2011年 10月	World Congress on Reproductive Biology (Second Scientific Meeting)	In vitro-maturation/fertilization derived porcine morulae can give rise to efficient piglet production following vitrification by the hollow fiber vitrification(HFV)-method	Maehara M, Honda K, Nakano K, Matsunari H, Takeuchi Y, Kanai T, Matsuda T, Hagiwara Y, Sasayama N, Shirasu A, Takahashi M, Watanabe M, Umeyama K, Hanazono Y, Nagashima H
2011年 10月	World Congress on Reproductive Biology (Second Scientific Meeting)	Establishment of in vitro fertilization protocol using cryopreserved epididymal sperm for proliferation of genetically modified pigs	Honda K, Takeuchi Y, Matsuda T, Kanai T, Maehara M, Matsunari H, Nakano K, Umeyama K, Watanabe M, Nakauchi H, Nagashima H
2011年 10月	World Congress on Reproductive Biology (Second Scientific Meeting)	【招待講演】The interface between reproductive biology and regenerative midicine	Nagashima H
2011年 9月	16th World Congress on In Vitro Fertilization	hHg is critical for the uterine decidual response in mice	Kawano H, Ezoe K, Kagawa N, Yabuuchi A, Ochiai K, Nagashima H, Osada H, Aono F, Takehara Y, Kato O
2011年 9月	第104回日本繁殖生物学会大会	シングルコピーの赤色蛍光蛋白Kusabira-Orange遺伝子を組み込んだトランスジェニックブタの系統確立	松成ひとみ, 金井貴博, 本田香澄, 前原美樹, 竹内靖浩, 渡邊將人, 梅山一大, 中野和明, 池澤有加, 高柳就子, 中内啓光, 長嶋比呂志
2011年 9月	第104回日本繁殖生物学会大会	遺伝子改変ブタの凍結精子を用いた体外受精による産仔作出	本田香澄, 竹内靖浩, 松田泰輔, 金井貴博, 前原美樹, 松成ひとみ, 中野和明, 梅山一大, 渡邊將人, 中内啓光, 長嶋比呂志
2011年 9月	第104回日本繁殖生物学会大会	中空糸法を用いてガラス化されたトウキョウX胚からの高効率産仔作出	中野和明, 松成ひとみ, 前原美樹, 竹内靖浩, 小川武甲, 松田泰輔, 金井貴博, 本田香澄, 萩原由以, 笹山典久, 白数昭雄, 太田久由, 高橋昌志, 長嶋比呂志

2011年 9月	第104回日本繁殖生物学会大会	中空糸法でガラス化されたブタ体外成熟・受精桑実胚からの高効率産仔作出	前原美樹, 本田香澄, 中野和明, 松成ひとみ, 竹内靖浩, 金井貴博, 松田泰輔, 萩原由以, 笹山典久, 白数昭雄, 高橋昌志, 渡邊將人, 梅山一大, 花園豊, 長嶋比呂志
2011年 9月	第29回日本受精着床学会総会	中空糸膜デバイスを用いた凍結融解操作後の卵子回収率の検討	天羽杏実, 橋本周, 右島理可, 山中昌哉, 中野和明, 長嶋比呂志, 高橋昌志, 笹山典久, 森本義晴
2011年 8月	第20回硬組織再生生物学会	クサビラオレンジブタ頭蓋骨由来骨芽細胞の単離とその生物学的評価	本田みちよ, 小西敏功, 水本みのり, 松成ひとみ, 長嶋比呂志, 相澤守
2011年 7月	Swine in Biomedical Research 2011	Transgenic pigs expressing the mutant insulin C93S for the study of pancreatic beta cell dysfunction	Renner S, Klymiuk N, Streckel E, Braun C, Landbrecht-Schessl C, Wunsch A, Kessler B, Kurome M, Nagashima H, Aigner B et al
2011年 7月	Swine in Biomedical Research 2011	Phenotypic characterization of diabetic INSC94Y transgenic pigs	Renner S, Braun C, Klymiuk N, Blutke A, Herbach N, Wunsch A, Kessler B, Kurome M, Puk O, Nagashima H et al
2011年 7月	Swine in Biomedical Research Conference 2011	Development of genetically modified pigs suitable for diabetes and its complications research	Umeyama K, Watanabe M, Matsunari H, Nakano K, Takeuchi Y, Honda K, Yokoo T, Nagashima H
2011年 7月	Swine in Biomedical Research Conference 2011	A challenge to developing humanized kidney using porcine renal anlagen as scaffold	Matsunari H, Yokoo T, Matsumoto K, Yokote S, Iwai S, Medin JA, Watanabe M, Umeyama K, Sato Y, Nakano K, Maehara M, Nagashima H, Kobayashi E
2011年 7月	Swine in Biomedical Research 2011	Tet-controlled transgene expression in large animal models	Klymiuk N, W. B, T. R, Bahr A, Wunsch A, Kessler B, Kurome M, Herbach N, Nagashima H, Schwinzer R et al

2011年10月	Swine in Biomedical Research 2011	Efficient targeting of genomic loci in the pig and production of knockout pigs by somatic cell nuclear transfer	Klymiuk N, Wunsch A, Wallner K, Burkhardt K, Kessler B, Kurome M, Nagashima H, Wolf E
2011年7月	Swine in Biomedical Research 2011	Determination of transgene integration loci by inverse PCR for multi-transgenic pig breeding	Bahr A, Klymiuk N, Kurome M, Kessler B, Nagashima H, Ayares D, Wolf E
2011年7月	Swine in Biomedical Research 2011	Establishment of LEA29Y transgenic donor pigs for xenotransplantation	Bahr A, Burck L, Wunsch A, Kurome M, Kessler B, Nagashima H, Seissler J, Klymiuk N, Wolf E
2011年6月	第122回無機マテリアル学会	ケイ素含有アパタイトセラミックス上でのクサビラオレンジブタ頭蓋骨由来骨芽細胞の骨分化過程の解析	本田みちよ, 小西□功,水本みのり, 松成ひとみ, 長嶋比呂志, 相澤守
2011年5月	第58回日本実験動物学会	糖尿病合併症研究に適した遺伝子改変ブタの開発	梅山一大, 渡邊将人, 松成ひとみ, 中野和明, 竹内靖浩, 本田香澄, 横尾隆, 長嶋比呂志
2011年3月	京都臓器保存セミナー	哺乳動物卵・初期胚および組織のガラス化保存について	前原美樹, 松成ひとみ, 中野和明, 落合恵子, 本田香澄, 竹内靖浩, 池澤有加, 池田有希, 長嶋比呂志
2011年3月	日本セラミックス協会	機械粉砕アパタイトとキトサン溶液を利用したキレート硬化型セメントの作製とその生体適合性	水本みのり, 吉川哲史, 小西敏功, 本田みちよ, 松成ひとみ, 竹内靖浩, 長嶋比呂志, 相澤守
2011年3月	日本セラミックス協会	クサビラオレンジ蛍光遺伝子を導入したブタを用いた二極化した細孔構造を備えたb-リン酸三カルシウム多孔体のin vivo評価	重光勇介, 本田みちよ, 水本みのり, 松成ひとみ, 竹内靖浩, 長嶋比呂志, 相澤守
2011年3月	日本セラミックス協会	クサビラオレンジブタ脛骨埋入による高強度化アパタイトファイバースキャフォルドの生体適合性評価	鴈本拓也, 島田愛生, 安富由美子, 本田みちよ, 水本みのり, 松成ひとみ, 竹内靖浩, 長嶋比呂志, 相澤守
2011年3月	第10回日本再生医療学会	ブタをscaffoldとする腎臓再生：IV.ネコにおけるエリスロポエチン療法の開発	岩井聡美, 横尾隆, 松成ひとみ, 田中友加, 寺岡義布史, 大段秀樹, 長嶋比呂志, 小林英司
2011年3月	第10回日本再生医療学会	ブタをscaffoldとする腎臓再生: III. Suicide geneを発現する遺伝子改変ブタの作出	松成ひとみ, 横尾隆, 岩井聡美, 渡邊将人, 梅山一大, Medin JA, 長嶋比呂志, 小林英司

2011年3月	第10回日本再生医療学会	ブタをscaffoldとする腎臓再生II：エリスロポエチン(EPO)産生組織の体内発生法の開発	横尾隆, 松成ひとみ, 岩井聡美, 松本啓, 辻収彦, 岡野James洋尚, 岡野栄之, 長嶋比呂志, 小林英司
2011年3月	第10回日本再生医療学会	ブタをscaffoldとする腎臓再生: I. クローンブタを利用した腎臓原基の発達能の検証	長嶋比呂志, 松成ひとみ, 横尾隆, 岩井聡美, 小林英司
2011年2月	Keystone Symposia: Stem Cells in Development, Tissue Homeostasis and Disease	The generation of "mouse-type" porcine induced pluripotent stem cells	Fujishiro S, Mizukami Y, Ishino R, Nishimura T, Matsunari H, Nakano K, Nagashima H, Hanazono Y
2011年1月	2011 Annual Conference of the International Embryo Transfer Society	Large-scale production of cloned transgenic pigs: efficiency and side effects	Kurome M, Kessler B, Klymiuk N, Wiensch A, Zackhartchenko V, Nagashima H, Wolf E
2011年1月	第8回北関東甲信越肝移植談話会	【招待講演】ブタ体細胞クローン技術を基盤とした移植・再生医学への取り組み	長嶋比呂志
2010年12月	5th International Symposium on Apatites and Correlative Biomaterials	Cell proliferation, morphology and differentiation of transgenic-cloned pig calvarial osteoblasts on the silicon-substituted hydroxyapatite ceramics fabricated via ultrasonic spray-pyrolysis technique	Honda M, Konishi T, Mizumoto M, Matsunari H, Nagashima H, Aizawa M
2010年12月	第33回日本分子生物学会年会	Zinc-finger nucleases(ZFNs)-driven gene disruption can work in porcine primary cultured cells	Watanabe M, Umeyama K, Matsunari H, Takayanagi S, Haruyama E, Nakano K, Fujiwara T, Ikezawa Y, Nakauchi H, Nagashima H
2010年12月	第33回日本分子生物学会年会	Characterization of transgene-free porcine iPS cell	正木英樹, 濱仲早苗, 脇山由起子, 山口智之, 長嶋比呂志, 中内啓光
2010年12月	第14回生体関連セラミックス討論会	クサビラオレンジブタ頭蓋骨より単離した骨芽細胞の骨分化過程の解析	本田みちよ, 水本みのり, 小西敏功, 松成ひとみ, 長嶋比呂志, 相澤守

2010年11月	2010 Seoul Forum on Xenotransplantation	Necessity and possibility of serial cloning in development of advanced genetically engineered pigs for xenotransplantation	Nagashima H, Matsunari H, Ikezawa Y, Nakano K, Kurome M, Watanabe M, Umeyama K, Miyagawa S
2010年10月	merican Society for Reproductive Medicine 66th Annual Meeting	A novel vitrification method using a microfiltration membrane(MFM) enables a simple manipulation of human embryos	Amo A, Hashimoto S, Nagashima H, Takahashi M, Sasayama N, Morimoto Y
2010年9月	第13回日本IVF学会	胚と中空糸膜をユニットとしたヒト胚の超急速凍結	天羽杏実, 橋本周, 右島理可, 山中昌哉, 中野和明, 長嶋比呂志, 高橋昌志, 笹山典久, 森本義晴
2010年9月	第103回日本繁殖生物学会	中空糸法を用いたブタMII期卵及び初期胚のガラス化保存	中野和明, 松成ひとみ, 前原美樹, 竹内靖浩, 小川武甲, 藤原主, 池澤有加, 本田香澄, 荻原由以, 笹山典久, 白数昭雄, 大田久由, 高橋昌志, 長嶋比呂志
2010年9月	第103回日本繁殖生物学会	トランジェニックブタ凍結精子の卵管内人工授精	本田香澄, 松成ひとみ, 藤原主, 竹内靖浩, 中野和明, 池澤有加, 前原美樹, 梅山一大, 渡辺将人, 長嶋比呂志
2010年9月	第103回日本繁殖生物学会	中空糸法によるマウス胚のガラス化保存	松成ひとみ, 前原美樹, 池澤有加, 中野和明, 落合恵子, 竹内靖浩, 本田香澄, 笹山典久, 白数昭雄, 荻原由以, 高橋昌志, 長嶋比呂志
2010年9月	第103回日本繁殖生物学会	臓器再生研究に向けた膵臓形成不全トランスジェニックブタの作出	松成ひとみ, 小林俊寛, 渡邊将人, 梅山一大, 高柳就子, 中野和明, 藤原主, 池澤有加, 本田香澄, 前原美樹, 竹内靖浩, 須磨崎亮, 中内啓光, 長嶋比呂志
2010年9月	第103回日本繁殖生物学会	糖尿病モデルトランスジェニッククローンブタの作出IV. 変異型ヒトHNF-1a遺伝子を導入したDominant-negative変異体の後代産仔作出	梅山一大, 渡邊将人, 松成ひとみ, 中野和明, 藤原主, 日高龍路, 竹内靖浩, 望月寛徳, 関口渓人, 長嶋比呂志

2010年9月	第103回日本繁殖生物学会	ブタ細胞におけるZinc finger nuclease (ZFN)によるEGFP遺伝子のノックアウト (KO)	渡邊将人, 梅山一大, 松成ひとみ, 高柳就子, 春山エリカ, 中野和明, 藤原主, 池澤有加, 中内啓光, 長嶋比呂志
2010年6月	26th Annual Meeting of European Society of Human Reproduction and Embryology	Increased longevity of old mice after allo-transplantation of young mice ovaries	Ikeda Y, Kagawa N, Kuwayama M, Nagashima H, Silber S, Kato K, Kato O
2010年6月	第28回日本受精着床学会総会・学術講演会	胚操作性の高い中空糸膜を用いた超急速凍結後のヒト胚の発育能力	天羽杏実, 橋本周, 右島理可, 山中昌哉, 中野和明, 長嶋比呂志, 高橋昌志, 笹山典久, 森本義晴
2010年6月	第28回日本受精着床学会総会・学術講演会	【招待講演】クローンブタのaging研究への応用の可能性	長嶋比呂志
2010年3月	日本畜産学会第112回大会	糖尿病発症遺伝子改変ブタの長期飼育に関する研究	日高龍路, 梅山一大, 望月寛徳, 関口渓人, 松成ひとみ, 中野和明, 藤原主, 渡邊將人, 長嶋比呂志
2010年3月	日本畜産学会第112回大会	自動冷却装置を用いた遺伝子改変ブタ精子の凍結保存	藤原主, 松成ひとみ, 梅山一大, 渡邊將人, 中野和明, 竹内靖浩, 中野貞雄, 長嶋比呂志
2010年3月	日本畜産学会第112回大会	Kusabira-Orange遺伝子導入トランスジェニッククローン(Tg-C)ブタの繁殖能力および遺伝子伝達	松成ひとみ, 竹内靖浩, 保谷美恵, 関口渓人, 望月寛徳, 日高龍路, 渡邊將人, 梅山一大, 高柳就子, 中野和明, 藤原主, 長嶋比呂志
2010年3月	日本畜産学会第112回大会	ブタにおける連続核移植の可能性：第6世代クローンの作出	松成ひとみ, 中野和明, 藤原主, 池澤有加, 小川武甲, 高柳就子, 渡邊將人, 梅山一大, 長嶋比呂志
2010年3月	第9回日本再生医療学会	異種胎仔組織を用いた再生腎臓誘導法の開発	横尾隆, 松成ひとみ, 岩井聡美, 松本啓, 長嶋比呂志, 小林英司
2010年3月	第9回日本再生医療学会	急性腎不全に陥った腎臓への胎児由来組織の移植効果—バイオイメージング・ラットを用いた検討	岩井聡美, 横尾隆, 杉本直美, 松成ひとみ, 長嶋比呂志, 小林英司
2010年3月	第9回日本再生医療学会	胎仔腎臓原基の器官培養を利用した幹細胞スクリーニング—手技の仔細と蛍光画像パターンによる分類—	杉本直美, 横尾隆, 増田茂夫, 花園豊, 竹内賢吾, 松成ひとみ, 長嶋比呂志, 土居雅子, 小林英司

2010年3月	第9回日本再生医療学会	膵臓特異的に緑色蛍光タンパク(Venus)を発現するトランスジェニック(Tg)ブタの作出.	松成ひとみ, 小林敏寛, 渡邊將人, 中野和明, 藤原主, 長屋昌樹, 中内啓光, 長嶋比呂志
2010年3月	第13回日本異種移植研究会	ブタ CMP-N-acetylneuraminic acid hydroxylase gene の解析	池田孔佑, 山本亜紀, 近藤昭宏, 松成ひとみ, 長嶋比呂志, 高間勇一, 上野豪久, 福澤正洋, 宮川周士
2010年3月	第13回日本異種移植研究会	α1,3 ガラクトース転移酵素遺伝子ダブルノックアウトブタの体細胞クローニングにおける Scriptaid の効果	松成ひとみ, 池澤有加, 渡邊将人, 梅山一大, 中野和明, 藤原主, 竹内靖浩, 本田香澄, 前原美樹, 高柳就子, 山田和彦, 宮川周士, 長嶋比呂志
2009年11月	平成21年度問題別研究会「体細胞クローン技術の現状と将来展望」	【招待講演】クローン動物とクローニング技術の医学・医療への応用. 体細胞クローン技術の取り扱いと利用方向	長嶋比呂志
2009年11月	第54回日本生殖医学会	妊孕性保存および QOL 向上を目的とした卵巣ガラス化保存・移植技術の有効性	香川則子, 桑山正成, 池田有希, 落合恵子, 長嶋比呂志, 加藤修
2009年10月	65th Annual Meeting of the American Society for Reproductive Medicine	Function of vitrified human ovarian grafts after xeno-transplantation	Kagawa N, Kuwayama M, Ikeda Y, Silber S, Nagashima H, Kato O
2009年10月	The Transplantation Society, IPITA-IXA 2009	Xeno-metanephros as a biocompetent scaffold for kidney regeneration	Yokoo T, Nagashima H, Matsunari H, Iwai S, Hosoya T, Kobayashi E
2009年10月	The Transplantation Society, IPITA-IXA 2009	Lectin microarray analyses of endothelial cells and fibroblasts from the α1,3 galactosyltransferase knockout pig	Miyagawa S, Yamamoto A, Ikeda K, Matsunari H, Nagashima H, Takeishi S, Yamada M, Fukuzawa M
2009年9月	第45回日本移植学会総会	レクチンブロット法による αGal-knockout ブタの糖鎖抗原の解析	山本亜紀, 徐恒傑, 武石俊作, 山田正雄, 三善英知, 池田孔佑, 松成ひとみ, 長嶋比呂志, 福澤正洋, 宮川周士

2009年 9月	第45回日本移植学会総会	ブタCMP-N-acetylneuraminic acid hydroxylase geneの解析	池田孔佑, 山本亜紀, 松成ひとみ, 中野和明, 藤原主, 長嶋比呂志, 福澤正洋, 宮川周士
2009年 9月	第102回日本繁殖生物学会大会	抗酸化機能強化培地がブタ体外生産胚の凍結生存性に及ぼす影響	中野和明, 中山順樹, 小川武甲, 松成ひとみ, 藤原主, 斉藤紗恵子, 池澤有加, 吉岡耕治, 星宏良, 長嶋比呂志
2009年 9月	第102回日本繁殖生物学会	若齢時凍結保存卵巣の自家移植による老齢不妊マウスの繁殖能力の回復および寿命の延長	香川則子, 桑山正成, 池田有希, 落合恵子, 長嶋比呂志, 加藤修
2009年 9月	第102回日本繁殖生物学会大会	糖尿病モデルトランスジェニッククローンブタの作出 III. 変異型ヒトHNF-1α遺伝子を導入したDominant-negative変異体の病態の詳細解析	梅山一大, 渡邊將人, 松成ひとみ, 黒目麻由子, 小川武甲, 中野和明, 藤原主, 三木敬三郎, 長嶋比呂志
2009年 9月	第102回日本繁殖生物学会	肝臓特異的赤色蛍光(Kusabira-Orange)発現を示す遺伝子改変ブタの作出	松成ひとみ, 渡邊將人, 梅山一大, 中野和明, 藤原主, 小川武甲, 池田有希, 春山エリカ, 塩田明, 長嶋比呂志
2009年 9月	東京電機大学公開講座 ME講座 第33回	トランスレーショナルリサーチに向けたクローンブタ・遺伝子改変ブタの開発	長嶋比呂志
2009年 8月	第36回豚の繁殖衛生セミナー	糖尿病モデル遺伝子改変ブタの生産と病態の特徴について	梅山一大, 渡邊將人, 松成ひとみ, 黒目麻由子, 小川武甲, 中野和明, 藤原主, 長嶋比呂志
2009年 8月	第27回日本受精着床学会	若齢時に凍結保存した卵巣の自家移植による老齢不妊マウスからの正常産子の作出	香川則子, 桑山正成, 池田有希, 落合恵子, 長嶋比呂志, 加藤修
2009年 8月	第27回日本受精着床学会	若齢時に凍結保存した卵巣の老齢マウスへの自家移植による繁殖能力の回復	池田有希, 香川則子, 落合恵子, 桑山正成, 加藤修, 長嶋比呂志.
2009年 8月	Yunnan Agricultural University	【招待講演】Recent advances in production of genetically modified pigs in Meiji University	Nagashima H
2009年 7月	自治医科大学CDAMTec内覧会	【招待講演】Tokyo Pig Center機構（構想）	長嶋比呂志
2009年 6-7月	25th Annual Meeting of the Europian Society of Human Reproduction and Embryology	Recovery of reproductive function and extension of life expectancy in old infertile mice by ovarian transplantation	Kagawa N, Kuwayama M, Ikeda Y, Nagashima H, Leibo S, Kato O

2009年6月	International Symposium Xenotransplantation	Recent advances in production of genetically modified pigs for xenotransplantation	Nagashima H
2009年5月	第50回日本哺乳動物卵子学会	Cryotop法を用いたマウス4細胞期胚および胚盤胞の再凍結保存後の生存性：凍結・再凍結保存時期の検討	池田有希, 松成ひとみ, 落合恵子, 香川則子, 桑山正成, 加藤修, 長嶋比呂志
2009年4月	バイオ・ナノテクフォーラムイブニングセミナー21	【招待講演】トランスレーショナルリサーチ推進のためのブタの遺伝子改変の現状	長嶋比呂志
2009年4月	自治医科大学先端医療技術開発センターシンポジュウム	【招待講演】遺伝子改変ブタの再生医療への応用	長嶋比呂志
研究分担者　石原美弥			
2012年1月	SPIE Photonics West Biomedical Optics	Continuous wavelet-transform analysis of photo-acoustic signal waveform to determine optical absorption coefficient	Hirasawa T, Ishihara M, Tsujita K, Hirota K, Irisawa K, Kitagaki M, Fujita M, Kikuchi M
2011年11月	Optics and Photonics Japan 201	光音響画像化技術の要素技術開発とシステム化:動物モデルによる性能検証	平沢壮, 石原美弥, 藤田真敬, 北垣学, 大谷直樹, 菊地眞
2011年10月	第32回日本レーザー医学会総会	光音響技術を利用した選択的イメージングの動物モデルによる検証	平沢壮, 石原美弥, 藤田真敬, 北垣学, 大谷直樹, 堀口明男, 菊地眞
2011年9月	BioOpto Japan 2011	【特別講演】光音響画像化技術の最新動向	石原美弥
2011年4月	第50回日本生体医工学会大会	深部組織の高分解能画像化に向けた光音響画像診断法の開発と評価	平沢壮, 石原美弥, 辻田和宏, 入澤覚, 北垣学, 藤田真敬, 菊地眞
2011年3-4月	CiRA国際シンポジウム	Development of methods to inhibit tumorigenesis after transplantation of differentiated iPS cells	Matsumura K, Ishihara M, Ichiki Y, Arai M, Ishihara M, Kobayashi Y, Kikuchi M
2011年1月	SPIE Photonics West Biomedical Optics, BiOS 2011	Analysis and verification of dominant factor to obtain the high resolution photo-acoustic imaging	Hirasawa T, Ishihara M, Kitagaki M, Bansaku I, Fujita M, Kikuchi M
2011年1月	SPIE Photonics West 2011 Biomedical Optics, BiOS 2011,	Multifunctional photoacoustic signals detected by P(VDF/TrFE) film sensor with a wide range of frequency	Ishihara M, Hirasawa T, Tsujita K, Manabu K, Bansaku I, Fujita M, Kikuchi M
2010年12月	光・量子デバイス研究会	光音響画像化技術の現状	石原美弥, 平沢壮, 菊地眞

2010年 10月	第31回日本レーザー医学会総会	光音響断層画像化技術による機能診断イメージングに関する研究	石原美弥, 平沢壮, 北垣学, 番作勲, 藤田真敬, 菊地眞
2010年 10月	第31回日本レーザー医学会総会	生体深部の高分解能画像化に向けた光音響画像化法の開発	平沢壮, 石原美弥, 北垣学, 番作勲, 藤田真敬, 菊地眞
2010年 9月	BioOpto Japan 2010	【特別講演】光音響原理を利用した非侵襲的診断法	石原美弥
2010年 9月	電気学会研究会光・量子デバイス研究会	光音響画像の高分解能化のための画像再構成手法の検証	平沢壮, 石原美弥, 北垣学, 藤田真敬, 菊地眞
2010年 6月	第49回日本生体医工学会大会	【シンポジウム】Development of the photoacoustic imaging system toward functional deep tissue imaging	石原美弥, 辻田和宏, 平沢壮, 番作勲, 佐藤良彰, 北垣学, 藤田真敬, 菊地眞
研究分担者　村井邦彦			
2009年 9月	第24回日本整形外科学会基礎学術集会	椎間板ヘルニアにおける免疫機能の関与	村井邦彦, 酒井大輔, 中井知子, 中村嘉彦, 持田譲治
2009年 4月	第38回日本脊椎脊髄病学会	椎間板ヘルニアの新治療に向けた基礎的研究 - 椎間板髄核細胞の特異的免疫特性について	村井邦彦, 酒井大輔, 中井知子, 中村嘉彦, 持田譲治
2009年 2月	第23回日本ペインクリニック学会東京地方会	椎間板ヘルニアの新治療に向けた基礎的研究—椎間板髄核細胞の特異的免疫特性について	村井邦彦, 鈴木英雄, 五十嵐孝, 瀬尾憲正, 酒井大輔, 中井知子, 中村嘉彦, 持田譲治
研究分担者　加藤玲子			
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2012年 3月	The 51st Annual Meeting of the Society of Toxicology	Cytotoxicity studies in A549 cells cultured on 2-methacryloyloxyethyl phosphorylcholine polymers	Miyajima-Tabata A, Kato R, SakaiK, Okada E, Matsuoka A
2012年 3月	The 51st Annual Meeting of the Society of Toxicology	Comparison of protein expression profiles in human mesenchymal stem cells cultured on surface-modified titanium with chemical treatments	Kato R, Haishima Y, Hasegawa C, Matsuoka A
2011年 11月	第33回日本バイオマテリアル学会	チタン系金属, 合成高分子等の医用材料上で培養したCHL細胞の細胞毒性および遺伝毒性	宮島敦子, 加藤玲子, 酒井恵子, 松岡厚子
2011年 11月	第33回日本バイオマテリアル学会	異なる表面処理を施したチタンプレート上で培養したヒト間葉系幹細胞のタンパク質発現解析	加藤玲子, 葩島由二, 長谷川千恵, 松岡厚子

Ⅳ．研究成果の刊行物・別刷

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Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model

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ABSTRACT

Lacking a blood supply and having a low cellular density, articular cartilage has a minimal ability for self-repair. Therefore, wide-ranging cartilage damage rarely resolves spontaneously. Cartilage damage is typically treated by chondrocyte transplantation, mosaicplasty or microfracture. Recent advances in tissue engineering have prompted research on techniques to repair articular cartilage damage using a variety of transplanted cells. We studied the repair and regeneration of cartilage damage using layered chondrocyte sheets prepared on a temperature-responsive culture dish. We previously reported achieving robust tissue repair when covering only the surface layer with layered chondrocyte sheets when researching partial-thickness defects in the articular cartilage of domestic rabbits. The present study was an experiment on the repair and regeneration of articular cartilage in a minipig model of full-thickness defects. Good safranin-O staining and integration with surrounding tissues was achieved in animals transplanted with layered chondrocyte sheets. However, tissue having poor safranin-O staining—not noted in the domestic rabbit experiments—was identified in some of the animals, and the subchondral bone was poorly repaired in these. Thus, although layered chondrocyte sheets facilitate articular cartilage repair, further investigations into appropriate animal models and culture and transplant conditions are required.

1. Introduction

Articular cartilage is hyaline cartilage featuring an extracellular matrix (ECM) consisting of an intricate collagen network and proteoglycans, and is highly resistant to mechanical loads. However, lacking a blood supply and having a low cellular density, articular cartilage has a minimal potential for self-repair. Therefore, wide-ranging cartilage damage rarely resolves spontaneously. Left untreated, cartilage damage in a load-bearing area causes secondary degeneration of the surrounding cartilage and ultimately exerts negative effects on routine activities. Numerous treatments have been developed to repair articular cartilage damage, typical examples being chondrocyte transplantation [1], mosaicplasty [2], and microfracture [3]. These treatments have produced satisfactory outcomes. However, the literature also documents treatment failures [4–8].

Autologous chondrocyte implantation (ACI), first reported by Brittberg et al. [1], has been performed over 20,000 times worldwide. However, two sites for the resection of cartilage and periosteum must be sacrificed to repair a single cartilaginous lesion. This is the largest drawback of ACI. Other problems include periosteal hyperplasia and incompatibility with surrounding tissues [5]. Problems associated with mosaicplasty include a limited number of donors of healthy cartilage and the need for long-term monitoring for damage at the harvest sites [2]. Microfracture induces cartilage regeneration by promoting the migration of mesenchymal cells from the marrow, but results in the production of fibrocartilage, which is mechanically weaker than hyaline cartilage [9].

Previously, we prepared highly adhesive layered chondrocyte sheets without a scaffold and with a short culture time using a temperature-responsive culture dish (Fig. 1a, b). On transplantation, these layered chondrocyte sheets suppressed degeneration in articular cartilage [10–12]. These temperature-responsive culture dishes have already been applied to research in various fields of regenerative medicine, including the regeneration of myocardium [13,14], vascular epithelium [15], cornea]16], hepatocytes [17], and renal cells [18]. This method has also been applied

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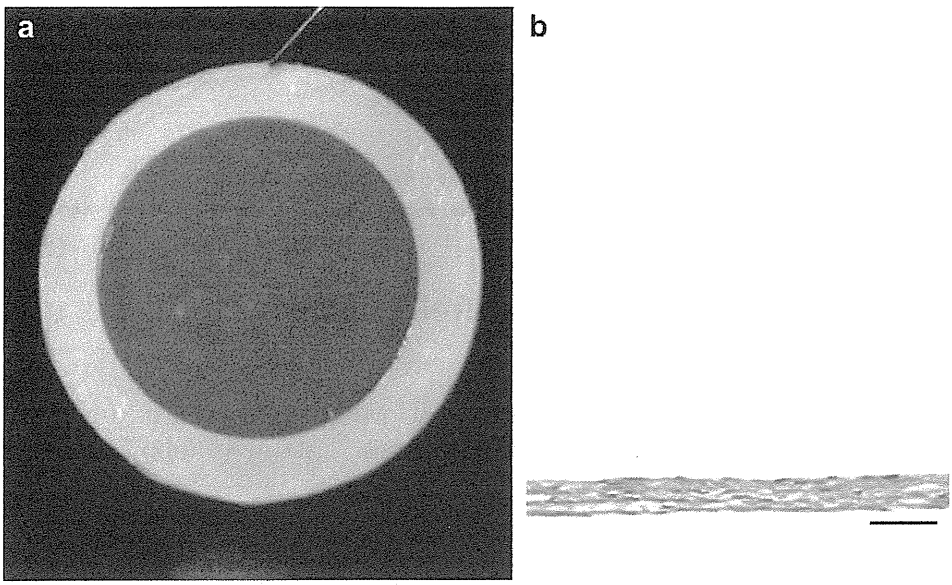

Fig. 1. (a) Layered chondrocyte sheet. Chondrocytes cultured on a temperature-responsive surface can be released from the dish (4.2 cm^2) by reducing the temperature without the need for proteolytic enzymes. Confluent cultured chondrocytes were harvested as a single contiguous cell sheet retaining cell–cell junctions, and extracellular matrix (ECM) was deposited on the basal side. (b) Chondrocyte sheets can be layered and thereafter adhere to other cell sheets. Culture of five-layered chondrocyte sheets can be continued for up to 1 week (scale bar = 150 μm).

clinically to the myocardium and cornea [16]. The surface of a temperature-responsive culture dish is coated with a polymer (poly(*N*-isopropylacrylamide)), which becomes hydrophilic or hydrophobic in a reversible manner, depending on the temperature [19]. The polymer has a low critical solution temperature of 32 °C, below which it becomes soluble in water. Based on this characteristic, the temperature-responsive culture dish has a weakly hydrophobic surface, similar to that of commercially available dishes, so it can be used to culture cells in a conventional manner when the temperature is 37 °C or higher. However, the surface of the dish becomes hydrophilic when the temperature falls below the critical solution temperature. Therefore, confluent sheets of cultured cells can be released spontaneously from the hydrophilic dish surface by reducing the temperature to below 32 °C [20]. Using this method, cultured cells can be harvested as a sheet without damaging cell–cell junctions and the ECM because it eliminates the need for conventional enzymatic harvesting with trypsin. Such cell sheets have been reported to have various advantages, including preservation of the normal phenotype and expression of adhesion proteins on the sheet base [21]. Furthermore, these cell sheets can also be superimposed to prepare layered 'tissue' because the ECM is preserved on the base, and such three-dimensional (3D) manufactured tissue has already been used successfully in transplantation [14]. We confirmed previously that such layered chondrocyte sheets are able to maintain a normal chondrocyte phenotype in the knee joints of rabbits. Moreover, they can be attached to injured cartilage, thereby acting as a barrier to prevent the loss of proteoglycan from these sites, while also protecting them from catabolic factors [10].

The objective of this study was to investigate the ability of layered chondrocyte sheets to repair and regenerate tissue in a minipig-based large animal model of full-thickness defects of articular cartilage.

2. Materials and methods

All animal experiments were approved and carried out following the Guidelines of Tokai University on Animal Use.

2.1. Temperature-responsive culture dishes

Specific procedures for the preparation of temperature-responsive culture dishes (provided by CellSeed, Tokyo, Japan) were as described [19]. Briefly, *N*-isopropylacrylamide (IPAAm) monomer solution was spread onto commercial tissue culture polystyrene dishes. These dishes were then subjected to electron beam irradiation, thus resulting in polymerization and covalent bonding of IPAAm to the dish surface. Poly-IPAAm (PIPAAm)-grafted dishes were rinsed with cold distilled water to remove ungrafted IPAAm. The dishes were then sterilized using ethylene oxide gas [22].

2.2. Chondrocytes from minipigs and proliferation on a temperature-responsive surface

Five minipigs aged 7–8 months and weighing 21.3–21.5 kg were used as the source of chondrocytes. Cartilage samples were collected from the femoral compartment of the knee joint and subjected to enzymatic processing; they were then seeded and cultured according to the method of Sato et al. [23]. Briefly, chondrocytes were digested for 1 h in Dulbecco's modified Eagle's medium/F12 (DMEM/F12; Gibco, Grand Island, NY, USA) containing 0.4% Pronase E (Kaken Seiyaku Inc., Tokyo, Japan) and for 4 h further in DMEM/F12 containing 5 mg/ml collagenase type 1/CLS1 (Worthington Inc., Lakewood, NJ, USA) at 37 °C in an atmosphere of 5% CO_2 and 95% air. Digested tissue was passed through a Falcon cell strainer (BD Biosciences, Franklin Lakes, NJ, USA) with a pore size of 100 μm. Cells were then seeded at high density (50,000 cells/cm^2) into temperature-responsive dishes (4.2 cm^2; provided by CellSeed, Tokyo, Japan) and were cultured in DMEM/F12 supplemented with 20% fetal bovine serum (FBS; Gibco), 50 μg/ml ascorbic acid (Wakojunyakukougyou Corp., Osaka, Japan) and 1% antibiotic–antimycotic mix (Gibco) at 37 °C in an atmosphere of 5% CO_2 and 95% air for a week. Culture dishes were removed from the incubator when the cells reached confluence and were left to stand at 25 °C for 30 min. After the culture medium had been removed, cell sheets were harvested as described by Kaneshiro et al. [10].

2.3. Measurement of chondrocyte proliferative activity

Chondrocytes isolated and cultured as above (3.0×10^4 cells) were cultured on 24-well plates and cell proliferation activity was measured using a thiazolyl blue tetrazolium bromide (MTT) assay on days 3, 5 and 7. MTT (Dojindo, Kumamoto, Japan) was added to each well of the 24-well plate and incubated for 2 h at 37 °C in the dark. The resulting crystals were solubilized in dimethyl sulfoxide. Absorbance was read using an enzyme-linked immunosorbent assay (ELISA) plate reader at 590 nm, with absorbance as a function of viable cell number. Data are expressed as the mean ± standard error of the mean.

Table 1
Variables used in the International Cartilage Repair Society (ICRS) grading system and the ICRS remodeling system for evaluating subchondral bone maintenance.

Variable	Comment
Ti:	**Tissue morphology**
	4 = Mostly hyaline cartilage
	3 = Mostly fibrocartilage
	2 = Mostly noncartilage
	1 = Exclusively noncartilage
Matx:	**Matrix staining**
	1 = None
	2 = Slight
	3 = Moderate
	4 = Strong
Stru:	**Structural integrity**
	1 = Severe integration
	2 = Cysts or disruptions
	3 = No organization of chondrocytes
	4 = Beginning of columnar organization of chondrocytes
	5 = Normal, similar to healthy mature cartilage
Clus:	**Chondrocytes clustering in implant**
	1 = 25–100% of cells clustered
	2 = <25% of the cells clustered
	3 = No clusters
Tide:	**Intactness of calcified cartilage layer, formation of tidemark**
	1 = <25% of the calcified cartilage layer intact
	2 = 25–49% of the calcified cartilage layer intact
	3 = 50–75% of the calcified cartilage layer intact
	4 = 76–90% of the calcified cartilage layer intact
	5 = Complete intactness of the calcified cartilage layer intact
Bform:	**Subchondral bone formation**
	1 = No formation
	2 = Slight
	3 = Strong
SurfH:	**Histologic appraisal of surface architecture**
	1 = Severe fibrillation or disruption
	2 = Moderate fibrillation or irregularity
	3 = Slight fibrillation or irregularity
	4 = Normal
FilH:	**Histologic appraisal defect filling**
	1 = <25%
	2 = 26–50%
	3 = 51–75%
	4 = 76–90%
	5 = 91–100%
Latl:	**Lateral integration of implanted material**
	1 = Not bonded
	2 = Bonded at one end/partially both ends
	3 = Bonded at both sides
Basl:	**Basal integration of implanted material**
	1 = <50%
	2 = 50–70%
	3 = 70–90%
	4 = 91–100%
InfH:	**Inflammation**
	1 = No inflammation
	3 = Slight inflammation
	5 = Strong inflammation
Hgtot	**Histologic grading system**
	Some of the histologic variables: tissue morphology (Ti), matrix staining (Matx), structural integrity (Stru), cluster formation (Clus), tidemark opening (Tide), bone formation (Bform), histologic surface architecture (SurfH), histologic degree of defect filling (FilH), lateral integration of defect filling tissue (Latl), basal integration of defect filling tissue (Basl) and histologic signs of inflammation
Remod:	**Subchondral bone remodeling (loose textures of highly cellular tissue composed mostly of fibroblasts)**
	1 = No remodeling
	2 = Discrete cellularity
	3 = Moderate cellularity
	4 = High cellularity

Presentation of the ICRS histological grading system (Hgtot) and remodeling scores (Remod) based on a modified ICRS grading scale [30] developed by O'Driscoll et al. [31] In Hgtot, 11 histologic categories were evaluated and scored. The total score ranged from 11 points (no repair) to 45 points (normal articular cartilage).

2.4. Transplantation of chondrocyte sheets

Before the surgery of implantation, 0.2 mg/kg dormicum (Midazolam 5 mg/1 ml, Astellas Pharma, Tokyo, Japan) and 40 μg/kg medetomidine (Domitor 1 mg/ml, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were given intramuscularly. Inhalation anesthesia was used during the operation with a combination of isoflurane, dinitrogen monoxide, and oxygen. A chondral defect measuring 6 mm in diameter and 5 mm deep was made in the area of the host animal's medial femoral condyle using a biopsy punch and the damaged cartilage was covered with a three-layered chondrocyte sheet, which was stabilized with a nylon suture until initial fixation was achieved. This was performed in the left knees of 12 minipigs (aged 7–8 months and weighing 21.5–25.0 kg) in the transplantation group. At the same time, the articular cartilage of the medial femoral condyle was holed similarly in the right knees of 12 minipigs in the control group, but not covered with a cell sheet. Cartilage was harvested after 3 weeks, fixed in 4% paraformaldehyde (PFA) for 1 week and decalcified with K-CX decalcifying solution (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) for 1 week. Specimens were then embedded in paraffin wax, sectioned and stained with safranin-O for evaluation. Histological scoring of these sections was carried out by two observers, using the International Cartilage Repair Society (ICRS) grading system and the ICRS remodeling system to evaluate subchondral bone maintenance (Table 1).

2.5. Statistical analysis

The Mann–Whitney nonparametric U test was used to analyze histological scores in assessing the efficacy of treatment. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Chondrocyte sheets

Multilayered chondrocyte sheets were prepared by superimposing sheets and then culturing them together. It was thus possible to continue culturing three-layered sheets. As shown in Fig. 1, the layered articular chondrocyte sheets cultured for 3 weeks maintained their original shape without shrinking when the polyvinylidene difluoride (PVDF) membrane was removed. As a result, it was possible to create round grafts that demonstrated a chondrocytic phenotype.

3.2. Measurement of chondrocyte proliferation activity

Cell proliferation activities examined by MTT assay on days 3, 5 and 7 are illustrated in Fig. 2. Proliferation increased gradually until day 7; the numbers of chondrocytes increased by approximately 2.4-fold by day 5 and by approximately 6-fold by day 7.

3.3. Gross findings in the repaired cartilage

No distinct evidence of infection, articular damage at uninvolved sites, or synovial proliferation was observed in the transplantation or control groups. All defects were filled with white cartilaginous tissue, but defect filling in the control group was insufficient. The surface layer in the transplantation group had been replaced with smooth cartilaginous tissue of a color resembling that of healthy cartilage, but the surface layer in the control group had been replaced with coarse cartilaginous tissue, while the subchondral bone was exposed in some locations (Fig. 3a–d).

3.4. Histological findings of repaired cartilage

Good safranin-O staining and integration with surrounding tissue was noted in the transplantation group, which achieved sufficient cartilaginous repair and regeneration. All animals in the control group exhibited poor safranin-O staining, and tissue repair and regeneration were insufficient (Fig. 4a, b). ICRS scores were compared between the two groups. The mean score in the transplantation group was significantly higher than in the control group

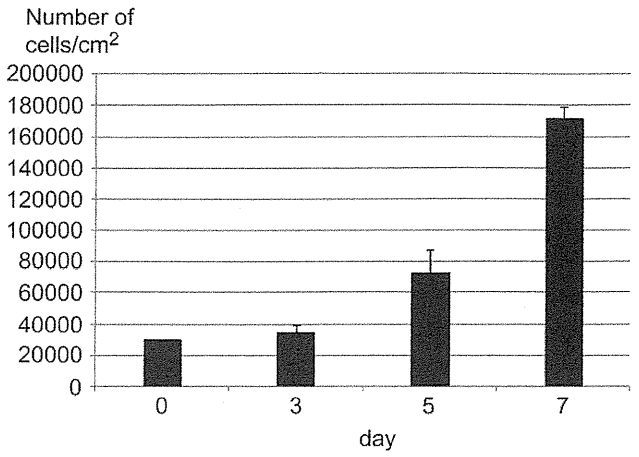

Fig. 2. Cell proliferation activity was examined by MTT assay. Cell proliferation increased steadily until day 7; the numbers of chondrocytes had increased approximately 2.4-fold by day 5 and approximately 6-fold by day 7.

(38.3 points versus 26.3 points, respectively, $p < 0.05$). Mean remodeling scores, an indication of subchondral bone condition, were significantly higher in the transplantation group (3.2 points) than in the control group (2.4 points; Fig. 5a, b). The ICRS scores in the transplantation and control groups are shown in Table 2.

In three of the 12 animals, a more detailed histological examination in the transplantation group revealed that, although tissue filling was present, safranin-O staining was poor and the subchondral bone in these animals was poorly repaired and regenerated (Fig. 4d). A comparison of ICRS scores indicated that subchondral bone repair was satisfactory (remodeling score 4 points) in the animals in the transplantation group that achieved adequate cartilage repair (histological score 42 points). However, subchondral bone repair was poor (remodeling score 2 points) in animals in the transplantation group with insufficient cartilage repair (histological score 29 points). These findings indicate that the degree of subchondral bone repair reflected that of cartilaginous tissue repair.

4. Discussion

Unless treated, full-thickness defects in knee cartilage cause secondary osteoarthritis and the resulting pain and poor joint function impact substantially on the activities of daily life. Recent advances in tissue engineering have prompted research on techniques to repair articular cartilage damage using a variety of transplanted cells. ACI was the first such technique investigated and is already used clinically in Western countries. However, the technique carries problems such as the potential for leakage of chondrocytes (implanted in suspension) outside the transplantation site, nonuniform distribution of transplanted cells [24] and damage to donor site tissues. Some have claimed that clinical outcomes are not superior to microfracture [25]. More effective treatments must therefore be developed. Ochi et al. [5], hypothesizing that the transplantation of 3D cartilaginous tissues composed of chondrocytes and matrix would yield better outcomes than chondrocyte transplantation, developed and clinically applied the transplantation of cultured chondrocytes embedded in atelocollagen gel. Furthermore, realizing the potential for bone marrow mesenchymal cells to differentiate, Wakitani et al. [26] developed and applied the transplantation of bone marrow mesenchymal cells embedded in collagen gel clinically. However, both types of implant were composites made of periosteum, a scaffold, bone marrow cells, cultured chondrocytes and numerous other elements and were thus unsuitable for creating an optimal environment for articular cartilage regeneration.

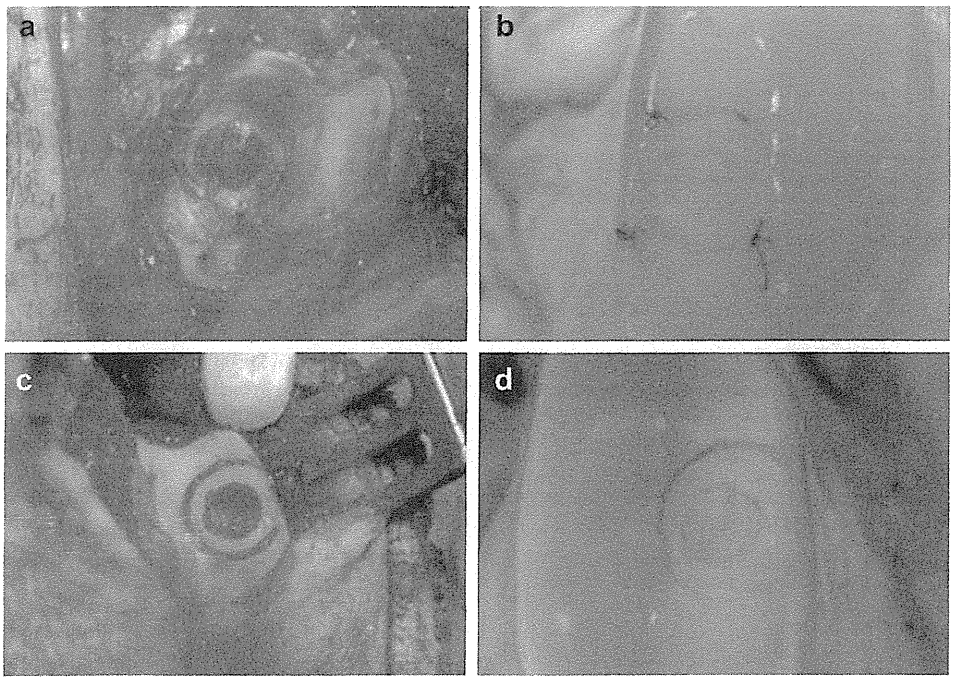

Fig. 3. Gross appearance (a, b, c, d) in the group receiving layered chondrocyte sheets (a, b) and the control group (c, d). Gross appearance is shown at transplantation (a, c) and 3 months later (b, d). The defect in the transplantation group (b) was filled with cartilaginous tissue, but filling in the defect of the control group (d) was insufficient and the subchondral bone was exposed partially. The defect of 6 mm in diameter and 5 mm deep was made, and outside circle of the defect is 8 mm in diameter, and is used for suturing chondrocyte sheet.

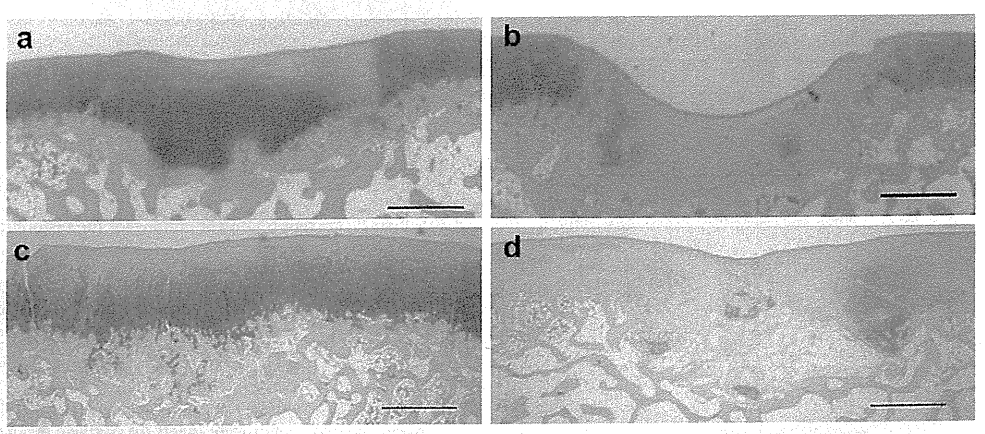

Fig. 4. Histology in the transplantation (a, c, d) and control groups (b). Safranin-O staining was robust and integration with the surrounding tissue was good in the transplantation group (a), but the control group (b) showed poor safranin-O staining and did not achieve satisfactory regeneration or repair. In (c), safranin-O staining was robust and subchondral bone repair and regeneration were good (International Cartilage Repair Society, ICRS histological grading system 42 points; remodeling score 4 points). Although the defect in (d) had been filled with tissue, safranin-O staining was poor and the subchondral bone was insufficiently repaired (ICRS histological grading system 29 points; remodeling score 2 points; scale bar = 2.0 mm).

Convinced that establishing an environment suited to tissue repair is essential for proper articular cartilage regeneration, we began basic research on repair and regeneration using only cells from bone marrow and cultured chondrocytes, without a scaffold or periosteum. Analysis of our layered chondrocyte sheets has shown that the chondrocytes maintain their phenotype, expressing aggrecan, collagen type II (COL2), SOX9 and COL27. The cells also express the adhesion molecules integrin α10 and fibronectin. Immunostaining confirmed the presence of COL2, integrin α10 and fibronectin in the cellular sheets [12,27]. Our findings indicate that these layered chondrocyte sheets demonstrate good adhesiveness and barrier functionality while maintaining a normal phenotype.

We also reported achieving robust tissue repair when covering only the surface layer with a layered chondrocyte sheet in research on partial-thickness defects in the articular cartilage of domestic rabbits [10]. This suggests that liquid factors from the layered sheets, in addition to the basic functionality of the sheets, contribute to repair.

In this study using chondrocytes from minipigs, which are large animals, we found that cultured chondrocytes had a high cell proliferation potential and that—as shown in previous studies—layered chondrocyte sheets created from these chondrocytes also contributed to the repair and regeneration of articular cartilage in this model of full-thickness defects. However, tissue having poor safranin-O staining, which was not noted in our domestic rabbit experiments, was identified in some of the animals (3/12) and the subchondral bone was poorly repaired in these animals. Vasara et al. [28] transplanted chondrocytes into a goat model of full-thickness defects. In animals with poor subchondral bone repair, the transplanted cells showed poor safranin-O staining, and integration with the surrounding tissue was insufficient. Muehleman et al. [29] transplanted chondrocytes into a minipig model of full-thickness defects and compared a group treated with risedronate to an untreated group using histology. Safranin-O staining of the transplanted cells was superior in the treated group and integration with the surrounding tissues was satisfactory,

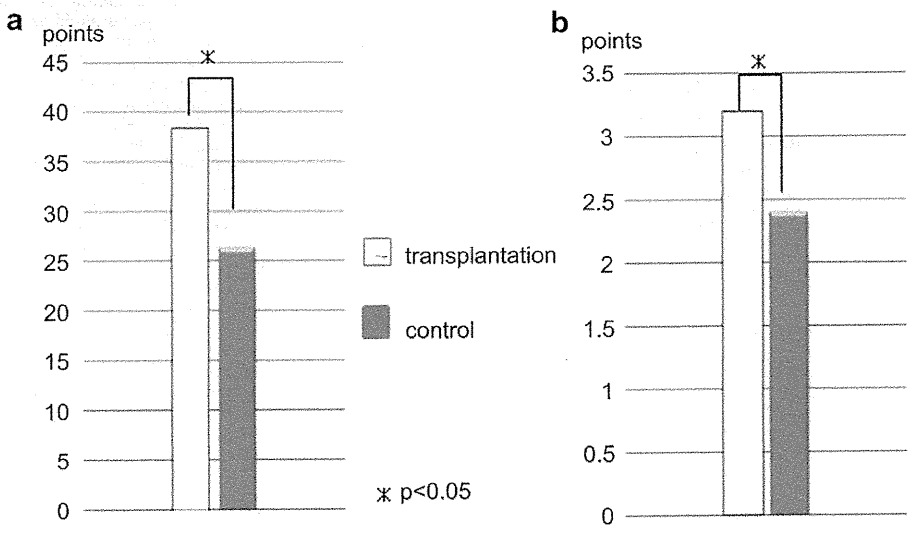

Fig. 5. ICRS histological grading system scores (a) and remodeling scores (b) in the transplantation and control groups. The mean histological score (a) in the transplantation group (38.3 points) was significantly higher than in the control group (26.3 points). Mean remodeling scores (b), an indication of subchondral bone condition, were significantly higher in the transplantation group than in the control group (3.2 points and 2.4 points, respectively).

Table 2
Results of ICRS histological grading system (Hgtot) and remodeling scores (Remod).

Case	Weight (kg)	Age (month)	Transplantation		Control	
			Hgtot	Remod	Hgtot	Remod
			(Points)		(Points)	
1	21.6	7	42	4	28	3
2	23.5	7	40	4	22	2
3	24.2	7	40	4	28	3
4	22.9	7	42	4	30	3
5	24.1	8	41	4	26	2
6	24.0	8	40	3	31	3
7	23.0	8	39	3	24	2
8	25.0	8	39	3	24	2
9	24.0	8	39	3	24	2
10	23.0	8	33	2	33	3
11	23.8	8	29	2	23	2
12	21.5	8	35	2	22	2
Average	23.4	7.7	38.3	3.2	26.2	2.4

These findings indicate that the points awarded in the ICRS histological grading system reflected the remodeling scores.

but greater than expected bone resorption occurred in this large animal model. Similarly, animals with poor subchondral bone repair in the transplantation group in the present study exhibited poor safranin-O staining in the regeneration tissue and insufficient integration with surrounding tissue. Risedronate administration and other measures to suppress bone resorption might thus be necessary in large animals used in future research to determine optimal transplantation conditions.

5. Conclusion

The use of layered chondrocyte sheets facilitated the repair and regeneration of tissue in a minipig model of full-thickness cartilaginous defects in the knee joints. Good safranin-O staining and integration with surrounding tissue was noted in the transplantation group, which achieved sufficient cartilaginous repair and regeneration. Some animals in the group receiving the layered chondrocyte sheets exhibited poor safranin-O staining of the repaired and regenerated tissue in the subchondral bone. Transplantation conditions and other factors must therefore be further investigated.

Acknowledgments

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軟骨再生

ラット膝関節内へ移植した細胞シートのBioluminescenceによる経時的評価

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In vivo Cell Tracking by Bioluminescence Imaging after Transplantation of Bioengineered Cell Sheets to the Knee Joint

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臨整外 47：27～31，2012

Key words：軟骨再生(cartilage regeneration)，生体発光イメージング(bioluminescence imaging)，細胞シート(cell sheet)

軟骨細胞シートは温度応答性培養皿を用いて酵素処理を必要とせずシート状に回収され，損傷した軟骨組織に対して優れた接着能をもち自己の軟骨修復能力を高める．すでに家兎およびミニブタを用いた動物実験において，膝関節症モデルへの同種細胞シート移植後の軟骨再生効果が実証されている．今回，細胞シートのラットにおける同種膝関節移植後の細胞動態について**bioluminescence imaging**(**BLI**)の手法を用いて検証したところ，関節内の長期生存を認め，移植細胞自身による軟骨再生効果が期待される結果となった．

はじめに

細胞シートは温度応答性培養皿を用いて酵素処理を必要とせずシート状に回収される．膝軟骨細胞は膝関節内を裏打ちする滑膜細胞が分泌する滑液(関節液)によって栄養されている．細胞シート作製時にこれらの細胞を共培養することで，軟骨細胞シートの作製期間の短縮が可能である．この組織工学的に構築された細胞シートによる関節軟骨の治療を目指している．いままでに家兎およびミニブタを用いた動物実験において，膝関節症モデルへの同種細胞シート移植後の軟骨再生効果が実証されている[6,8]．近年，生物発光(bioluminescence) imaging(BLI)が移植細胞の*in vivo*追跡に用いられているが，中でもホタルの発光遺伝子ルシフェラーゼ(*luc*)が最も頻用されている．発光基質ルシフェリンとの反応により放出される光量子を高感度charge-coupled device(CCD)カメラで捕捉しイメージ化することで，実験動物を殺

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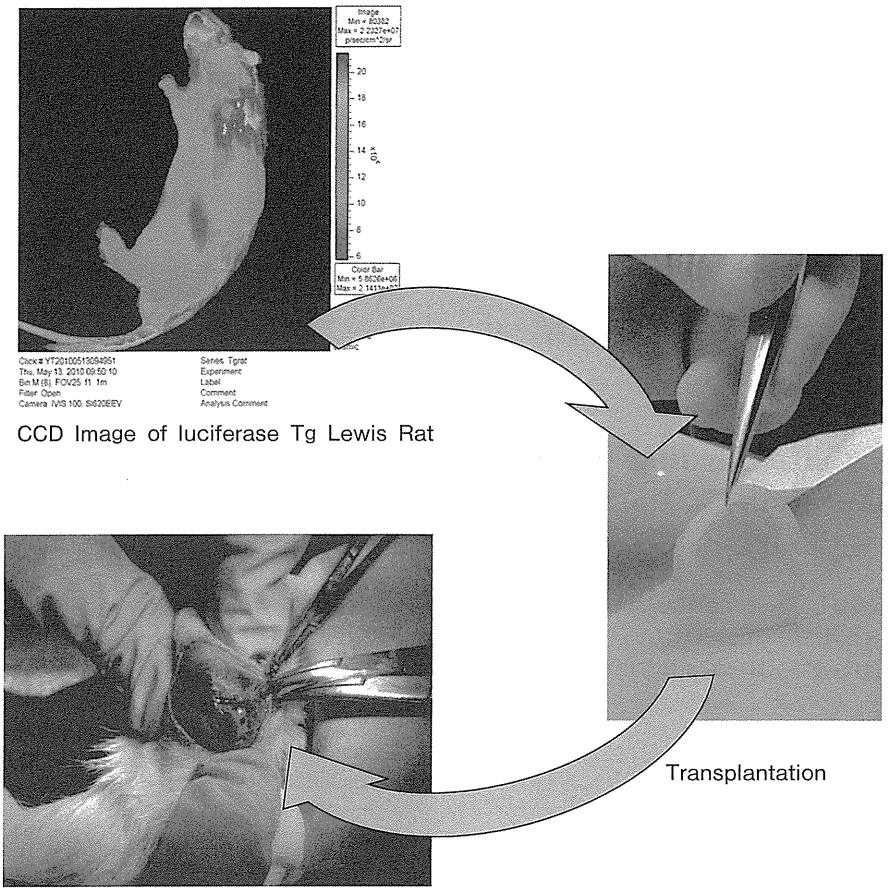

図1　Luc^{+} 軟骨細胞シートの移植
ルシフェラーゼを全身に発現するトランスジェニックラットの軟骨，滑膜組織から細胞シートを作製後，野生型 Lewis ラットの骨軟骨欠損部に移植した．

すことなく非侵襲的に同一個体を繰り返し経時的に長期追跡することが可能である．今回われわれはルシフェラーゼ遺伝子を導入したトランスジェニックラットより組織工学的に構築した細胞シートを用いて，ラット同種膝関節移植後の移植細胞動態を観察することができたので紹介する．

BLI

Bioluminescence とは，生体内で励起分子を作り出し，そのエネルギーにより発光する現象である．電気など他の化学発光に比べ発光量子効率に優れるため，熱を伴わない冷光と呼ばれ，発光スペクトルなども異なる．発光生物のなかでもホタルの発光量子効率が高く，発光基質ホタルルシフェリンの大量生産が可能になったことからバイオ研究のなかで最も頻用されている．発光基質ルシフェリンは発光酵素ルシフェラーゼと Mg^{2+} イオンを触媒として ATP と反応し発光する[3,5]．BLI とは発光する際に放出される光量子を高感度 CCD カメラで補足し画像イメージングする手法である．ルシフェラーゼ遺伝子を導入した遺伝子や細胞を用いて腫瘍細胞の観察，再生医学における移植細胞や免疫担当細胞の追跡，治療遺伝子の発現評価など，医学研究において近年飛躍的に広がりつつある[1,2,9]．

トランスジェニックラット

Kobayashi ら[4]は，ルシフェラーゼ遺伝子を発現するトランスジェニックラットを開発した(Rosa/Luciferase transgenic Lewis rats)．プロモーターに ROSA26 を有し，安定してルシフェラーゼを発現する．発光基質ルシフェリン投与すると，生体内のルシフェラーゼと ATP を使い自ら発光する．発光強度は細胞内 ATP や Mg^{2+} などの細胞環境に影響されるため，細胞種ごとに異なるが，同一種細胞においては投与したルシフェリン量に比例する．そのため，半定量的に生存細胞を追跡することも可能となる．ラット移植実験の細胞ソースとして有用である．

対象と方法

16週齢のRosa/Luciferase transgenic Lewis rats由来の軟骨細胞，滑膜細胞からLuc$^+$細胞シートを作製後，同週令の野生型Lewisラットの右膝大腿骨膝蓋面に18G針を用いて骨軟骨欠損を作製(ϕ；3mm)，同部位にLuc$^+$細胞シートを同種移植した(図1)．群の作製としてLuc$^+$軟骨細胞(AC)シートとLuc$^+$滑膜細胞(SY)シートの2枚ずつの組み合わせによる群を作製した．すなわち，Luc$^+$軟骨細胞シート単独群(AC-AC group)とLuc$^+$滑膜細胞(SY-SY group)シート単独群，両シート併用群(AC-SY group)の3群を作製した(各n＝4)．各種細胞シートの膝関節内滞在期間の影響について比較検討を試みた．D-luciferin(potassium salt；Biosynth)150mg/kgを肩甲骨下から皮下投与後，CCDカメラを搭載したIVISシステム(Xenogen Corp；Alameda，CA)(図2)を用いて繰り返し経時的にイメージングを行った．イメージングでは最も強いルシフェラーゼ発光強度を測定した．

結果

Luc$^+$移植細胞は1年以上イメージングにより追跡可能であった．ルシフェラーゼの発光シグナルは移植した右膝関節部位のみで検出され，他の部位では検出されなかった(図3)．

発光強度はAC-AC group，AC-SY group，SY-SY groupの順に強く測定された．この順序は移植後1カ月以降は変化しなかった．各群とも移植後3カ月頃から発光強度は安定し，明らかな増減を認めず，また1年の経過中にすべての個体イメージにおいて右膝のルシフェラーゼ発光は消失しなかった(図4)．

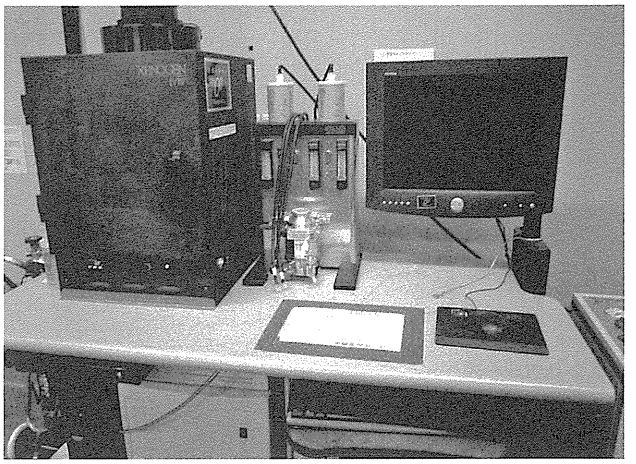

図2　IVISシステム(Xenogen社)
小動物体内におけるルシフェラーゼ遺伝子からの微弱な光を高感度CCDカメラで検出し，同時に被検体の普通写真を撮影した後，発光画像を重ね合わせてルシフェラーゼ発現の分布像を得る．

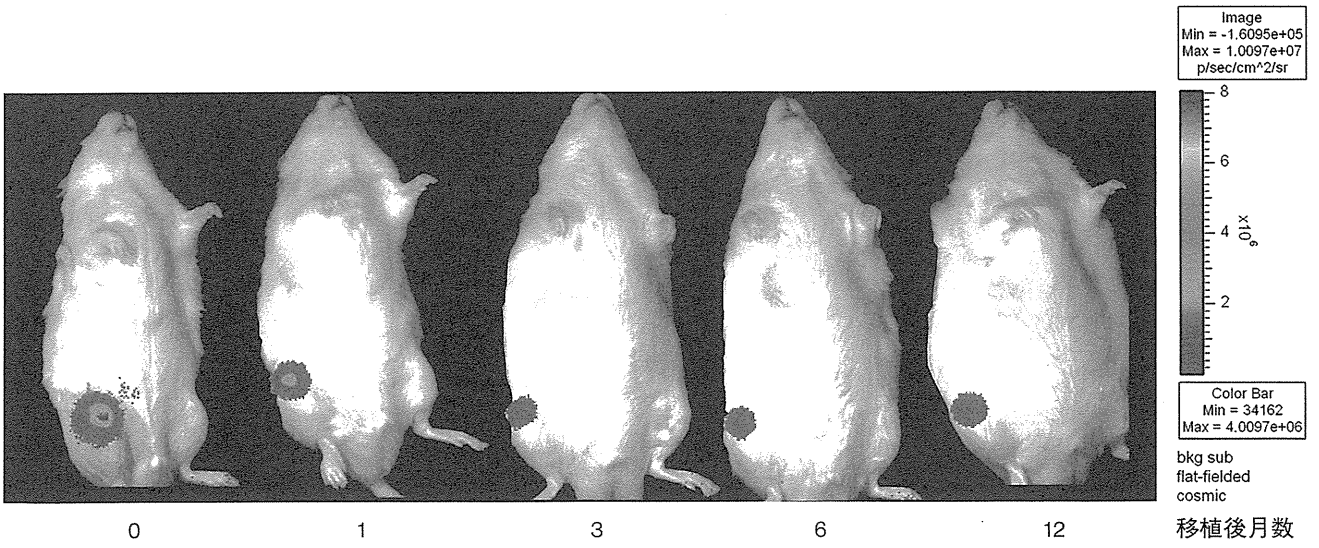

図3　AC-AC groupの細胞シート移植後の同一個体におけるCCDイメージ画像の継時的変化
カラー標識は発光強度を示す．ルシフェラーゼ発光を右膝部分にのみ認める．AC-AC group，AC-SY group，SY-SY groupの個体すべてに同様のイメージングが得られた．