉系幹細胞を用いた治療

近年各組織由来のMSCを用いた治療も検討されている．MSCは自己複製能と多分化能(脂肪，骨，軟骨分化)を有し，適切な環境下で培養されれば，軟骨細胞に分化しうる[42]．よって軟骨細胞移植において指摘されている高齢者におけるドナー組織の質の問題，細胞増殖能の低さと，培養中の脱分化などの問題をクリアできる利点がある．MSCを用いた軟骨再生については多くの研究がなされているが，まだ臨床での報告は少ない[43,44]．

我々はアスコルビン酸存在下に滑膜由来MSCを高密度培養することによって形成されるMSCとその細胞外マトリックスのみからなるスキャフォールドフリー3次元人工組織(tissue-engineered construct：TEC)を用いた軟骨再生に取り組んでいる．TECはファイブロネクチンを発現しており軟骨欠損部に接着するため[45]，移植の際に固定の必要がなく，スキャフォールドの使用によってしばしば問題となる免疫反応の可能性も少ない．ブタの軟骨欠損モデルにて硝子軟骨様の組織修復を認め，力学的試験でも良好な結果を報告している(図2)[46]．平成24年度より厚生労働省ヒト幹細胞臨床研究を開始している．

おわりに

膝関節軟骨損傷に対する治療法は発展してきたが，いまだゴールデンスタンダードとなる治療法は確立されていない．病態のメカニズム解明の進歩，さらには早期診断技術の開発による，新規治療法の開発，早期治療および科学的エビデンスに基づいたリハビリテーションプログラムの導入が今後期待される．

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半月板変性に対する治療法：国際的現況

Current status on the preserving intervention of degenerative menisci

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抄録▶膝関節の恒常性を維持するためには半月板は必要不可欠であるが，変性断裂した半月板に対しての有効な治療法は確立されていない．国際的には半月板機能の回復のために研究レベルで多くの生物学的アプローチやバイオマテリアルの開発，さらに臨床では同種半月板移植が数多く応用されている．本稿ではこれらの治療研究の現況について紹介する．

Key Words 半月板，変性，修復，成長因子，バイオマテリアル，同種半月板移植

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はじめに

これまで述べられてきたように，半月板は膝の安定性に寄与し，衝撃吸収機能や荷重分散機能を担う．半月板の血流供給は辺縁1/3までしか認めず，したがって無血管野に損傷が生じると十分な生体修復反応は得られない．

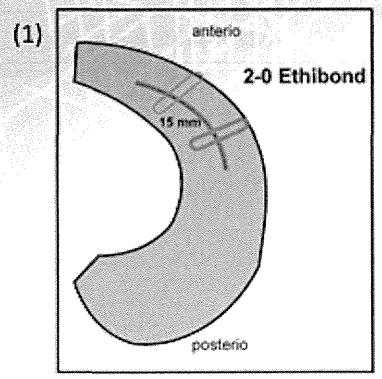
この無血管野に生じた断裂，とりわけ半月板の変性を基盤として生じた断裂は高頻度に切除される．しかし生体力学的な解析により，たとえ部分切除であっても関節の接触圧が有意に上昇することが明らかとなっている[1]．さらに，半月板を全切除すると，関節の接触面積は約50％に減少し，衝撃吸収機能は約20％にまで減少するとされている[2]．さらに，これまで多くの動物実験や臨床報告から，半月板の部分切除もしくは全切除により二次性に変形性関節症が進行することが示唆されている．その意味で半月板の温存のための治療戦略は重要である．

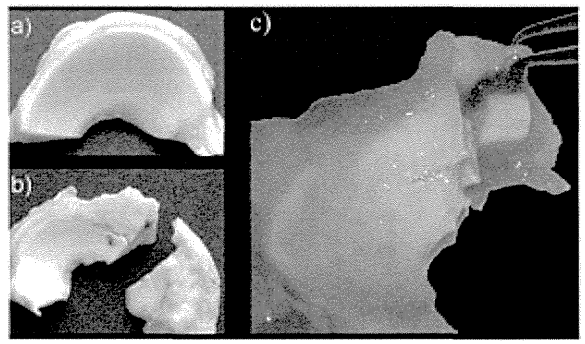
本稿では現在臨床および研究の場において試みられている半月温存のためのアプローチについて紹介する．

半月板修復の augmentation

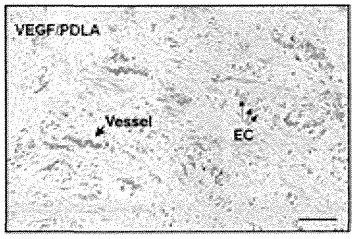
自然治癒能力に乏しい無血管領域の半月板を単純に縫合するだけでは縫合部に十分な修復反応は生じず，長期的な治療効果は期待できない．そこで半月板の修復能を向上させることを目的として，サイトカインを用いた修復向上を狙った研究が報告されている．

成田らはウサギ半月板辺縁に関節外から作製した水平断裂部にFGF-2（Fibroblast growth factor-2）を添加したgelatin hydrogelを留置することで，術後4週間にわたって細胞増殖の促進と細胞死の抑制が認められ，結果として修復部の細胞密度が上昇し，組織修復が促進されたことを報告した[3]．Heらは，ウサギ半月板の無血管領域から採取・培養した線維性軟骨細胞にCTFG（connective tissue factor）を添加するとⅠ型・Ⅱ型コラーゲンおよびVEGF（vascular en-





(3)



(表1)

	Ethibond (N = 6)	PDLLA (N = 6)	VEGF/PDLLA (N = 6)
No healing	3 (50%)	4 (66.7%)	5 (83.3%)
Partial healing	3 (50%)	1 (16.7%)	1 (16.7%)
Complete healing	0	1 (16.7%)	0

図1　ヒツジの半月板縫合術においてVEGF/PDLLAコーティング縫合糸による治療成績への影響の検討実験
(1)断裂・縫合モデル
(2)手術2カ月後の各群での典型的な肉眼所見
a)正常半月板，b) EthibondもしくはPDLLAコーティング縫合糸群，c) VEGF/PDLLAコーティング縫合糸群：縫合糸周囲に組織が溶解したようなゼラチン様組織がみられる．
(3)手術2カ月後のVEGF/PDLLAコーティング縫合糸周囲組織の第Ⅷ因子免疫染色：他群より有意に血管内皮細胞の増生がみられた．
(表1)手術2カ月後の各群での治癒率：VEGF/PDLLAコーティング縫合糸群で有意に治癒率が低かった．
(文献5より引用)

dothelial growth factor)の産生が上昇することを示し，またウサギ半月板に縦断裂を作製後，縫合処置にCTFGを添加したfibrin glueを加えることで，損傷部周囲のⅠ型・Ⅱ型コラーゲンおよびVEGFの発現が上昇し，組織修復が促進されたと報告している[4]．また，Petersenらはヒツジ半月板の無血管領域に縦断裂を作製し，血管内皮細胞増殖因子であるVEGFをコーティング加工した縫合糸(エチボンド)の修復効果を検討したが，VEGFコート群ではかえって修復が抑制され，血管新生のみならず局所炎症反応の惹起が修復組織の質を低下させることが示唆された(図1)[5]．

PRP(Platelet-rich plasma)により複数のサイトカインを介して組織反応を惹起させる研究も報告されている．石田らはPRPが濃度依存性にウサギ半月板由来細胞の生存率と硫酸プロテオグリカンの産生を向上させ，ウサギ半月板部分欠損の修復を有意に促進させることを報告している[6]．しかしPRPは作成方法により品質特性が異なり，高濃度のPRPでは半月板細胞の増殖が逆に阻害されるという報告もある[7]．以上に示したようにPRPを含めたサイトカイン治療は，投与の目的と異なりanabolicな反応よりもcatabolicな反応が優位に惹起されてしまう危険性がある．投与方法や投与量によってそのバランスが変化する可能性があり，安定した治療効果の予測は現状では困難で，そのためか，いま

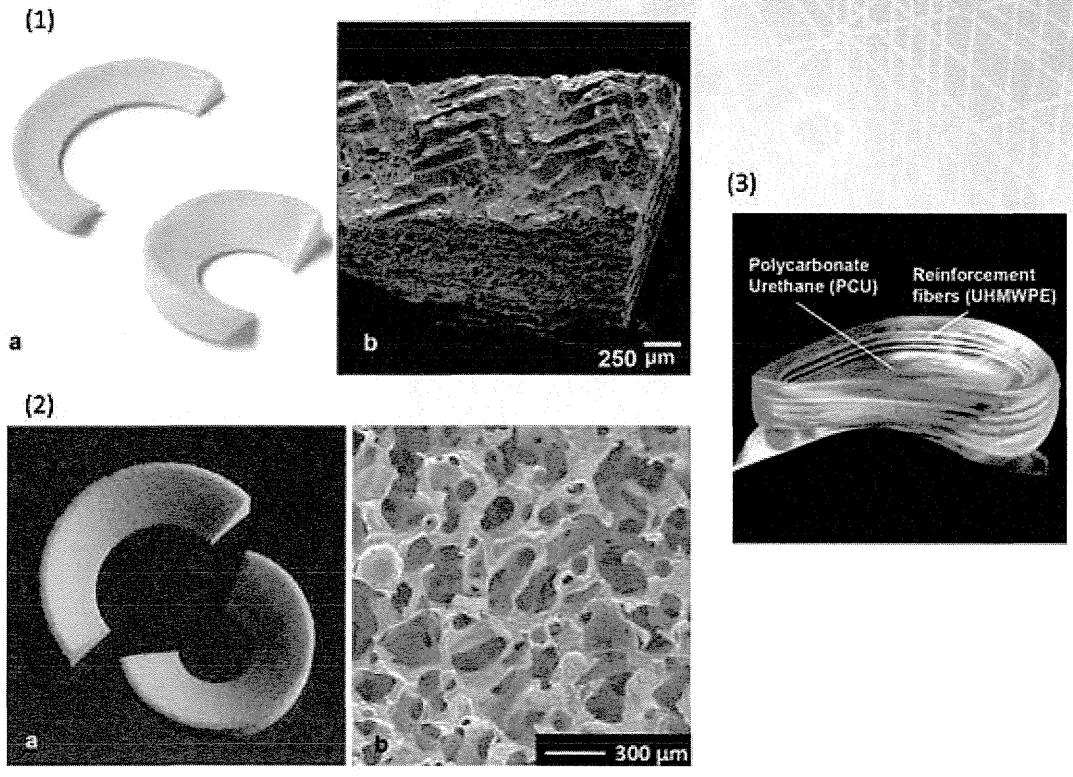


図2

(1) a：CMI®外観：内側(左)，外側用(右)
　　b：電子顕微鏡像，薄層の重層構造
(2) a：Actifit®外観：内側(左)，外側用(右)
　　b：電子顕微鏡像，連通孔をもつ多孔性の構造
(3) NU surface®：内側半月板全切除症例に使用．円盤状でFT関節に留置し，固定しない．
(Int Orthop 37：291–299, 2013より引用)

だヒト半月板修復への臨床応用の報告はみられない．

実際の臨床の場においては，自己末梢血より作成できるfibrin clotが，血小板由来のサイトカインを含有することに加え，fibrin由来マトリックスが修復の足場となりうることから，半月板縫合のaugmentationとして併用されている．van Trommelらは，辺縁部にまで達する外側半月の横断裂を認めた5症例(平均年齢20歳)に対して，関節鏡視下にfibrin clotを断裂部に挟むようにしてoutside-in法で水平縫合したところ，その全例が術後4カ月の再鏡視検査で修復反応を示し，術前と同様のスポーツレベルまで復帰したと報告している[8]．またHenningらは，半月板縫合時にfibrin clotを併用した群ではfailure rateが8％であり，使用しなかった群の41％に対して有意に低かったと報告している[9]．

人工半月板移植術

これまで，polyvinyl alchohol-dydrogelやpolyurethaneから成る人工半月板を用いた動物実験がいくつか報告されているが[10,11]，現在のところ半月板部分断裂症例に対して諸外国で臨床応用の機会を得たdeviceには以下の2つがある．ともに生体分解性の多孔体であり，1つはウシアキレス腱由来Ⅰ型コラーゲンからなるCMI®(collagen meniscus implant現在はMenaflex®に改名；ReGen Biologics)(図2-1)であり，もう1つはpolycaprolactone(80％)とurethane(20％)からなるActifit®(Orteq)(図2-2)である．直視

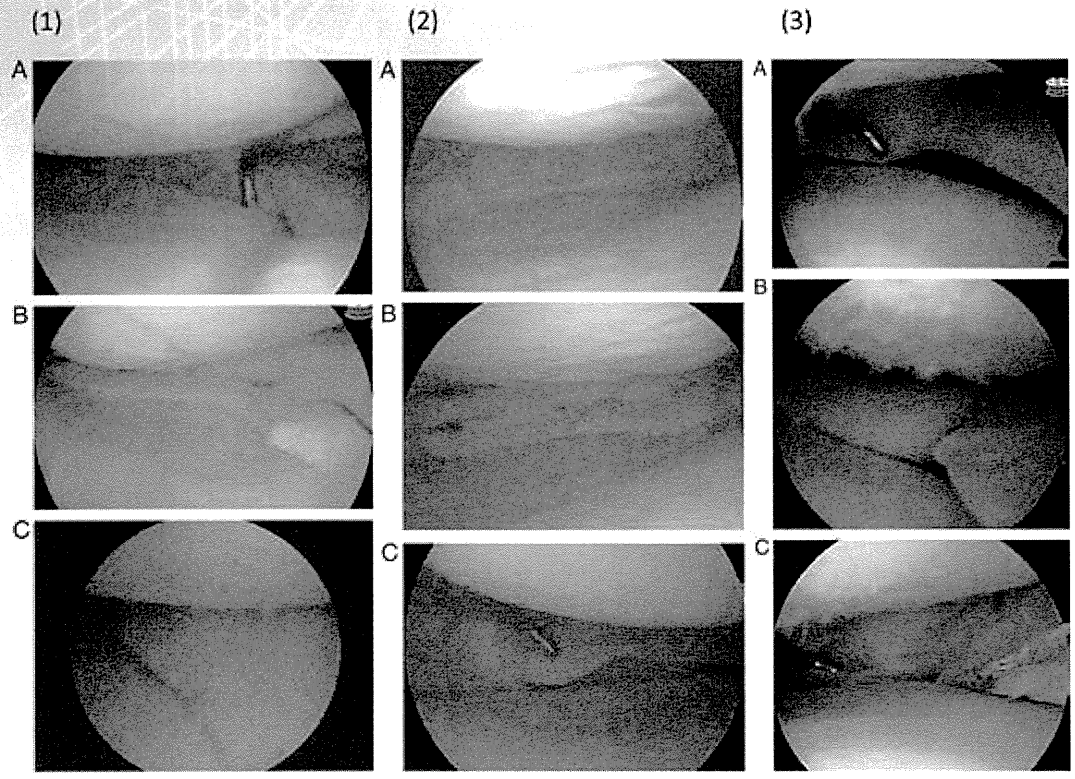


図3　関節鏡所見

A：初回手術時（移植前）
B：初回手術時（移植後）
C：再鏡視手術時（時期については後述）
（1）Actifit使用　5カ月後：充填率50%以上，（2）Actifit使用　10カ月後：充填率ほぼ100%
（3）Menaflex使用　2.5年後：充填率50%未満
（文献10より引用）

下もしくは関節鏡視下に半月板欠損のサイズを計測し，それに合ったサイズのimplantと残存半月板とを縫合糸で端端縫合して移植する．

Rodkeyらは311例の内側半月板損傷例を無作為にCMI移植群と半月板部分切除群とに群分けし，平均59ヵ月後にそれぞれの治療成績を比較した．術後1年での再鏡視所見では，移植群で有意に移植半月板への組織充填率が高く，移植部をBiopsyした組織学的検査では，全例で周囲組織から移植片へのintegrationや，移植片内部に半月板様の線維軟骨組織が認められた．また臨床的には，慢性期症例においてのみ有意な活動性（Tegner index）の改善がみられた[12]．Zaffagniniらは内側半月板損傷例に対して，CMIを移植した群と部分切除術を行った群のうち，10年以上follow-up（平均133カ月）可能であった33症例（移植群：17例，部分切除群：16例）について検討している．無作為試験でない点や，急性期・慢性期症例に分けていない点がRodkeyらとは異なるが，移植群では有意に臨床成績が良好で，またXpでの内側関節裂隙の患健側差が有意に小さかったと報告している[13]．しかし，術後10年以後に撮影されたMRIでは，4例に移植片の縮小，2例に消失が認められた．Spencerらは内側半月板に限定せず，内外側半月板損傷例に対してMenaflexもしくはActifitを移植した症例の術後成績を報告している[10]．術後平均19.4カ月〔Menaflex群（18～27カ月），Actifit群

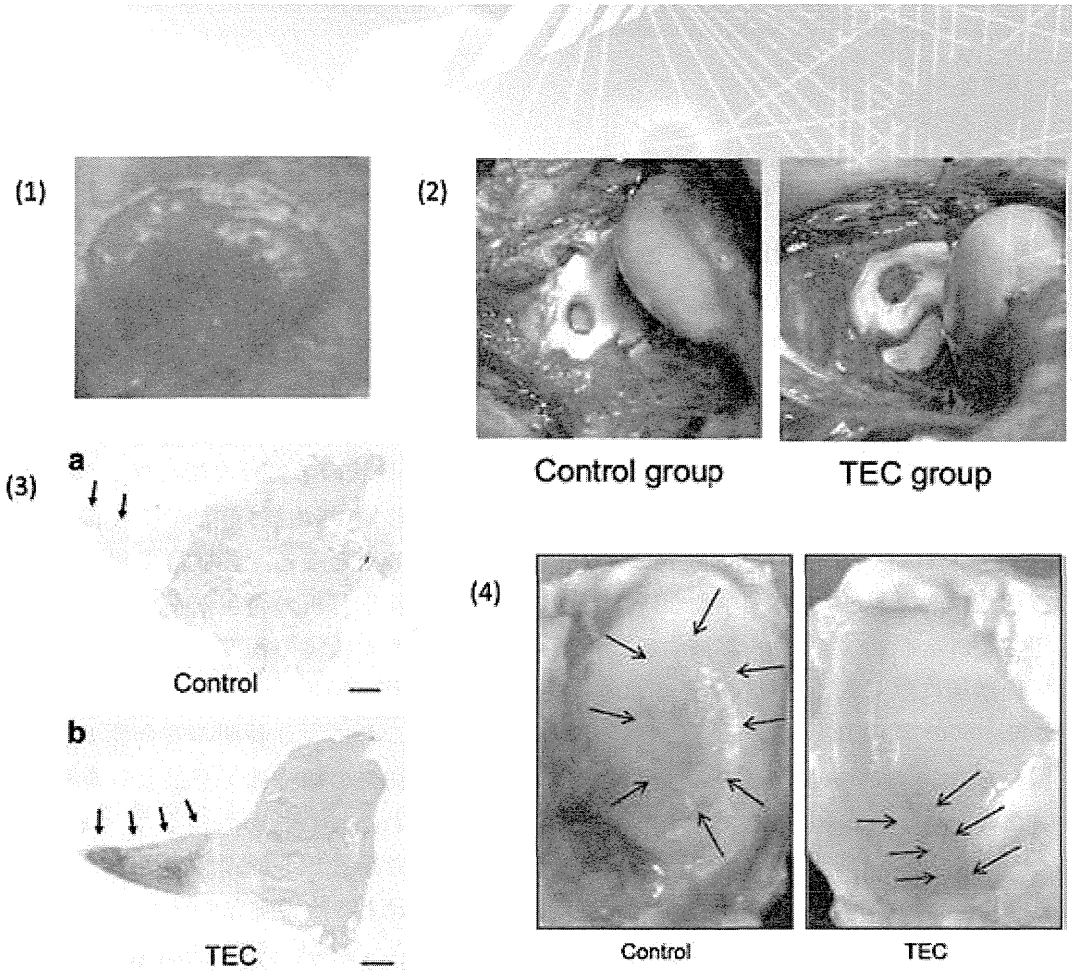


図4　TECによるウサギ半月板再生実験
(1) TEC外観
(2)内側半月板前角に作成した径4mmの欠損(左：欠損のみ，右：TEC移植)
(3)手術6カ月後の組織学的所見：TEC群では正常と同様の線維軟骨再生がみられる．
(4)手術6カ月後の脛骨内側プラトーの軟骨損傷の肉眼所見：TEC群で有意に抑制できた．
(文献23より引用)

(12～18カ月)〕時点で，両群合わせた23例中21例(91.3%)で臨床スコアの改善がみられ，群間には差がなく，また両群ともにMRIで軟骨損傷の進行もみられなかったと報告している．しかし，術後平均12.8カ月で施行した再鏡視所見では移植片の50%以上に組織の充填を認めたものはActifit群で5例中4例であったが，Menaflex群では9例中4例のみであった(図3)．Menaflex・Actifitともに短・中期的な安全性と有効性が示されつつあるが，その適応は限定的であり，今後長期追跡を含むさらなる検証が必要である．

最近では，この人工半月板に骨髄穿刺濃縮細胞(BMAC：Bone Marrow Aspiration Concentrate)を含ませて移植するといった，細胞治療とのHybridな治療方法も紹介されており(Verdonk P：ICRS Focus Meeting, 2013)，今後の成果が望まれる．

また，半月板全切除症例に対して，polycarbonate urethane製の非解剖学的なNU surface®というインプラントが現在開発されている(図2-3)．この形態は半月板の解剖学的形態とは異なりdisc状で，周囲組織と固定はせずに大腿脛骨関節内に留置するだけという全く新しいコンセプトに基づいたインプラントである．ヒツジの内側半月板と置換した前臨床試験(この試験では，解剖学的なpolycarbonate urethane製インプラントを使用)で安全性を確認した後，現在臨床試験が行われている[15]．

半月板移植術

諸外国では他家半月板移植術が1984年にMilachowskiらによって始められて以来[16]，広く行われている．移植半月板の殺菌・保存方法として主に凍結乾燥法(lyophilized graft)や低温保存法(cryopreserved graft)急速冷凍法(deep frozen graft)があげられているが，総じて凍結乾燥された移植片の成績は不良で[17]，最近はこの方法による処理は行われずfresh allograftが広く用いられている．また，生体力学的な研究により適切でないサイズの移植片を移植することは接触圧の増加に繋がることが判明しており[18]，適切なサイズの選択が必須である．こうした殺菌・保存方法や術式(移植片のサイズ選択など)といった複合的な因子を加味して検証する必要はあるが，低温保存処理されたfresh allograftを用いた移植術後の中～長期的な調査では，臨床的にはおおむね有意な症状の改善が報告されており，画像的にも術後10年で変形性関節症の進行がみられないことを報告した論文もあり，その有効性が確かめられてきている[19,20]．しかし，allograftでは有害事象としてウィルスや異常プリオン蛋白感染の危険性がある．これまでallograft半月板移植による感染症の報告はなされていないが，整形外科領域においても他家移植組織によるHIVやHCVといったウィルス感染症の報告は過去に存在する．本邦ではこうした安全性の担保に加え，倫理的問題が加味され組織バンキングシステムの整備が遅れている．マトリックス組成を考えた場合Allograftはある意味究極の組織工学製品であり，わが国においても早期の導入が待たれるところである．

組織工学による半月板修復

半月板治療に対する細胞治療も動物実験ではいくつかの報告がなされている．詳細は他稿に譲るが，自家もしくは他家半月板由来細胞や間葉系幹細胞などがcell sourceとして使用されている[21,22]．われわれは，滑膜由来の間葉系幹細胞と，それ自身が産生する細胞外基質のみから構成される3次元人工組織TEC(Tissue engineered construct)を開発し，骨成熟ミニブタ半月板欠損モデルに対しての治療効果を検討した[23]．内側半月板の前角に作製した径4 mmの円柱状の半月板欠損に対して，TEC移植群と非移植(無治療)群とで半年後の治療成績を比較したところ，移植群で組織学的に有意に良好な半月板の再生が得られ，脛骨プラトーへの軟骨損傷の程度を有意に軽減させることを確認した(図4)．スキャフォールドを使用しないことにより長期の安全性や作成コストの点で従来の組織加工製品より有利であると考えられる．われわれは現在，TECを用いて，厚生労働省ヒト幹細胞臨床研究により軟骨損傷に対する治療研究を行っている(研究概要：https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=search&action=input　検索ID：UMIN000008266)．今後は半月板治療への臨床応用の拡大が計画されている．

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TISSUE ENGINEERING: Part A
Volume 00, Number 00, 2014

DOI: 10.1089/ten.tea.2013.0414

Original Article

Osteochondral Repair Using a Scaffold-Free Tissue-Engineered Construct Derived from Synovial Mesenchymal Stem Cells and a Hydroxyapatite-Based Artificial Bone

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For an ideal osteochondral repair, it is important to facilitate zonal restoration of the subchondral bone and the cartilage, layer by layer. Specifically, restoration of the osteochondral junction and secure integration with adjacent cartilage could be considered key factors. The purpose of the present study was to investigate the feasibility of a combined material comprising a scaffold-free tissue-engineered construct (TEC) derived from synovial mesenchymal stem cells (MSCs) and a hydroxyapatite (HA) artificial bone using a rabbit osteochondral defect model. Osteochondral defects were created on the femoral groove of skeletally mature rabbits. The TEC and HA artificial bone were hybridized to develop a combined implant just before use, which was then implanted into defects ($N=23$). In the control group, HA alone was implanted ($N=18$). Histological evaluation and micro-indentation testing was performed for the evaluation of repair tissue. Normal knees were used as an additional control group for biomechanical testing ($N=5$). At hybridization, the TEC rapidly attached onto the surface of HA artificial bone block, which was implantable to osteochondral defects. Osteochondral defects treated with the combined implants exhibited more rapid subchondral bone repair coupled with the development of cartilaginous tissue with good tissue integration to the adjacent host cartilage when assessed at 6 months post implantation. Conversely, the control group exhibited delayed subchondral bone repair. In addition, the repair cartilaginous tissue in this group had poor integration to adjacent cartilage and contained clustered chondrocytes, suggesting an early osteoarthritis (OA)-like degenerative change at 6 months post implantation. Biomechanically, the osteochondral repair tissue treated with the combined implants at 6 months restored tissue stiffness, similar to normal osteochondral tissue. The combined implants significantly accelerated and improved osteochondral repair. Specifically, earlier restoration of subchondral bone, as well as good tissue integration of repair cartilage to adjacent host tissue could be clinically relevant in terms of the acceleration of postoperative rehabilitation and longer-term durability of repaired articular surface in patients with osteochondral lesions, including those with OA. In addition, the combined implant could be considered a promising MSC-based bio-implant with regard to safety and cost-effectiveness, considering that the TEC is a scaffold-free implant and HA artificial bone has been widely used in clinical practice.

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Introduction

OSTEOARTHRITIS (OA) is a common disease that causes joint pain, joint deformity, and functional disability, and it could potentially affect the quality of life of elderly populations worldwide.[1] There are several clinical options for the treatment of OA such as total joint replacement, osteotomy, and osteochondral transplantation, according to the severity of the joint destruction. Moreover, several biological approaches such as the use of biologics and tissue-engineered materials have been recently evaluated.[2–6]

For an ideal repair of osetochondral lesions with the involvement of subchondral bone pathology, it is important to regenerate subchondral bone, and to facilitate zonal restoration of cartilage and subchondral bone, layer by layer.[5,7,8] As a strategy to regenerate these structures layer by layer, biphasic or triphasic constructs have been developed.[9–17] These constructs have been reported to contribute to good osteochondral repair *in vivo*, while there are still several concerns associated with the complicated process of manufacturing implants such as cell seeding, cell differentiation and combining materials, and the long-term safety of these constructs due to the involvement of chemical- or animal-derived materials. Therefore, a novel construct that overcomes such potential problems is preferable for clinical applications. The process of manufacturing implants should be simplified. The use of chemical- or animal-free materials could be considered an ideal method to meet such requirements.

Artificial bones generated from hydroxyapatite (HA) or beta-tricalcium phosphate (β-TCP) have been widely used for clinical treatment of bone defects after fractures or after resection of bone tumors.[18–20] We have developed a novel fully interconnected HA artificial bone with a sufficient initial strength, as well as an excellent bone-formation capacity,[19,21] and previously reported the feasibility of this implant to repair subchondral bone.[18] In addition, we have developed a scaffold-free three-dimensional tissue-engineered construct (TEC) composed of allogenic mesenchymal stem cells (MSCs) derived from the synovium and extracellular matrices (ECMs) synthesized by the cells,[22] and demonstrated the feasibility of the resultant TEC to facilitate cartilage repair in a large animal model.[23,24] These TEC are developed without an artificial scaffold, and, thus, their implantation could eliminate or minimize the risk of potential side effects induced by extrinsic chemical or biological materials. Furthermore, such TEC are highly adherent to cartilage matrix, and secure integration of the TEC to adjacent cartilage tissue is observed after implantation.[23–25] Therefore, combined constructs of TEC and the fully interconnected HA-based artificial bone may effectively repair an osteochondal lesion with zonal restoration. The purpose of the present study was to test this hypothesis using a rabbit osteochondral defect model.

Materials and Methods

All procedures of this study followed the Declaration of Helsinki principles.

Harvest of synovial tissue and isolation of the cells

All animal experiments were approved by the Animal Laboratory of our institute. Rabbit synovial membranes were obtained aseptically from the knee joints of skeletal mature (24 weeks of age) female rabbits within 12 h of death. The cell isolation protocol was essentially that which was previously used for the isolation of human synovial-derived MSC.[22] Briefly, synovial membrane specimens were rinsed with sterile phosphate-buffered saline (PBS), minced meticulously, and digested with 0.4% collagenase XI (Sigma-Aldrich, St. Louis, MO) for 2 h at 37°C. After neutralization of the collagenase with growth medium containing high-glucose Dulbecco's modified Eagle's medium (HG-DMEM; Wako, Osaka, Japan) that was supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT) and 1% penicillin/streptomycin (Gibco BRL, Life Technologies, Inc., Carlsbad, CA), the cells were collected by centrifugation, washed with PBS, re-suspended in growth medium, and plated in culture dishes with growth media mentioned earlier. The characteristics of the rabbit cells were similar to those of the human synovium-derived MSC with regard to morphology, growth characteristics, and multipotent differentiation capacity (to osteogenic, chondrogenic, and adipogenic lineages).[22,26] For expansion, cells were cultured in the growth medium at 37°C in a humidified atmosphere of 5% CO_2. The medium was replaced once per week. After 7–10 days of primary culture, when the cells reached confluence, they were washed twice with PBS, harvested by treatment with trypsin-EDTA (0.25% trypsin and 1 mM EDTA; Gibco BRL, Life Technologies, Inc.), and replated at 1:3 dilutions for the first subculture. Cell passages were continued in the same manner with 1:3 dilutions when cultures reached near confluence. Cells at passages 3–7 were used in the present studies.

Development of the TECs

Synovial MSCs were plated on six-well plates (9.6 cm^2) at a density of 4.0×10^5 cells/cm^2 in growth medium containing 0.2 mM ascorbate-2-phosphate (Asc-2P), an optimal concentration from earlier studies.[22–24] Within a day, the cells became confluent. After an additional 7–14 days in culture, a complex of the cultured cells and the ECM synthesized by the cells was detached from the culture dish by the application of shear stress using gentle pipetting. The detached monolayer complex was left in suspension to form a three-dimensional structure by active tissue contraction, and kept in cultured medium until implantation. This tissue was termed a basic scaffold-free three-dimensional TEC. Such TECs were sufficiently strong to be sustained against surgical handling as shown in our previous study.[22]

Development of the combined implant made of the TEC and artificial bone

A fully interconnected porous synthetic HA [5 mm in diameter, 4 mm in height (NEOBONE®; MMT Co. Ltd., Osaka, Japan)] was prepared as an artificial bone. The HA ceramics have 75% porosity and an interconnected porous structure, with more than 90% of the pores being connected by channels that are large enough for cells or tissue to penetrate. The surfaces of the pores are very smooth and the HA particles are tightly bound together, which provides a rather high mechanical compressive strength of about 10 MPa despite the porosity of this substance. This material displays good osteoconduction and bone ingrowth in animals and is also in clinical use.[18,19,21]

We prepared individual TEC to be hybridized with an artificial bone. TEC were detached from culture dishes just before the animal surgery, and combined with the artificial bone without any adhesive, to create a biphasic construct (Fig. 1a). The TEC immediately bonded to the surface of the artificial bone block and developed a stable complex that was maintained throughout the experiment.

Implantation of the combined implants to osteochondral defects

Forty one skeletal mature New Zealand White rabbits were kept in individual cages and had free access to food pellets and water. The rabbits were anesthetized by an intravenous injection of 1 mL of pentobarbital [50 mg/mL (Nembutal®; Dainippon Pharmaceutical Co. Ltd., Osaka, Japan)] and an intramuscular injection of 1 mL of xylazine hydrochloride [25 mg/mL (Seractal®; Bayer, Germany)]. After shaving, disinfection, and draping, a straight 3 cm-long medial parapatellar incision was made over the right knee; the patella was gently dislocated laterally; and the femoral groove was exposed. Full-thickness articular osteochondral defects, 5 mm in diameter and 6 mm in depth, were created mechanically in the femoral groove of the right distal femur using a drill at moderate speed, while irrigating the site with a room temperature saline solution, so as to prevent thermal damage to the surrounding bone and cartilage (Fig. 1b). The TEC and artificial bone was combined just before implantation as described earlier, and then, the combined constructs were implanted into the defects in 23 right knees by a press-fit technique (TEC group). In the control defect group, the defects were implanted with the artificial HA bone alone for 18 right knees (Fig. 1c). All animals were immobilized for 7 days, and euthanized under anesthesia at 1, 2, and 6 months after surgery. The distal femur of the animals, including the grafted site (18 specimens from the TEC group and 13 specimens from the control group), was used for histological analysis. The other specimens (five specimens from the TEC group and five specimens from the control group) were subjected to biomechanical testing. Five left knees were used as untreated normal controls for the biomechanical testing.

Histological evaluation of repaired tissue

For histological evaluation, tissue was fixed with 10% neutral buffered formalin, decalcified with K-CX (Falma, Tokyo, Japan), and embedded in paraffin, and 3 μm sections were prepared. The sections were stained with hematoxylin and eosin (H&E) and Toluidine Blue staining.

The histology of repaired tissue at 1, 2, and 6 months was evaluated by the modified O'Driscoll score for cartilage and subchondral bone repair.[27–29] The category "Toluidine Blue staining" was substituted for "Safranin O staining." Moreover, new criteria categories "cellular morphology" and "exposure of subchondral bone" were implemented in addition to the categories associated with subchondral bone repair. With regard to the "cellular morphology" category, normal subchondral bone repair was given a score of 2, a repair tissue mixed with cartilage-like tissue was a score of 1, and a repair tissue mixed with fibrous tissue was a score of 0. With regard to the "exposure of subchondral bone" category, no subchondral bone exposure was a score of 2, subchondral bone exposure at one side of the borders between repair tissue and adjacent cartilage was a score of 1, and subchondral bone exposure at both sides was a score of 0. The repair tissue was divided into three parts of 2 mm width, which consisted of the center area and both border areas, and then each area was evaluated by the modified O'Driscoll score. Based on these scores, each category was evaluated for "overall evaluation," which averaged the center area and both border areas. Moreover, the score of the central area as "central area" was also evaluated, and the average score of both border areas as "border area." The categories "bonding to adjacent cartilage," "freedom from degeneration of adjacent cartilage," and "exposure of subchondral bone," which do not involve spatial differences, were evaluated only as "overall evaluation."

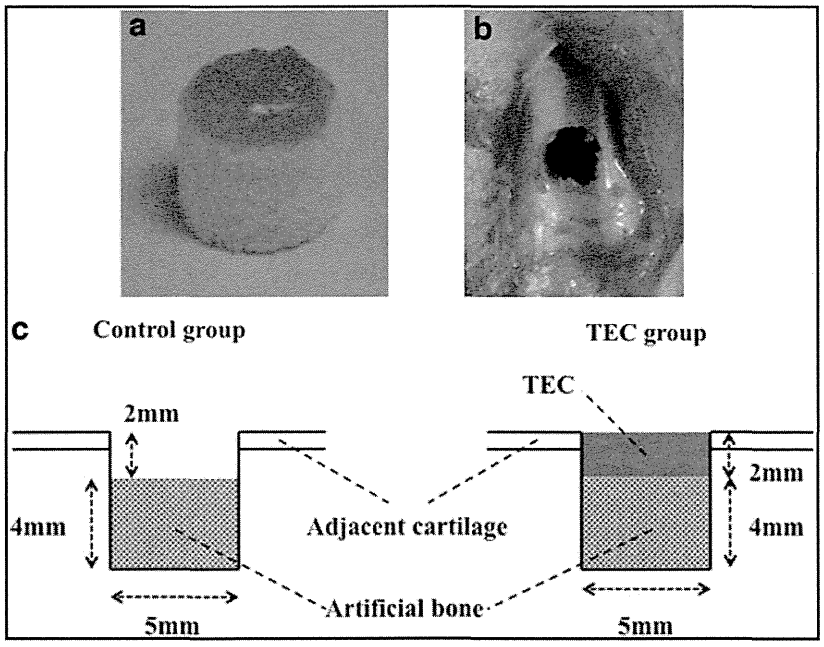

FIG. 1. **(a)** The combined implant generated with a tissue-engineered construct (TEC) and an artificial bone. **(b)** Osteochondral defects in the femoral groove of the rabbit knee. **(c)** Schematic representation of implanted materials in the control (hydroxyapatite, HA-bone alone) and the TEC-HA bone group. Color images available online at www.liebertpub.com/tea

Biomechanical testing

Cylindrically shaped specimens that were 4 mm in diameter and 5 mm in depth were removed from the graft sites of defects from both the TEC group and the control group. Similarly, cylindrically shaped specimens were removed from the central femoral groove of untreated normal knees. Micro-indentation testing was performed on the specimens using an Atomic Force Microscope (AFM) (Nanoscope IIIa; Veeco Instruments, Santa Barbara, CA) and a silicon nitride probe (spring constant: 0.06 N/m, DNP-S; Veeco Instruments). Each specimen was mounted on the sample stage of the AFM and soaked in saline solution at room temperature. Micro-indentation testing was performed on the specimens at an indentation rate of 5.12 μm/s.

Statistical analysis

Statistical analysis was performed using analysis of variance followed by *post-hoc* testing for the postoperative changes of total histological scores and biomechanical testing (Figs. 6a, b and 7). The comparison of results for other parameters between the control and TEC groups was analyzed by the Mann–Whitney *U* test (Tables 1 and 2). The results are presented as mean ± SD. The data were analyzed with JMP 9 (SAS Institute, Cary, NC), and significance was set at $p < 0.05$.

TABLE 1. HISTOLOGICAL EVALUATION FOR CARTILAGE REPAIR

Histological score description	*1 month postop*			*2 months postop*			*6 months postop*		
	Control (N=4)	*TEC (N=6)*	p *value*	*Control (N=4)*	*TEC (N=7)*	p *value*	*Control (N=5)*	*TEC (N=5)*	p *value*
Cellular morphology									
Overall evaluation	0	1.22±0.50	**0.0073**	1.67±0.39	3.14±0.63	**0.0109**	1.47±1.28	3.20±0.30	**0.0343**
Central area	0	0.33±0.82	0.4142	1.00±1.15	2.86±1.57	0.0716	1.60±2.19	3.60±0.089	0.1202
Border area	0	1.67±0.52	**0.0062**	2	3.29±0.76	**0.0152**	1.40±1.14	3.00±0.71	**0.0393**
Toluidine Blue staining									
Overall evaluation	0	0.92±0.37	**0.0073**	1.09±0.42	2.14±0.51	**0.0171**	1.27±0.86	2.67±0.47	**0.0196**
Central area	0	0.33±0.52	0.2207	0.50±0.58	2.29±1.11	**0.0309**	1.20±1.64±	3	**0.0495**
Border area	0	0.92±0.38	**0.0073**	1.38±0.48	2.07±0.61	0.0716	1.30±0.84	2.10±0.42	0.0827
Surface regularity									
Overall evaluation	0.42±0.50	1.89±0.66	**0.0131**	1.83±0.43	2.24±0.42	0.1420	1.20±0.65	1.80±1.30	0.0731
Central area	0.25±0.50	2.17±0.75	**0.0114**	2.75±0.50	2.71±0.49	0.9029	2.40±0.89	2.80±0.45	0.4386
Border area	0.50±0.58	1.75±0.69	**0.0159**	1.38±0.48	2.00±0.58	0.0977	0.60±0.65	1.70±0.91	0.0723
Structural integrity									
Overall evaluation	0.42±0.50	1.50±0.36	**0.0131**	1.09±0.42	1.52±0.38	0.1001	0.73±0.43	1.53±0.38	**0.0174**
Central area	0.50±0.58	1.83±0.41	0.0109	2	2	1.0000	1.60±0.89	2	0.3173
Border area	0.38±0.48	1.33±0.41	0.0201	0.63±0.63	1.29±0.57	0.1001	0.30±0.27	1.30±0.57	**0.0170**
Thickness									
Overall evaluation	0.50±0.58	1.39±0.57	0.0765	1.83±0.34	1.52±0.38	0.1862	0.80±0.65	1.40±0.28	0.1071
Central area	0.50±0.58	1.50±0.55	**0.0372**	2	1.71±0.49	0.2598	1.20±1.10	1.80±0.45	0.3662
Border area	0.50±0.58	1.33±0.61	0.0765	1.75±0.50	1.43±0.45	0.2621	0.60±0.55	1.20±0.27	0.0652
Bonding to adjacent cartilage									
Overall evaluation	0.13±0.25	1.50±0.45	**0.0089**	0.38±0.48	1.64±0.48	**0.0109**	0.30±0.27	1.50±0.61	**0.0167**
Hypocellularity									
Overall evaluation	1.00±1.28	2.89±0.27	**0.0121**	3	3	1.0000	1.80±1.12	2.74±0.15	0.0837
Central area	0.25±0.50	2.67±0.82	**0.0078**	3	3	1.0000	1.80±1.30	3	0.0539
Border area	1.25±1.50	3	**0.0177**	3	3	1.0000	1.80±1.15	2.60±0.22	0.1797
Chondrocyte clustering									
Overall evaluation	0	0.17±0.41	0.4142	0.42±0.50	1.24±0.46	**0.0325**	0.40±0.37	1.53±0.30	**0.0074**
Central area	0	0.17±0.41	0.4142	0.25±0.50	1.29±0.76	**0.0453**	0.80±0.84	2	**0.0177**
Border area	0	0.17±0.41	0.4142	0.5±0.58	1.21±0.39	**0.0482**	0.20±0.27	1.30±0.45	**0.0072**
Freedom from degeneration of adjacent cartilage									
Overall evaluation	2.88±0.25	3	0.2207	2.38±0.25	2.71±0.27	0.0763	1.60±0.22	2.40±0.22	**0.0073**
Total score	5.34±2.59	14.22±2.02	**0.0103**	13.67±2.10	19.16±2.33	**0.0179**	9.56±5.17	19.03±2.15	**0.0119**

Bold values show statistically significant differences between control group and TEC group.

TABLE 2. HISTOLOGICAL EVALUATION FOR SUBCHONDRAL BONE REPAIR

	1 month postop			*2 months postop*			*6 months postop*		
Histological score description	*Control (N=4)*	*TEC (N=6)*	p *value*	*Control (N=4)*	*TEC (N=7)*	p *value*	*Control (N=5)*	*TEC (N=5)*	p *value*
Subchondral bone alignment									
Overall evaluation	0	0	1.0000	0.67±0.54	1.67±0.34	**0.0144**	0.73±0.87	1.27±0.43	0.3305
Central area	0	0	1.0000	0.25±0.50	1.57±0.79	**0.0249**	0.80±0.84	1.00±0.71	0.6501
Border area	0	0	1.0000	0.88±0.85	1.71±0.39	0.0904	0.70±0.97	1.40±0.55	0.2328
Bone integration									
Overall evaluation	0	0	1.0000	0.75±0.57	1.79±0.39	**0.0130**	1.47±0.84	1.87±0.18	0.4189
Central area	0	0	1.0000	0.25±0.50	1.43±0.98	0.0601	1.20±0.84	1.60±0.55	0.4189
Border area	0	0	1.0000	1.00±0.91	1.57±0.79	0.2150	1.60±0.89	2	0.3173
Bone infiltration into defect area									
Overall evaluation	0	0	1.0000	0.67±0.54	1.48±0.50	**0.0437**	1.60±0.55	1.80±0.18	0.7290
Central area	0	0	1.0000	0.25±0.50	1.29±0.95	0.0831	1.20±0.84	1.40±0.55	0.7290
Border area	0	0	1.0000	0.88±0.85	1.79±0.39	0.0629	1.80±0.45	2	0.3173
Tidemark continuity									
Overall evaluation	0	0	1.0000	0	0.67±0.67	0.0763	0.40±0.37	1.20±0.38	**0.0192**
Central area	0	0	1.0000	0	0.86±1.07	0.1432	0.60±0.89	1.20±0.84	0.2685
Border area	0	0	1.0000	0	0.57±0.53	0.0708	0.30±0.27	1.20±0.27	**0.0071**
Cellular morphology									
Overall evaluation	0	0	1.0000	0.84±0.33	1.76±0.32	**0.0104**	1.47±0.51	1.60±0.28	0.8266
Central area	0	0	1.0000	0.25±0.50	1.43±0.79	**0.0355**	1.00±1.00	1.40±0.55	0.5023
Border area	0	0	1.0000	1.13±0.63	1.93±0.19	**0.0281**	1.70±0.45	1.70±0.45	1.0000
Exposure of subchondral bone									
Overall evaluation	1.00±1.15	1.67±0.52	0.2708	2	1.71±0.49	0.2598	0.80±0.84	1.80±0.45	0.0539
Total score	1.00±1.15	1.67±0.52	0.2708	4.92±1.91	9.07±1.84	**0.0140**	6.47±3.20	9.54±1.26	0.0937

Bold values show statistically significant differences between control group and TEC group.

Results

Macroscopic evaluation of repair tissue

At 1 month after surgery, bare artificial bones were exposed at the surface of the implanted area in all subjects of the control group (Fig. 2a, arrow heads). Conversely, the defects were uniformly covered with repair tissue in the TEC group. The periphery of the repair tissue was white and in contrast, the center area was translucent (Fig. 2b). At 2 months after surgery, the defects were covered with a white colored repair tissue in both groups. However, more precise observation revealed that the repair tissue in the control group exhibited surface cracks or subchondral bone exposure between the repair tissue and the adjacent cartilage (Fig. 2c, arrow heads). In the TEC group, although the margin line was obvious, there were no overt cracks or subchondral bone exposure detected within the repair tissue (Fig. 2d). At 6 months post surgery, obvious cracks or

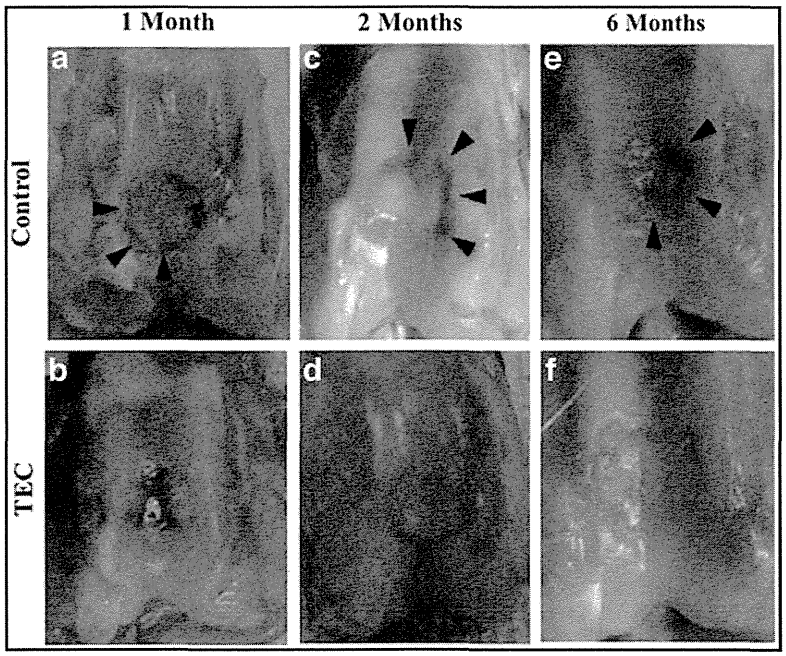

FIG. 2. Macroscopic view of repair tissues at 1, 2, and 6 months after surgery treated with artificial HA bone alone **(a, c, e,** respectively) or the TEC-HA combined implant **(b, d, f,** respectively). At 1 month after surgery, bare artificial bones were exposed at the surface of the implanted area in the control group **(a)**. At 2 and 6 months, the control group showed obvious cracks or subchondral bone exposure between repair tissue and the adjacent cartilage **(c, e,** arrow heads). Conversely, such defects were covered with repair tissue in the TEC-HA group out to 6 months **(b, d, e)**. Color images available online at www.liebertpub.com/tea

subchondral bone exposure between repair tissue and the adjacent cartilage were still observed in the control group samples (Fig. 2e, arrow heads). In contrast, the repair tissue in the TEC group consistently showed a continuous surface beyond the surface of adjacent cartilage. The margin line between the repair tissue and the adjacent cartilage was less distinguishable (Fig. 2f).

Histological evaluation of repair tissue

At 1 month after surgery, bare artificial bone was exposed partially at the surface of implanted area without repair tissue in the control group (Fig. 3a, arrows). Conversely, the defects were consistently repaired with thick fibrous tissues with good integration to the adjacent host tissue in the TEC group (Fig. 3b). In higher magnification views, new bone formation was observed at the bilateral peripheral margin of implanted TEC adjacent to the surrounding host bone marrow and the surface of the artificial bone (Fig. 3c, arrow heads). Notably, the development of immature chondrogenic tissue with round-shaped cells in lacuna were simultaneously observed within the implanted TEC surrounding the area of new bone formation (Fig. 3d), while fibrous tissue was observed in the center area of the TEC (Fig. 3e).

At 2 months, defects were filled with a fibrous-like tissue with moderate Toluidine blue staining, but bone formation was rarely observed on the surface of the artificial bone in the control group samples (Fig. 4a, b). In contrast, new bone formation within the TEC further extended from the bilateral peripheral border toward the central area on the surface of the artificial bone (Fig. 4c, arrows). It should be noted that the level of the upper surface of the newly synthesized bone was similar to that of the adjacent uninjured subchondral bone (Fig. 4c, d, dotted lines). In higher magnification views, there was poor integration of the repair tissue with the adjacent host cartilage in the control group samples (Fig. 4e). The repair tissue in the control group contained round-shaped cells in lacuna, but with weak Toluidine Blue-stained ECM, and, thus, the development of chondrogenic tissue appeared insufficient or less advanced (Fig. 4f, j). Conversely, the repair tissue in the TEC group samples exhibited hyaline cartilage-like repair (Fig. 4h, k) with good tissue integration to the adjacent host cartilage (Fig. 4g). Similar to 1 month post implantation, chondrogenic tissue with Toluidine Blue-positive ECM was observed to have developed in contact with newly synthesized bone (Fig. 4i).

At 6 months, osteochondral repair had progressed in the control group (Fig. 5a, b); however, the repair tissue still

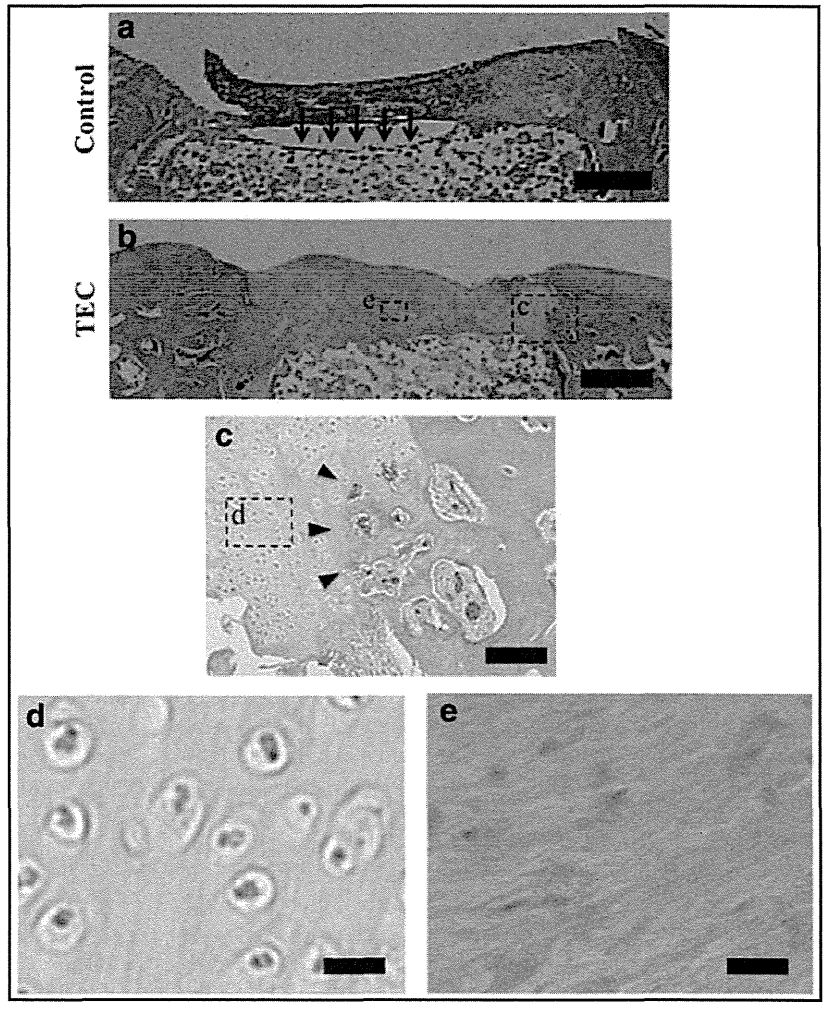

FIG. 3. Hematoxylin and eosin (H&E) staining of repair tissues implanted with artificial bone alone **(a)** or the combined implant **(b)**. The osteochondral defects treated with the combined implants were repaired with a thick fibrous-like tissue. Arrows show that bare artificial bone was exposed at the surface of implanted area without repair tissue in the control group **(a)**. Bar = 1 mm. Higher magnification views showed that ossification was partially observed inside the implanted TEC adjacent to host bone marrow on the surface of the artificial bone **(c**, arrowheads). Bar = 100 μm. Notably, the development of an immature chondrogenic tissue with round-shaped cells in lacuna was simultaneously observed within the implanted TEC surrounding the area of new bone formation **(d)**, while fibrous tissue was observed in the center of the TEC **(e)**. Bar = 20 μm. Color images available online at www.liebertpub.com/tea

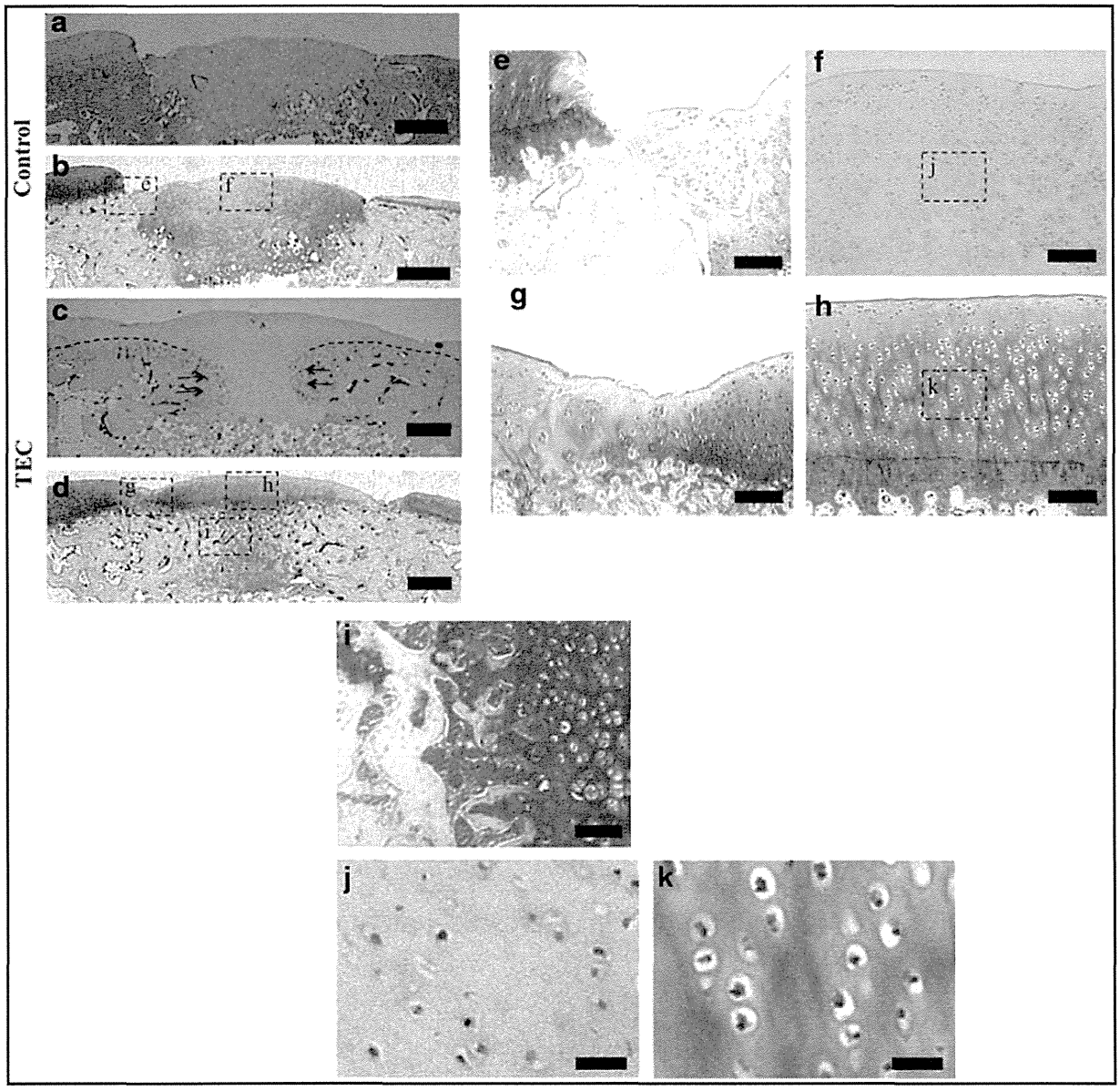

FIG. 4. Hematoxylin and eosin (H&E) staining and Toluidine Blue staining of repair tissues from defects treated with artificial bone alone **(a, b)** or the combined implant **(c, d)**. Bar = 1 mm. Arrows show that new bone formation within the TEC further extended from the bilateral peripheral border toward the central area on the surface of the artificial bone **(c)**. Light magnification view at the border **(e, g)**, center **(f, h)** area of the repair tissue, and cartilage-like tissue in contact with newly synthesized bone **(i)**. Bar = 100 μm. Note that the defect treated with the combined implant was repaired with osteochondral tissue and exhibited good tissue integration to the adjacent host tissue. Higher magnification views at the center area of the repair tissue **(j, k)**. Bar = 20 μm. Cellular morphology of defects treated with the combined implant shows round-shaped cells in lacuna. Color images available online at www.liebertpub.com/tea

exhibited poor integration with the adjacent host cartilage (Fig. 5a, arrows, e). In contrast, the TEC group samples showed complete osteochondral repair (Fig. 5c, d), and good tissue integration of the repair tissue to the adjacent host tissue persisted (Fig. 5c, arrows, g). However, some subjects exhibited overgrowth of the subchondral bone with the thinning of cartilage (Fig. 5c, d). Higher magnification views revealed that the repair tissue and the border with the adjacent cartilage contained a number of cell clusters in the control group, and the distribution of chondrocytes was disorganized throughout the matrix (Fig. 5f, i, j), suggesting the involvement of some pathological condition within the repair tissue and the adjacent host cartilage in this group. Conversely, in the TEC group, the repair tissue exhibited hyaline-like cartilage without cells in clusters, and the chondrocytes were arranged in longitudinal columns in the center area of the samples (Fig. 5h, k, l).

Histological score for cartilage repair

The total histological scores for cartilage repair were significantly higher in the TEC group samples compared with those in the control group at 1 month (14.22 ± 2.02 vs. 5.34 ± 2.59, $p = 0.010$), 2 months (19.16 ± 2.33 vs. 13.67 ±

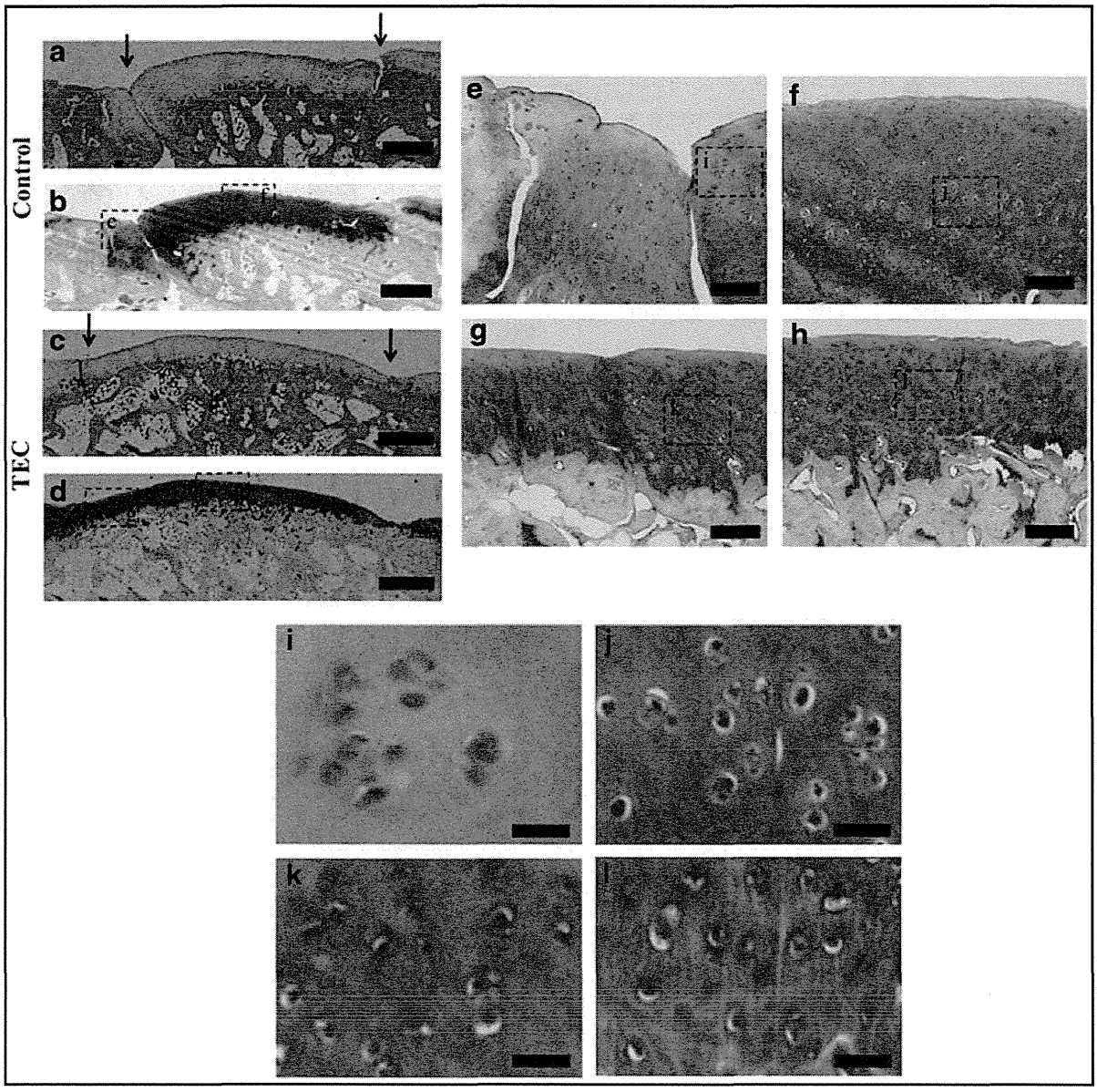

FIG. 5. H&E staining and Toluidine Blue staining of repair tissues treated with artificial bone alone **(a, b)** or the combined implant **(c, d)**. Bar = 1 mm. Light magnification view at the border **(e, g,** arrow) and center **(f, h)** area of the repair tissue. Bar = 100 μm. Note that the repair tissue in defects treated with the combined implant sustained good tissue integration to the adjacent host tissue, while that of artificial bone alone showed poor integration. Higher magnification views at the border **(i, k)** and center areas **(j, l)** of the repair tissue. Bar = 20 μm. Cellular morphology in defects treated with the combined implant showed round-shaped cells in lacuna, while that in defects treated with artificial bone alone showed cell clustering in lacuna. Color images available online at www.liebertpub.com/tea

2.10, $p = 0.018$), and 6 months (19.03 ± 2.15 vs. 9.56 ± 5.17, $p = 0.012$) after surgery (Fig. 6a).

The categories "cellular morphology" and "toluidine blue staining" in the TEC group were significantly higher than those in the control group samples out to 6 months postsurgery, and these results suggested the promotion of cartilage repair with the combined implants. With regard to the category "bonding to adjacent cartilage," the histological scores in the TEC group samples were significantly higher compared with those in the control group throughout the studies. In addition, the category "structural integrity," especially the subcategory "border area," values for the TEC group were significantly higher than those of the control group at 6 months, suggesting that secure integration to adjacent host tissue contributed to the proper development of the border area of the repair cartilage. In contrast, the categories "chondrocyte clustering" and "freedom from degeneration of adjacent cartilage" showed significantly lower scores in the control group at 6 months, findings that suggest the involvement of a pathological process within the repair tissue (Table 1).

Histological score for subchondral bone repair

At 1 month after surgery, subchondral bone repair was not observed in either the TEC group or the control group. Therefore, all categories except for the category "exposure of