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第12章 ティッシュエンジニアリング

到達目標

1．再生医療の概念を説明できる
2．ティッシュエンジニアリングの三大因子について説明できる
3．現行の歯周治療の問題点を説明できる
4．歯周組織再生療法について概説できる

A 再生医療

今世紀の医学・生物学の分野におけるキーワードの1つに「再生医療」があげられる．疾患や外傷により一度失った組織・臓器の形態と機能を，あたかもプラナリアやイモリの四肢のように，もう一度みずからの細胞の働きにより再生させるということはほとんど不可能なことと従来考えられてきた．いまどうして「再生」ということばが脚光を浴びるようになったのであろうか．日本再生医療学会によると，再生医療とは「機能障害や機能不全に陥った生体組織・臓器に対して，細胞を積極的に利用して，その機能の再生をはかるもの」と定義されている．「細胞を積極的に利用」するとは，具体的にどのような医療をさすのであろうか．

1 再建医療・移植医療の問題点

今日に至るまで，種々の疾患あるいは外傷により薬剤などでは治療できないほど大きな組織や臓器の欠損が生じた場合には，人工臓器を中心とした再建外科医療によって治療されてきた．しかしながら，人工臓器は人工材料であるがゆえの生体異物反応や，その代行機能の経時的低下などの欠点がある．他方，臓器移植により対応されるケースも考えられる．しかしながら，慢性的なドナー不足の問題や脳死判定の問題などもあり，わが国で臓器移植法が施行されてからの経緯をみても，その恩恵にあずかれるのはまだまだまれなケースといわざるを得ない．

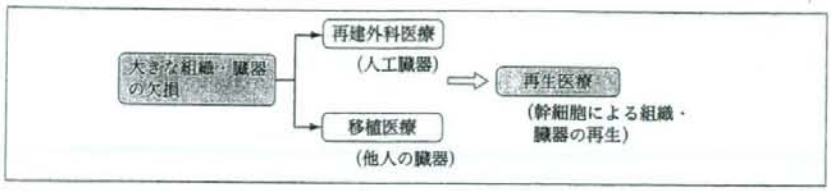

図 2-71　大きな組織・臓器欠損を対象とした医療
現在，大きな組織・臓器の欠損が生じた場合には人工臓器を中心とした再建外科医療，あるいは適当なドナーが見つかれば臓器移植を対象とした移植医療が施される．これらの方法は医療上あるいは倫理上の問題を有しており，幹細胞の潜在能力を引き出すことによる再生医療の発展が期待されている．

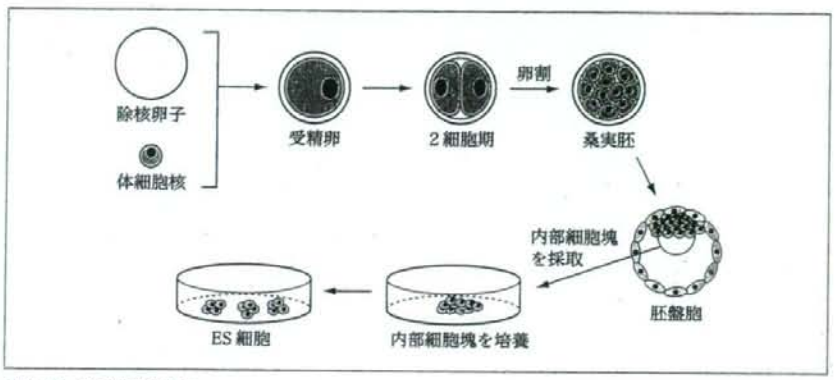

図 2-72　ES 細胞の樹立
除核卵子に体細胞核を移植することにより受精卵をつくり，これを培養すると細胞分裂の結果，胚盤胞が形成される．この胚盤胞内にある内部細胞塊を取出して培養することにより ES 細胞株が得られる．

2　幹細胞，前駆細胞

近年，成人の生体組織のなかにも多分化能を保有した幹細胞 stem cells や，特定の成熟細胞への分化能を保有した前駆細胞が，成人になっても存在し続けていることが証明された．さらに，個体がいかに発生するか，創傷治癒の過程において個々の細胞がどのように集合体を形成して組織・臓器を形づくるかといった研究が分子レベルで進められた結果，これらの情報を活用し，組織再生を人為的に促進しようとする再生医療に対するさまざまな試みが始められるようになった（図 2-71）．

3　胚性幹細胞の樹立

このような時代背景のなか，1998 年には多分化能を保有するヒト胚性幹細胞（ES 細胞 embryonic stem cell）が樹立された．ES 細胞は，受精卵が胎児になる前に胚盤胞から内部細胞塊を取り出して，その細胞を株化したものである．ヒト由来 ES 細胞は拒絶反応を起こせない免疫不全マウスに移入されると，骨，軟骨，筋肉，消化管など，人体のあらゆる組織に成熟，分化する能力を備えていることが認められ，このことより多能性細胞とよばれている（図 2-72）．この ES 細胞を試験管内にて神経前駆細胞にまで分化させた細

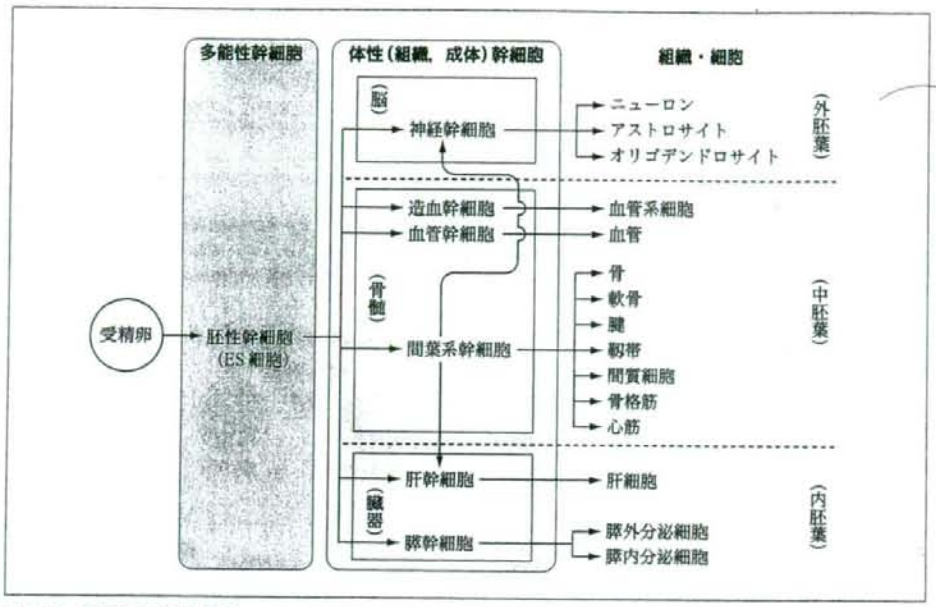

図 2-73　幹細胞の分類と階層
受精卵に端を発する多能性幹細胞からさまざまな体性幹細胞となり，さらに分化を遂げることによって外胚葉，中胚葉，内胚葉といった身体のあらゆる組織細胞が形成される．

胞を移入することにより Parkinson 病や Alzheimer 病の治療に応用することが想定されている．このように考えると，あらゆる細胞に分化して望みの組織・臓器を形成することが可能な多能性幹細胞としての ES 細胞は，再生医療にとって夢の細胞といえるであろう．しかしながら，ヒトの ES 細胞はヒトの受精卵つまり胚由来であり，倫理上の問題が存在するのみならず，非自己細胞であるがゆえに免疫学的拒絶反応をいかに抑制するかという医学的な問題も残されている．

4　体性幹細胞の利用

幹細胞には大きく分類して 2 種類あり，多能性細胞としての胚性幹細胞（ES 細胞）のほかに分化の程度が進んで，特定の組織・臓器を再構築する能力を有する体性（組織，成体）幹細胞が存在する（図 2-73）．

体性幹細胞は，生体における組織・臓器の恒常性の維持や損傷時の再生を可能にするために成人の生体内にもなお存在する細胞である．体性幹細胞のうち骨髄中には造血幹細胞のみならず間葉系幹細胞が存在することも明らかにされている．このことは患者本人の骨髄から幹細胞を得ることにより，拒絶反応の問題，倫理的問題を克服した再生療法が実現可能であることを示唆している．

このように，種々の組織の再生を可能にする幹細胞の情報や単離技術が近年急速に進歩している．そして，これらの幹細胞に加えて，種々の生体材料をも研究対象として取り入

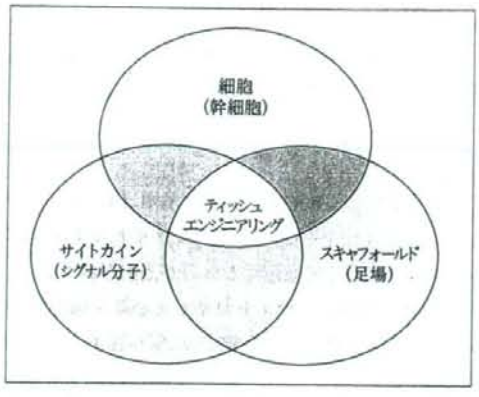

図 2-74　生体組織工学における三大因子
生体組織工学は，三大因子である細胞（幹細胞），スキャフォールド（足場）およびサイトカイン（シグナル分子）を用いて，損なわれた組織・臓器の形態と機能を再生し，維持し，改善することを目的とする学問である．

れた再生医学に支えられた再生医療は，一躍 21 世紀の夢の医療技術として，医学的のみならず社会的関心をも集めるに至っている．

B ティッシュエンジニアリング

1　ティッシュエンジニアリングの三大因子

成人の体内にも種々の体性幹細胞が存在していることは先にも紹介したが，生体の自然な治癒にすべてをゆだねると，幹細胞の働きを十分に引き出すことができず，理想的な組織再生は果たされない．

幹細胞を十分に機能させ組織・臓器を生体内で再生，あるいは生体外で再構築するためティッシュエンジニアリング tissue engineering（生体組織工学）を用いた治療法が研究されている．ティッシュエンジニアリングとは「工学と医学・生物学の原理を統合することによって，損なわれた組織・臓器の形態と機能を再生し，維持し，改善することを目的とする学問」と定義される．ティッシュエンジニアリングは，①組織を再生するための「幹細胞 stem cells」，②幹細胞が三次元的に遊走・増殖・分化するための「スキャフォールド scaffold（足場）」，および③幹細胞の増殖・分化を制御する「サイトカイン cytokine（シグナル分子）」の三大因子により構成されている（図 2-74）．

2　足場としての細胞外マトリックス

血球細胞を除くすべての正常細胞は，何らかの物質表面に接着することにより分裂するため，細胞の増殖には接着するための“足場”が必要となる．通常，生体内において足場の機能を果たすのは細胞外マトリックスである．大きな組織欠損がある場合には細胞成分のみならず足場までもが失われているため，幹細胞のみの移植では組織の再生は見込めない．そこで，生体細胞がみずから細胞外マトリックスのネットワークを再構築するようになるまで，生体内の環境を模倣し細胞を望みどおりの三次元的立体構造に配置して（すな

わち，再生を期待する組織・臓器の形態を幹細胞に伝え），その増殖・分化を誘導するための人工的な足場を欠損部に提供する必要がある．さらに，この足場は適当な時期に生体組織と置き換わる生体吸収性を有することが求められる．

3　サイトカインと薬物配送システム

幹細胞自身の再生誘導能を高めるために，幹細胞の遊走，増殖，あるいは分化を促進する刺激（サイトカイン，シグナル分子）が必要となる．どのサイトカインを用いるかは，どの組織・臓器を再生誘導するのかによって最も適切なものが選択されることになる．また，組織再生効果を維持・向上させるために，サイトカインを必要な場所で必要な期間，有効濃度を保持させる必要が生じることもある．その際には，増殖因子の基剤（キャリア）やスキャフォールドに薬物配送システム（DDS）の技術を導入することも必要となるかもしれない．また，近年では興味深いことに細胞外マトリックスの量的および質的変化によっても細胞の分化・増殖が制御されることも示唆されており，スキャフォールドとしての細胞外マトリックス自身がシグナル分子としても機能しうるものと考えられている．

C　ティッシュエンジニアリングによる歯周組織の再生

1　原因除去療法の限界

検査・診断→歯周基本治療→再評価→メインテナンスまたはサポーティブペリオドンタルセラピーと続く歯周治療の流れに従って，スケーリング・ルートプレーニングを中心とした原因除去療法を的確に施行することにより，歯周病の進行を阻止し，歯周組織の治癒を促すことができる．しかしながら，重度の歯槽骨吸収を伴った歯周炎の場合には，原因除去療法後にも歯周ポケットが残存したり，たとえ歯周ポケットが消失しても，著しい歯肉退縮を伴った審美的にも機能的にも好ましくない治癒形態をとることがある．すなわち，通常の原因除去療法や歯周外科治療を行っても，歯槽骨やセメント質の新生を伴った歯周組織の再生は望めない．そこでティッシュエンジニアリングを用いた再生療法 tissue regenerative therapy に大きな期待が寄せられている．

2　GTR 法とエナメルマトリックスタンパク質応用の意味

近年，歯根膜組織中に骨芽細胞やセメント芽細胞へ分化しうる間葉系幹細胞が成人になっても存在することが示され，歯根膜組織の生物学的機能を十分に発揮させることにより，歯周組織の再生を誘導することが生物学的に可能であることが明らかにされている．現在，臨床応用されている GTR 法（組織再生誘導法）（☞第 3 編第 2 章）や，エナメルマトリックスタンパク質（エムドゲイン®）（☞第 3 編第 3 章）もこの理論に基づき歯根膜組織のなかに存在する歯周組織の幹細胞を積極的に活用することにより，組織再生を果たそうとした歯周組織再生療法と位置づけることができる．

GTR 法に用いられる GTR 膜は，歯周組織を構成する細胞の遊走をコントロールする

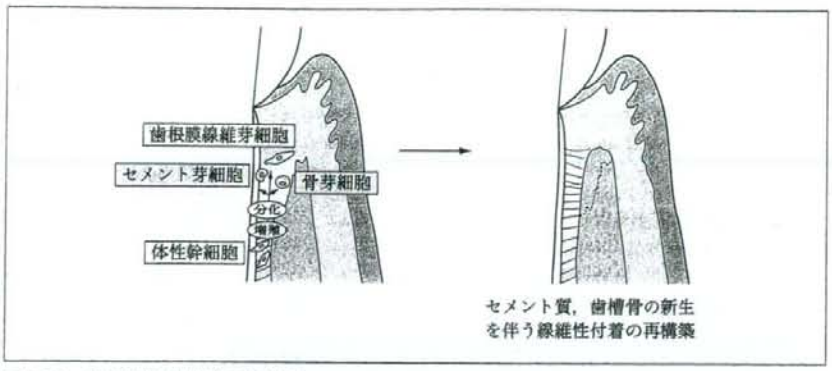

図 2-75　歯周組織再生療法の基本概念
歯根膜組織にはセメント芽細胞，骨芽細胞，歯根膜線維芽細胞に分化しうる体性幹細胞が成人になっても存在しており，体性幹細胞の生物学的可能性を最大限に引き出すことにより線維性付着を再構築した歯周組織再生が理論上可能と考えられている．

ことで歯周組織の再生を促しているという観点からティッシュエンジニアリングの三大因子のスキャフォールド（足場）に分類される．また，エムドゲイン®はセメント芽細胞への分化を促進するタンパク質を含有した医療材料であると考えると三大因子のシグナル分子に分類することができ，これらの歯周組織再生療法は今日の臨床の場において一定の成果をあげている．

再生医療全般の歴史を振り返ってみると，1982 年にヒトに応用された GTR 法に関する論文発表がなされて以来，歯科医療は他分野に遅れることなく（臨床応用という点ではむしろ他分野に先んじて）再生療法に取り組んできたといえる．しかしながら，現行の歯周組織再生療法にも，①部分的な再生しか期待できない，②術式が困難，③適応症が限られている，④十分な予知性に欠けるなど，克服されるべき問題点が残されている．

3　サイトカイン療法

われわれの生体を構成している細胞から産生され，産生した細胞を含めてその周囲の細胞に増殖・分化などの制御に関する種々のシグナルを伝達するタンパク質をサイトカインとよぶ．近年，次世代の歯周組織再生療法としてサイトカインを用いた治療法（サイトカイン療法）の研究が進んでいる．歯周組織の再生誘導を考えると，①歯周組織欠損部の歯根面に歯根膜由来細胞が選択的，優先的に誘導されること，②歯根膜由来細胞中に含まれる未分化間葉系細胞 undifferentiated mesenchymal cell が多分化能を保有したまま増殖し，硬組織形成細胞（骨芽細胞 osteoblast やセメント芽細胞 cementoblast）や歯根膜線維芽細胞として部位特異的な分化を遂げること，③歯根膜線維芽細胞によって産生されたコラーゲン線維束が骨芽細胞やセメント芽細胞により新生された骨組織，セメント質に埋入され，新付着が獲得されること，が必要となる（図 2-75）．

サイトカイン療法とは歯周組織欠損部への歯根膜細胞の遊走・定着や，同欠損部における細胞増殖および硬組織形成細胞への分化の過程を種々のサイトカインを局所投与す

表 2-23　サイトカインによる歯周組織再生誘導

以下は，動物実験において有意な歯周組織再生を誘導することができると報告されているサイトカインである．

1．血小板由来増殖因子（platelet-derived growth factor；PDGF）＋インスリン様増殖因子（insulin-like growth factor-I；IGF-I）
2．骨形成タンパク-2（bone morphogenetic protein-2；BMP-2）
3．トランスフォーミング増殖因子-β（transforming growth factor-β；TGF-β）
4．骨形成タンパク-7（bone morphogenetic protein-7；BMP-7, osteogenic protein-1；OP-1）
5．塩基性線維芽細胞増殖因子（basic fibroblast growth factor；FGF-2，bFGF）
6．脳由来神経栄養因子（brain-derived newrotrophic factor；NGF）
7．血管内皮増殖因子（vascular endothelial growth factor；VEGF）

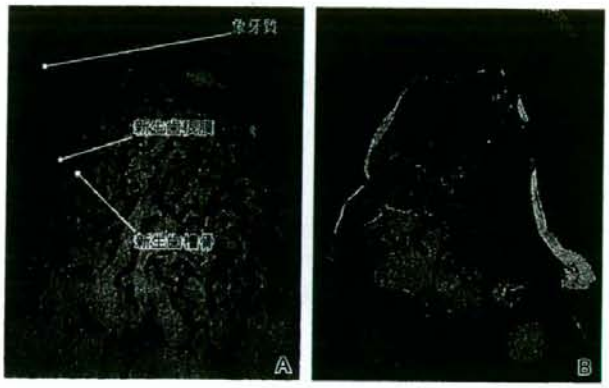

図 2-76　塩基性線維芽細胞増殖因子（FGF-2）を投与したビーグル犬 2 級根分岐部病変に認められた歯周組織再生

ビーグル犬の下顎白歯に実験的に作製した 2 級根分岐部病変に FGF-2 を投与後，6 週目の組織学的観察結果（アザン染色）を示す．FGF-2 投与側（A）では，新生歯槽骨，新生歯根膜，新生セメント質の再生が確認されるが，対照側（B）では，歯肉上皮の下方増殖が生じており，歯槽骨の再生は限定的である．また，FGF-2 投与側では骨性癒着や歯根吸収は認められない．

ることで活性化し，歯周組織の再生を積極的に促進するものである．現在までに，実験動物に作製された人工的歯槽骨欠損部に表 2-23 に示すようなサイトカインを局所投与することにより，同部の歯周組織再生が誘導，促進されたとの報告がなされており，次世代の歯周組織再生療法を担う有望な選択肢の一つとして大いに期待されている（図 2-76）．

4　幹細胞とスキャフォールドの組合せ

先に述べたようにヒト骨髄中に間葉系幹細胞が存在していることが明らかになっていることから，骨髄から幹細胞を採取して（場合によっては生体外でその数を増やし），スキャフォールドとともに歯槽骨欠損部へ細胞移植することにより，歯周組織再生を促す試みも始まっている（☞第 3 編第 5 章）．また，シート状に細胞外マトリックスを加工したスキャフォールドのなかで歯根膜細胞を増殖させ，歯周組織再生を期待する部位にその細胞シートを移植する試みもなされている．このような治療法は生体組織工学の三大因子の幹細胞とスキャフォールドを組み合わせたものと考えられ，大きな歯周組織欠損に対応する再生療法の一つとして期待されている．

5　再生療法の夢多き課題

歯周治療の最も理想的なゴールは，歯周病の進行によって失われた歯周組織を元どおり

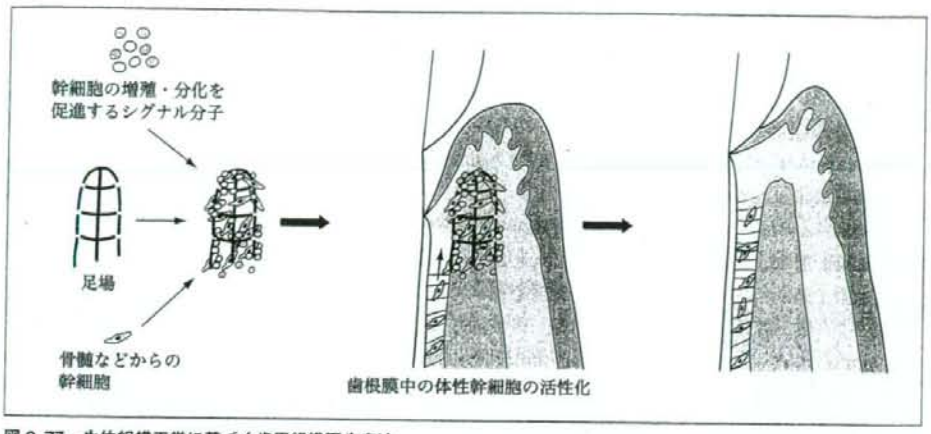

図 2-77　生体組織工学に基づく歯周組織再生療法
歯根膜に内在する体性幹細胞と他部位の骨髄などから採取された間葉系幹細胞，そしてそれら幹細胞のセメント芽細胞，骨芽細胞，歯根膜線維芽細胞への増殖，分化を誘導するサイトカイン（シグナル分子），さらに歯周組織再生を期待する空間を保持（スペースメイキング）し，その形態を伝える能力を有したスキャフォールドを組み合わせることにより理想的な歯周組織再生が達成される．

に再生することであろう．その目的に向かって，ティッシュエンジニアリングの考え方を導入した新しい歯周組織再生療法の開発が現在積極的に進められている．しかしながら，生体組織工学の三大因子のすべてを最良の条件で組み合わせた歯周組織再生療法はいまだに確立されていない（図 2-77），このことは，21 世紀初頭の歯周治療分野における大きな，そして夢多き課題として残されているのである．

チェック項目
1．胚性幹細胞と体性幹細胞について述べよ
2．ティッシュエンジニアリングの目的を述べよ
3．ティッシュエンジニアリングの三大因子を説明せよ
4．歯周組織再生療法の理論を説明せよ
5．現行の歯周組織再生療法の問題点について述べよ
6．歯周組織再生におけるサイトカイン療法について述べよ
7．生体組織工学に基づく歯周組織再生療法について述べよ

The development of a bioengineered organ germ method

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To bioengineer ectodermal organs such as teeth and whisker follicles, we developed a three-dimensional organ-germ culture method. The bioengineered tooth germ generated a structurally correct tooth, after both *in vitro* organ culture as well as transplantation under a tooth cavity *in vivo*, showing penetration of blood vessels and nerve fibers. Our method provides a substantial advance in the development of bioengineered organ replacement strategies and regenerative therapies.

The approaches that have been adopted in regenerative medicine are influenced by our understanding of embryonic development, stem-cell biology and tissue-engineering technology[1–3]. To restore the partial loss of organ function, stem cell transplantation therapies have been developed[1–3]. The ultimate goal of regenerative therapy, however, is to develop fully functioning bioengineered organs that can replace lost or damaged organs after disease, injury or aging[1–3]. Almost all organs arise from the organ germ, which is induced by the reciprocal interactions between the epithelium and mesenchyme in the developing embryo[4–7]. Therefore, it has been suggested that to properly reproduce the developmental process of organogenesis, it will be necessary to fully reconstitute these events in an artificially bioengineered organ[7].

The purpose of our study was to improve bioengineering methods for three-dimensional organ germs using completely dissociated epithelial and mesenchymal cells. For this purpose, we adopted the tooth and whisker follicle germs as model ectodermal organs. Although previous studies have demonstrated three-dimensional reconstruction of an artificial organ germ from dissociated single cells *in vitro*, improvement in bioengineering technology is needed before reconstitution of a primordial organ precisely replicates tooth organogenesis as observed in embryonic development[7–11]. The first step in multicellular aggregation of epithelial and mesenchymal cells is multicellular assembly by self-reorganization in each cell type through cell movement and selective cell adhesion until the cells reach an equilibrium configuration[12]. Next the reciprocal interactions between epithelial and mesenchymal cell layers initiate organogenesis and regulate differentiation and morphogenesis[5,6]. The cell potential for self-reorganization and tissue reconstitution, however, is different among cell types of various organs[10]. Here we describe a bioengineered organ germ method with cell compartmentalization *in vitro*, which is applicable to not only *in vitro* organ culture but also *in vivo* transplantation. Our model improves our understanding of the principles by which organ reconstitution can be achieved with tissues that have been bioengineered *in vitro* and increases the potential for the use of bioengineered organ replacement in future regenerative therapies.

We first investigated the possibility of developing a bioengineered tooth germ using completely dissociated single cells from epithelial and mesenchymal tissues of incisor tooth germ at cap stage from the lower jaw in ED14.5 mice (**Fig. 1a**, **Supplementary Methods** online and **Supplementary Fig. 1** online). The explants reconstituted by epithelial or mesenchymal cells alone generated keratinized oral epithelium-like structures or bone, respectively, but not a complete tooth (**Supplementary Fig. 1**). The explants that reconstituted the cell compartmentalization between epithelial and mesenchymal cells at a low-cell density ($0.5–1 \times 10^8$ cells/ml) or that did not form cell compartmentalization at high-cell density (5×10^8 cells/ml), also could not generate a correct tooth structure (**Supplementary Fig. 1**). To reconstitute a bioengineered tooth germ with the correct cell compartmentalization between epithelium- and mesenchyme-derived single cells, we injected the cells in turn at high cell density (5×10^8 cells/ml) into adjacent regions within a collagen gel drop (**Fig. 1a** and **Supplementary Methods**). Within 1 d of organ culture, we observed formation of a tooth germ with the appropriate compartmentalization between epithelial and mesenchymal cells and cell-to-cell compaction (**Fig. 1b**). We then performed transplantations of this bioengineered tooth germ into subrenal capsules in mice and over a 10-d period observed by histology that this primordium could generate plural incisors, in which tissue elements such as odontoblasts, dentin, dentinal tubules, ameloblasts, enamel, Tomes' process, dental pulp, root, blood vessels, alveolar bone and periodontal ligaments, were arranged appropriately when compared with a natural tooth (**Fig. 1c**, **Supplementary Fig. 1** and **Supplementary Fig. 2** online). We also found that this occurred with a frequency of 100% in 50 separate transplants. *In situ* hybridization analysis of the reconstituted teeth showed mRNA for dentin sialoprotein, amelogenin and periostin, specific markers for odontoblasts, ameloblasts and periodontal ligaments, respectively (**Supplementary Fig. 1**). We next examined the origin of the cell types derived from epithelium or mesenchyme using bioengineered tooth germ from GFP-transgenic mice. GFP-transgenic mouse–derived mesenchymal cells generated

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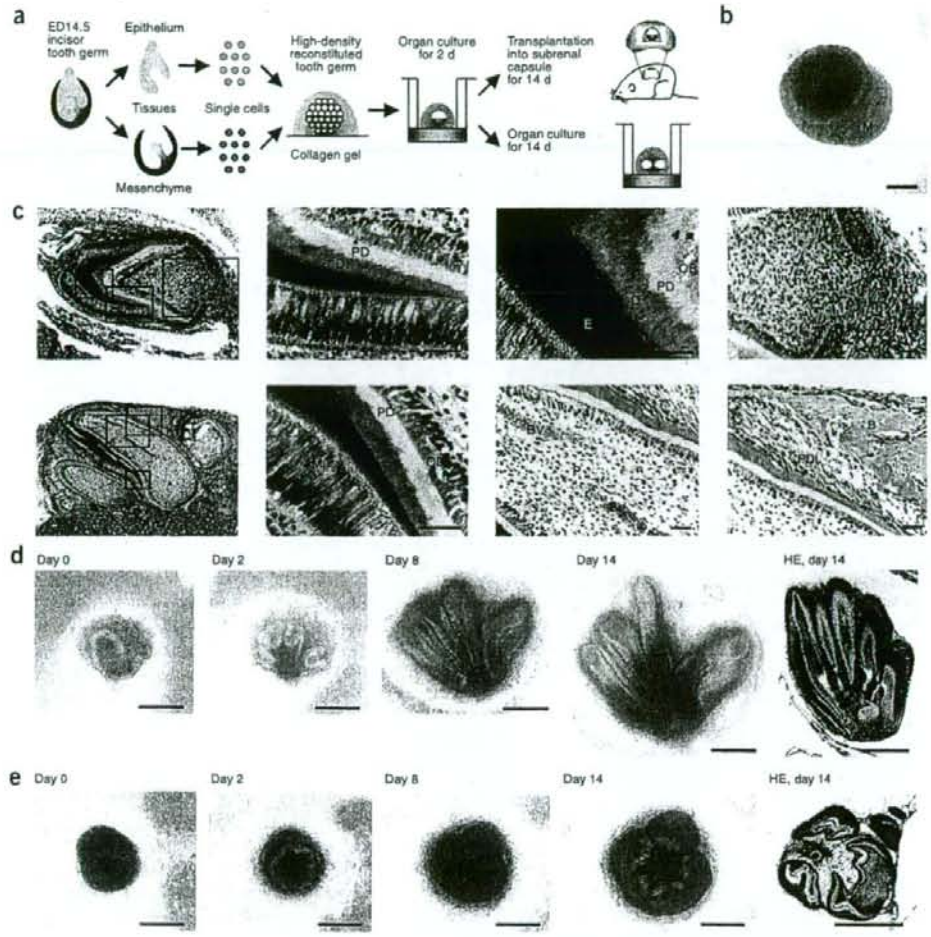

Figure 1 | Generation of a whole tooth using bioengineered tooth germ derived from dissociated single cells *in vivo*. (**a**) Schematic of the bioengineering technology used for the generation of a reconstituted tooth germ. The epithelial and mesenchymal tissues isolated from incisor tooth germ of ED14.5 mice were completely dissociated into single cells. The bioengineered incisor tooth germ was then reconstituted using these dissociated cells that showed cell compartmentalization at a high cell density. The explants were either transplanted beneath a subrenal capsule or were continuously cultured. (**b**) Phase-contrast image of bioengineered incisor tooth germ after 1 d of cultivation. E, epithelial cells; M, mesenchymal cells. Scale bar, 250 μm. (**c**) Histological analysis of the reorganized tooth germ under a subrenal capsule for 10 d (top) and 14 d (bottom) after transplantation. AM, ameloblasts; B, alveolar bone; BV, blood vessels; PD, pre-dentin; D, dentin; E, enamel; OD, odontoblasts; P, pulp cells ; PDL, periodontal ligaments. Scale bars, 50 μm. (**d**,**e**) Time course images of a bioengineered incisor and molar tooth germ in an *in vitro* organ culture. Bioengineered incisor (**d**) and molar (**e**) tooth germ were reconstituted between dissociated epithelial and mesenchymal cells isolated from each incisor and molar tooth germ of ED14.5 mice, respectively. Multiple induction of primordia can be observed by phase contrast microscopy (IX70 Olympus). Bioengineered incisor and molar that had been cultured for 14 d were also analyzed by hematoxylin-eosin staining (HE). Scale bars, 500 μm.

not only odontoblasts and dental pulp derived from dental papilla, but also alveolar bone and periodontal ligaments, which are derived from dental follicles (**Supplementary Fig. 1**). GFP-transgenic mouse–derived epithelial cells generated ameloblasts (**Supplementary Fig. 1**). We also determined that bioengineered tooth germ reconstituted with epithelial and mesenchymal cells isolated from incisor tooth germ at bell stage in ED16.5 mice could not develop into teeth (data not shown). This result indicates that the developmental stage of the tooth germ is critical for the application of this method.

We next examined whether our bioengineered tooth germ had the capability of generating teeth via *in vitro* organ cultures (**Supplementary Methods**). Notably, our experiments indicate that bioengineered tooth germ successfully develops teeth *in vitro*, and that these structures have correctly placed mineralized tissue and cell types (**Fig. 1d,e** and **Supplementary Fig. 2**). Using

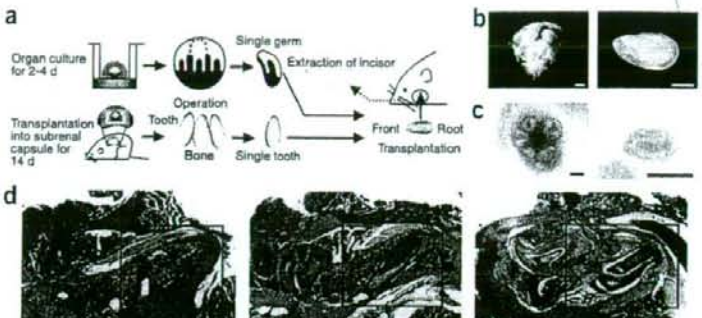

Figure 2 | Development of the bioengineered incisor in the tooth cavity of adult mice. (**a**) Schematic representation of the transplantation procedure. A bioengineered incisor developed under a subrenal capsule and reconstituted incisor tooth germ cultured for 2 d were each separated surgically into single primordia. These explants were then transplanted into a tooth cavity generated by the extraction of a mandibular incisor from the vestibular surface of an adult mouse. (**b**) Representative phase contrast images showing a bioengineered incisor developed in a subrenal capsule environment for 14 days (left) and a tooth separated from reconstituted tissue in the subrenal capsule and used for transplantation (right). Scale bars, 500 μm. (**c**) Separation of individual primordia (dotted circle) from bioengineered tooth germ that had been cultured for 2 d. Scale bars, 250 μm. (**d**) Histological analysis of the explants at 14 d after transplantation into a tooth cavity. Images from the control experiment (left) and transplants isolated from a single incisor primordium (center) and a single tooth developed in the subrenal capsule (right) are shown. Boxes indicate the area shown at a higher magnification in the lower panels. Scale bars, 1 mm.

time-course images, we observed that both incisor and molar-derived bioengineered tooth germ reconstituted using dissociated epithelial and mesenchymal cells from the tooth germ of an incisor or a molar, respectively, gave rise to multiple primordia from the periphery of the boundary surface between epithelial and mesenchymal cells after 2–3 d of growth. Thereafter we observed development of plural teeth containing natural tooth materials and correct cell placement from each primordium (**Fig. 1d,e**). Notably, in the tooth germ cultures, the epithelial cells invaginated and multiplied prominently in the mesenchymal aggregates, in combination with cells isolated from normal and GFP-transgenic mice (**Supplementary Fig. 1**). Furthermore, the development of chimeric bioengineered tooth germs, prepared with epithelial and mesenchymal cells of normal and GFP-transgenic mice in various conditions, clearly demonstrated that the chimeric primordia were reconstituted by completely dissociated single cells (**Supplementary Fig. 1** and **Supplementary Note** online).

Direct cell-to-cell communication induced by high cell density and cell compartmentalization is essential in tooth organogenesis[13] and possibly that of other organs. Beta-1 integrin, CD29, is expressed at the boundary surface between epithelial and mesenchymal cells[14]. Treatment of dissociated epithelial and mesenchymal cells by neutralizing CD29 monoclonal antibodies inhibited tooth formation in a subrenal capsule assay at a frequency of 90% (9/10; **Supplementary Methods** and **Supplementary Fig. 1**) and the expression of dentin sialoprotein and amelogenin mRNA could not be detected (data not shown). These results suggest that CD29 is essential for the epithelial-mesenchymal interactions involved in tooth organogenesis. Hence, our present method is also a useful model system for the analysis of molecular mechanisms that function in organogenesis.

We next examined whether a bioengineered primordial organ replicates the tooth organogenesis observed in embryonic development[4–6]. We analyzed the expression profile of the gene networks that regulate the early development of our bioengineered tooth germ into multiple teeth (**Fig. 1d,e**). The multiple enamel knots were induced by epithelial-mesenchymal cell interactions in our bioengineered tooth germ (**Supplementary Note** and **Supplementary Fig. 3** online). The spatial delineation of the enamel knots in the bioengineered tooth germ is regulated by the expression of both ectodin, belonging to the Dan/Cerberus family of secreted BMP antagonists and ectodysplasin receptor (Edar; **Supplementary Note** and **Supplementary Fig. 3**). The expression of patched homolog 1, *Ptch1*, and the growth arrest–specific gene, *Gas1*, also regulates the regionalization of the odontogenic mesenchyme during development in our bioengineered tooth germ (**Supplementary Note** and **Supplementary Fig. 3**). These observations are

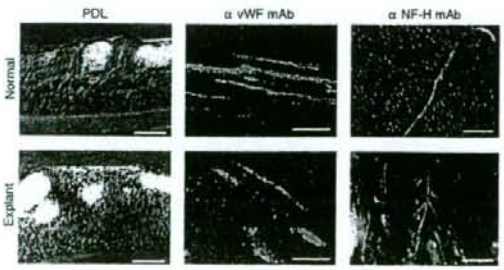

Figure 3 | Analysis of periodontal ligaments, endothelial cells of tooth blood vessels and neural filaments in a bioengineered tooth. Incisors isolated from 3 day-post-birth normal mice (normal; top) and a bioengineered tooth (explant; bottom) are shown. Periodontal ligaments (PDL) were morphologically analyzed by haematoxylin-eosin staining (left), and the endothelial cells of blood vessels (vWF mAb; center) and neurons (NF-H mAb; right) were analyzed by immunoreactivity with specific antibodies for von Willebrand factor (vWF) and neurofilament-H (NF-H), respectively. Fluorescence and differential interference contrast (DIC) images were merged. Scale bar, 50 μm.

evidence that our present tooth germ model reproduces the interaction between epithelial and mesenchymal cells in early tooth organogenesis. These results also suggested that the cell compartmentalization, which mimics multicellular assembly and equilibrium configuration between epithelial and mesenchymal cells, is effective for the initiation of organogenesis in an artificially bioengineered organ primordium.

To examine the potential application of this bioengineering technique to the reorganization of other ectodermal organs, we examined the reconstitution of epithelium- and mesenchyme-derived single cells isolated from whisker follicles of ED14.5 mice with positive results (**Supplementary Note** and **Supplementary Fig. 4** online).

Finally, we examined if either a bioengineered tooth germ or a developing tooth in a subrenal capsule could be successfully transplanted and redevelop in a tooth cavity after the extraction of a mandibular incisor in an 8-week-old adult mouse (**Fig. 2a** and **Supplementary Methods**). We allowed bioengineered teeth to develop in a subrenal capsule for 2 weeks and bioengineered tooth germ to develop in organ culture for 2 d. We dissected individual teeth and primordia from each culture and determined their mean length to be 1.5 ± 0.3 mm (**Fig. 2b**) and 250 ± 50 μm (**Fig. 2c**), respectively. The individual primordia could generate a single tooth after 14-d culture in a subrenal capsule or *in vitro* (**Supplementary Fig. 5** online). Just after dissection, we engrafted these individual bioengineered teeth or primordia into the tooth cavities in a cusp-to-root direction. In a nontransplant control experiment, we could not detect the incisor in the cavity, and we observed prominent ossification and cell infiltration (**Fig. 2d**). At 14 d after transplantation both the single primordia isolated from cultured tooth germ and the single teeth isolated from explants in the subrenal capsules developed in the tooth cavities and formed a correct tooth structure comprising enamel, dentin, root, dental pulp, blood vessels and bone by histological observations at frequencies of 17/22 and 23/27, respectively (**Fig. 2d**). The lengths of each of the bioengineered teeth developed from either tooth or tooth germ were found to be 1.6 ± 0.3 mm (1.1-fold increase in length) or 2.0 ± 0.5 mm (8.0-fold increase in length), respectively. Moreover, the transplantation of explants reconstituted in combination with normal epithelial cells and GFP-transgenic mouse–derived mesenchymal cells clearly demonstrated that the bioengineered tooth had developed from the explants in the cavity (**Supplementary Fig. 5** online).

Periodontal ligaments could also be partially observed in the areas around the dentin in the explants (**Fig. 3**). Blood vessels and nerve fibers were also detectable in the pulp of not only an incisor isolated from 3 day-post-birth mice (normal) but also the developing bioengineered tooth (explant) in the oral cavity by immunohistochemical analysis with antibodies against von Willebrand factor and neurofilament-H, respectively (**Fig. 3**). These results indicate that both bioengineered tooth germ and teeth reconstituted by single cell-processing are transplantable, and the bioengineered tooth germ can develop a normal tooth with a complete structure. Furthermore, these data also strongly suggest that the replacement of biological and functional teeth is possible by reconstitution in the tooth cavity of an adult animal.

It has been expected that reconstitution of an entire organ from a single cell would be required to allow regenerative therapy in addition to stem cell transplantation[1–3]. Our reconstituted tooth germ generates a complete and entirely bioengineered tooth, not only in *in vitro* organ cultures, but also in a tooth cavity *in vivo* after the extraction of a mandibular incisor followed by the transplantation of either early primordia or a tooth that had partially developed in a subrenal capsule. This study thus provides the first evidence of a successful reconstitution of an entire organ via the transplantation of bioengineered material. Our results therefore make a substantial contribution to the development of bioengineering technologies and the future reconstitution of primordial organs *in vitro*. Our present findings should also encourage the future development of organ replacement by regenerative therapy.

Note: Supplementary information is available on the Nature Methods website.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

K.N. was involved in each of the experiments described in this study. R.M. analyzed the explants that were formed by a combination of normal and GFP-transgenic mouse-derived cells and performed the immunohistochemical analysis shown in **Figure 3**. Y.S. performed and analyzed the transplantation experiments in both the subrenal capsule and the tooth cavity. K.I. and M.S. performed the *in situ* hybridization analysis. Y.T. performed the subrenal capsule transplantation experiments and histological analysis. M.O. maintained the *in vitro* organ cultures and performed whole-mount analysis of the chimeric bioengineered tooth germ. K.N. and T.T. prepared the manuscript. K.N., M.S., Y.T. and T.T. discussed the results and also contributed to the preparation of this manuscript. T.T. designed the experiments.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Supplementary Information

Supplementary Notes

Analysis of the Development of a Chimeric Bioengineered Tooth Germ in *in Vitro* Organ Culture. We investigated whether our current bioengineered tooth germ is reconstituted by completely dissociated single cells from both epithelial and mesenchymal tissues of incisor tooth germ. We developed a chimeric bioengineered tooth germ analysis using the single cells dissociated from tooth germs of normal and GFP-transgenic mice. Each dissociated cells of epithelial and mesenchymal cells of normal or GFP-transgenic mice were prepared as described (see **Supplementary Methods**). At first, we analyzed the development of the chimeric bioengineered tooth germ reconstituted between normal mice-derived epithelial cells and the mixed mesenchymal cells isolated from normal and GFP-transgenic mice with cell compartmentalization at high-cell density. The mesenchymal cells were premixed with normal and GFP-transgenic mice-derived cells at the chimerism of 95% and 5%, respectively (see **Supplementary Fig. 1**). Whereas the green fluorescence could not be detected in epithelial cell-derived ameloblast, the fluorescence was observed and distributed in dental mesenchymal-derived cell types, pulp and odontoblast. The number of GFP-positive cells correlated to the chimerism between normal and GFP transgenic mice-derived cells in this chimeric tooth germ (data not shown). Furthermore, we also examined the chimeric germ reconstituted between epithelial and mesenchymal cells each containing of equally number of normal and GFP-transgenic mice-derived single cells with cell compartmentalization at high-cell density (see **Supplementary Fig. 1**). GFP-positive cells largely distributed in the bioengineered tooth according to the chimerism and detected in the all cell types of ameloblast, pulp and odontoblast. These results indicate that our current tooth germ method successfully reconstituted by single cell manipulation.

Multiple Tooth Induction in a Bioengineered Incisor Tooth Primordium by Gene Expressions of Signalling Network. We analyzed the expression profile of signalling networks that play essential roles both in early tooth development and in morphogenesis[1, 2]. The expression of the enamel knot marker genes[3], Shh and Wnt10b, were detectable in plural sites and at the boundary surface between epithelial and mesenchymal cells in our bioengineered incisor tooth germ (see **Supplementary Fig. 3**). Activin βA and Fgf3 transcripts were also mainly detectable in the mesenchyme that had formed in the region adjacent to the epithelial Shh-expression site (see **Supplementary Fig. 3**). Msx1 transcript was additionally observed throughout the entire mesenchyme, as seen in normal tooth germ (see **Supplementary Fig. 3**).

Ectodin inhibits the expression of p21, through its antagonistic effects upon BMP4 signalling and is critical for the spatial delineation of enamel knots and cusps[4, 5, 6]. Consistent with this, Ectodin and p21 were found to be inversely expressed at the boundary surfaces between the epithelial and mesenchymal cells (see **Supplementary Fig. 3**). Furthermore, the signalling between ectodysplasin (Eda) and its receptor, Edar, is thought to induce enamel knot formation[7] and the expression pattern of Edar was identical to those of Shh and p21 in the reconstituted tooth germ (see **Supplementary Fig. 3**).

The Shh signalling network molecules, patched 1 (Ptc1) and growth arrest-specific gene (Gas1) are thought to regulate the regionalization of the odontogenic mesenchyme in the mandibular arch[8]. Ptc1 and Gas1 transcripts were detectable in the proximal and distal mesenchyme, respectively, of the boundary surface between the epithelium and mesenchyme, and the strong expression of Ptc1 transcripts could be observed in the regions adjacent to the enamel knot (see **Supplementary Fig. 3**). These observations are clearly indicated the evidences that our current tooth germ model reproduce the interaction between epithelial and mesenchymal cells in early tooth organogenesis.

Generation of a Reconstituted Whisker from a Bioengineered Follicle. We investigated whether it would be possible to a bioengineered mouse whisker using our developed bioengineering technology for the reconstitution of artificial primordial organs. Tissues containing whisker follicles were dissected from the cheeks of ED14.5 mice and the epithelial and mesenchymal tissues were completely dissociated to single cells via the same enzymatic method used for tooth germ regeneration (see **Supplementary Fig. 1**). Epithelial and mesenchymal cells were also reconstituted with cell compartmentalization at a high-cell density in a collagen gel, and the bioengineered whisker follicle was transferred to a cell culture insert. After one day of incubation, the explant was then transplanted into a subrenal capsule for 14 days (see **Supplementary Fig. 4**). At post-transplantation day 14, the explants in the subrenal capsule were found to regenerate a whisker at a 100% frequency (20/20; see **Supplementary Fig. 4**). Histological analysis of these explants also revealed the expected tissue morphologies, such as the whisker shaft (ws), inner root sheath (irs) and outer root sheath (ors). Moreover, these tissues were arranged normally, when compared with a natural whisker (see **Supplementary Fig. 4**).

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Supplementary Methods

Animals

C57BL/6 mice were purchased from CLEA Japan Inc (Tokyo, Japan). C57BL/6-TgN (act-EGFP) OsbC14-Y01-FM131 mice were obtained from RIKEN Bioresource Center (Tsukuba, Japan). Mouse care and handling conformed to the NIH guidelines for animal research. All experimental protocols were approved by the Tokyo University of Science Animal Care and Use Committee.

Reconstitution of bioengineered primordial organs from single cells

Tooth germs derived from incisors or molars were dissected from the mandibles of ED14.5 mice. The extra tissues surrounding of tooth germ should be carefully removed. The purity of tooth germ cells affect to the frequency of tooth formation. Isolated tooth germs were incubated in 1.2 U/ml dispase II (Roche, Mannheim, Germany) and 20 U/ml DNase I (Takara Bio, Shiga, Japan) for 12.5 min at room temperature. The epithelial and mesenchymal tissues were separated using a fine needle. The epithelial tissues were treated twice at 37°C for 20 min in 100 U/ml collagenase I (Worthington, Lakewood, NJ)-PBS(-), then in Ca^{2+}- and Mg^{2+}-phosphate-buffered saline (PBS(-)) supplemented with 0.25% trypsin (Sigma, St. Louis, MO) and 20 U/ml DNase I (Takara Bio) for 5 min at 37°C, and dissociated into single cells by gentle pipetting. Single cells of mesenchymal origin were also prepared by treatment with PBS(-) supplemented with 0.25% trypsin (Sigma), 50 U/ml collagenase I, and 20 U/ml DNase I (Takara Bio) at 37°C for 10 min. Each epithelial and mesenchymal cells were precipitated by centrifugation in a siliconised microtube and the supernatant was removed completely using a GELoader Tip 0.5-20 µl (Eppendorf, Hamburg, Germany). The cell density of the precipitated epithelial and mesenchymal cells after the removal of supernatants reached at the concentration of 5 x 10^8 cells/ml. We defined the cell concentration (5 x 10^8 cells/ml) as a "high-cell density". To prepare bioengineered primordial organs with the correct cell compartmentalization between the epithelial and mesenchymal cells at a high-cell density (5 x 10^8 cells/ml), the precipitated mesenchymal cells were mixed without further dilution and injected (0.2 µl) using 0.1-10 µl pipette tip (Molecular Bio Products, San Diego, CA) into a 30 µl gel drop of Cellmatrix type I-A (Nitta gelatin, Osaka, Japan), which is acid-soluble collagen isolated from tendon of pig, formed on a siliconised dish. Epithelial single cells were then mixed without further dilution and injected (0.1-0.2 µl) into an area adjacent to the mesenchymal cell aggregate to enable direct cell contact (Fig. 1a). To prepare bioengineered tooth germ without cell compartmentalization between the epithelial and mesenchymal cells at a high-cell density (5 x 10^8 cells/ml), the cell suspensions of epithelial and mesenchymal cells were premixed and precipitated. The cell precipitate were mixed and injected (0.3-0.4 µl) into a collagen gel drop. The bioengineered tooth germs were incubated for 5 min at 37°C, placed on a cell culture insert (0.4 µm pore diameter; BD, Franklin Lakes, NJ), and the explants were then incubated at 37°C in a humidified atmosphere of 5% CO_2. For the neutralization of CD29, each epithelial and mesenchymal cell preparation were preincubated with 100 µl of 50 µg/ml of an anti-CD29 monoclonal antibody (αCD29 mAb; clone 9EG7, BD, San Diego, CA) in PBS(-) in the absence of NaN_3. The reconstituted tooth germ was prepared in collagen gel containing 50 µg/ml of αCD29 mAb. The explants were then subjected to a subrenal capsule assay as described below.

Organ cultures

Reconstituted explants were cultured for 2 days for up to 14 days on cell culture inserts in 12-well cell culture plates (BD) containing 380 µl/well Dulbecco's modified eagle medium (D-MEM; Sigma) supplemented with 10% FCS (JRH), 100 µg/ml ascorbic acid (Sigma), 2 mM L-glutamine (Sigma). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and the culture medium was changed at 2-day intervals.

Subrenal Capsule Assays

After 2 days cultivation, the reconstituted germs of tooth or whisker follicles were transplanted into a subrenal capsule for 14 days using 8 week-old male mice as the host, according to the method of Bogden and co-workers[1].

Tissue Preparation and Immunohistochemistry

The tissues were removed and immersed in 4% paraformaldehyde in PBS(-). After fixation, the tissues were decalcified in 4.5% EDTA (pH 7.4) for 1-10 days at 4°C. To store frozen samples, the specimens were immersed in a series of graded sucrose solutions and embedded in Tissue-Tek O.C.T. (Sakura-Finetek USA, Torrence, CA). For immunohistochemistry, the primary antibodies used were anti-von Willibrand factor (αvWF pAb; 1:100, Chemicon, Temecula, CA)[2] and anti-neurofilament H (αNF-H pAb; 1:100, Chemicon)[3] polyclonal antibodies. Immunoreactivity was detected using fluorescence-conjugated goat anti-rabbit IgG (1:200, Chemicon). The sections were observed using an Axio Imager A1 (Carl Zeiss, Jena, Germany) with an AxioCAM MRc5 (Carl Zeiss) and processed with AxioVision software (Carl Zeiss). Fluorescent images were acquired using an Axiovert 200M (Carl Zeiss) with an AxioCAM MRm (Carl Zeiss).

In Situ Hybridization Analysis

In situ hybridizations were performed using 10 µm frozen sections. Digoxgenin-labelled probes for specific transcripts were prepared by PCR with primers designed using published sequences (GenBank Accession Numbers; Shh: NM009170, Msx1: NM010835, Fgf3: NM008007, Wnt10b: NM011718, Activin βA: NM008380, Periostin: NM015784, Amelogenin: NM009666, Dspp: NM010080, Ectodin: NM025312, Edar: NM010100, p21: NM007669, Ptc1: NM008957, Gas1: NM008086). After hybridization, the expression patterns for each mRNA were detected and visualized by immunoreactivity with an anti-digoxigenin alkaline phosphatase-conjugated Fab fragments (Roche), according to the method of Iseki and co-workers[4].

Transplantation

After incubation, reconstituted explants were separated into the individual primordium using a fine needle. The mandibular bones around the right incisors of 8 week-old adult male mice were surgically removed and the right incisors were then

extracted under deep anaesthesia. After 3 days, the vestibular surface of the right mandibular ramus was surgically exposed also under deep anaesthesia. An incision of about 8 mm in length was then made through the skin with a fine scissors to access the muscle layer, according to an imaginary line joining the auditory meatus to the lip commissure. The fibres of the masseter were separated along their longitudinal axis with a scalpel blade. The underlying bony surface of the ramus was then exposed and a scalpel blade was used to create a hole through the alveolar bone. The bony window was placed approximately 2 mm anterior to the posterior border of the ramus and slightly superior to the bony elevation at the apical end of the incisor. A sample was then inserted through the bony window. The animals were then sutured and the surgical site was cleaned.

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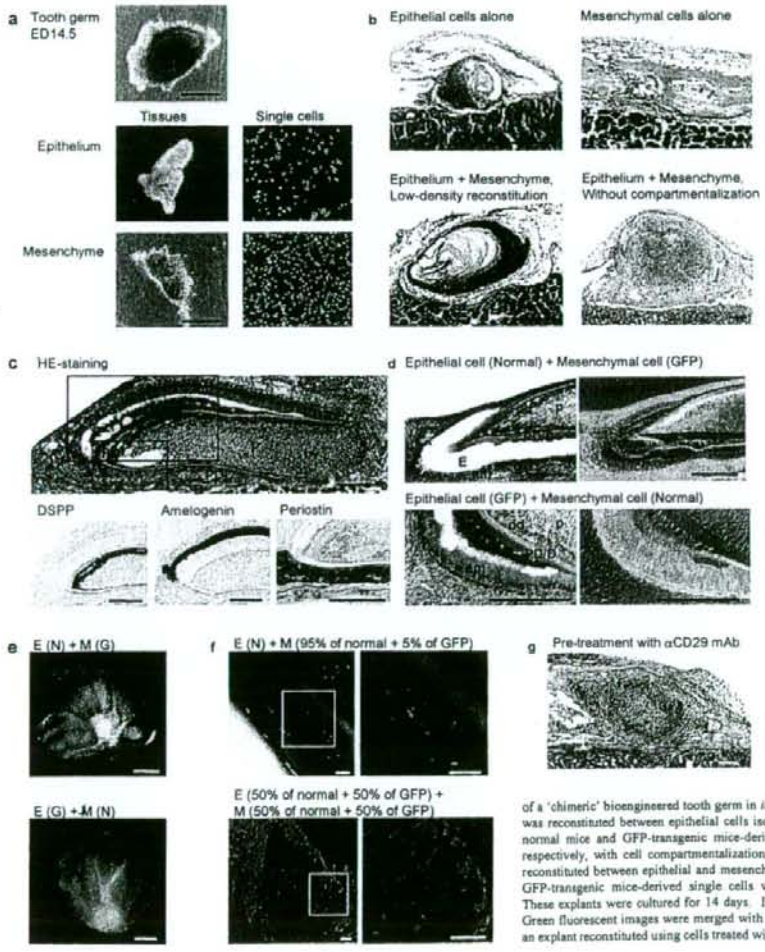
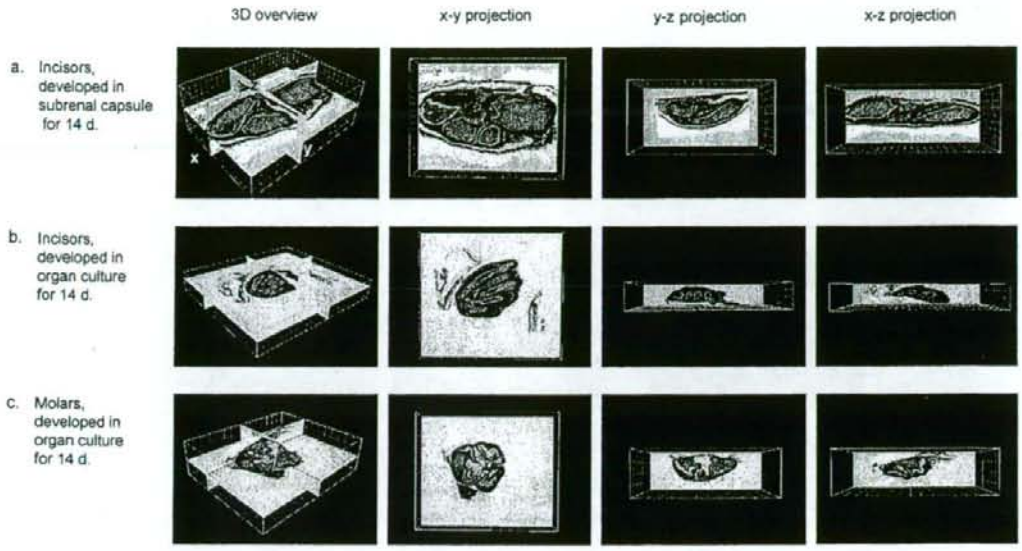
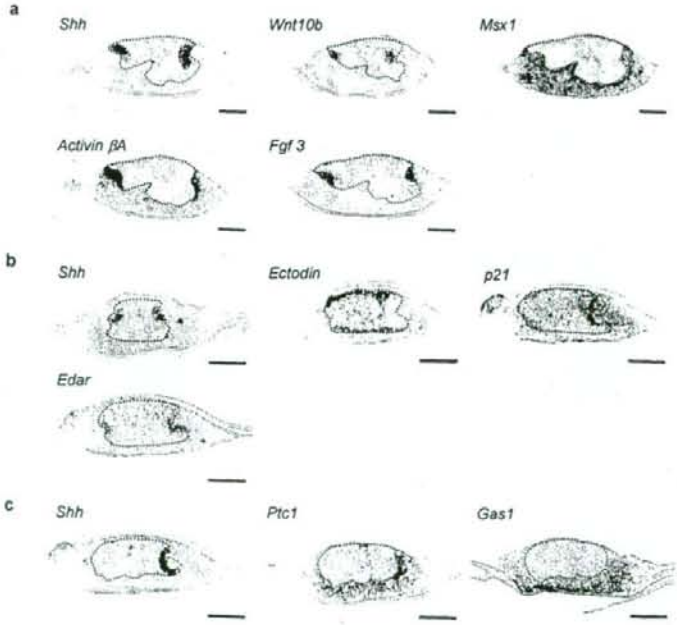


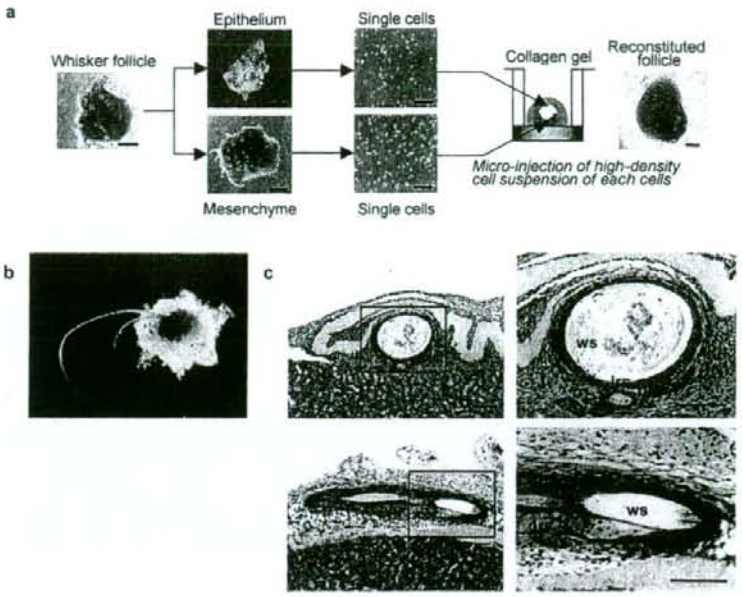
Supplementary Fig. 1 Effects of cell density and cell compartmentalization between epithelial and mesenchymal cells upon the generation of bioengineered teeth. (**a**) Phase contrast images of tooth germ, tissues and dissociated single cells as described (see **Supplementary Methods**. Scale bar, 200 μm. (**b**) Histological analysis of the explants under various conditions. The reconstituted tooth germs were prepared from epithelial cells only (upper left), mesenchymal cells only (upper right), a combination of epithelial and mesenchymal cells showing compartmentalization at a low-cell density (lower left) and a combination of epithelial and mesenchymal cells in the absence of compartmentalization at a high-cell density (lower right). For the preparation of a low-density reconstituted structure, the cells were suspended in medium of four volumes to the packed cell volume. To prepare tooth germ in the absence of compartmentalization between the epithelial and mesenchymal cells, a equal number of each cell type was premixed, followed by reconstitution. The reconstituted germs were grown for 2 days by *in vitro* organ culturing and then subjected to the subrenal capsule assay. Scale bar, 200 μm. (**c**) Histological analysis of the expression of typical differentiation markers for ameloblasts, odontoblasts and periodontal ligaments in a bioengineered incisor at post-transplantation day 14 in the subrenal capsule. The regenerated tooth displays normal cellular and tissue components in the correct location, including ameloblasts (am), odontoblasts (od), pulp cells (p), blood vessels (bv), enamel (E), dentin (D), pre-dentin (PD), periodontal ligaments (PDL) and alveolar bone (B). Gene expression patterns were detected by *in situ* hybridization of sequential sections of bioengineered incisor. Scale bar, 250 μm. (**d**) Analysis of the cell types that have differentiated from the epithelial and mesenchymal cells in the bioengineered incisor tooth germ. The origins of the epithelial and mesenchymal cells are indicated above the panels. Green fluorescent images were merged with DIC images. The abbreviations used are identical to those shown in (**c**). Scale bar, 250 μm. (**e**) Wholemount analysis of bioengineered tooth germ, which was reconstituted using a combination of epithelial (E) or mesenchymal cells (M) isolated from normal mice (N) or GFP-transgenic mice (G), and cultured for 4 days. The origins of the epithelial and mesenchymal cells are indicated above each panel. Green fluorescent images were merged with those of DIC. Images were acquired using a SteREO Lumor V12 (Carl Zeiss). Scale bar, 200 μm. (**f**) Analysis of development of a 'chimeric' bioengineered tooth germ in *in vitro* organ culture. The chimeric bioengineered tooth germ was reconstituted between epithelial cells isolated from normal mice and mesenchymal cells mixed with normal mice and GFP-transgenic mice-derived mesenchymal cells at the chimerism of 95% and 5%, respectively, with cell compartmentalization at high-cell density (upper). The chimeric tooth germ was reconstituted between epithelial and mesenchymal cells each containing of equally number of normal and GFP-transgenic mice-derived single cells with cell compartmentalization at high-cell density (lower). These explants were cultured for 14 days. Images were acquired using a LSM 510 META (Carl Zeiss). Green fluorescent images were merged with those of DIC. Scale bar, 50 μm. (**g**) Histological analysis of an explant reconstituted using cells treated with CD29 antibodies. Scale bar, 200 μm.



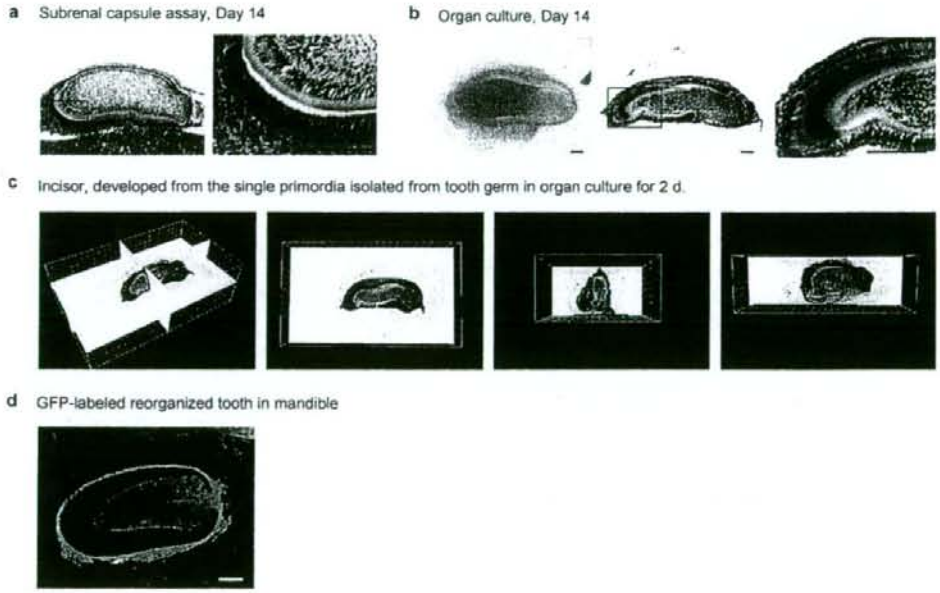
Supplementary Fig. 2 Three-dimensional histological analysis of bioengineered teeth under various developmental conditions. Bioengineered incisors developed in a subrenal capsule (a), via *in vitro* organ culturing (b), bioengineered molars developed via *in vitro* organ culturing (c) were sectioned and stained with haematoxylin-eosin. The sections were then observed by an Axio Imager A1 (Carl Zeiss, Jena, Germany) with an AxioCAM MRc5 (Carl Zeiss) and processed with AxioVision software (Carl Zeiss). Serial images were compiled and aligned using an LSM 5 Image Browser (Carl Zeiss) and AutoAligner (Bitplane AG, Zurich, Switzerland), and analyzed using Imaris 4 software (Carl Zeiss). A three-dimensional overview, x-y projection, y-z projection and x-z projection are demonstrated.



Supplementary Fig. 3 Expression of the regulatory genes that function during early tooth development in a bioengineered incisor tooth germ. Gene expression in bioengineered incisor tooth germ that had been cultured *in vitro* for 2 days was detected by *in situ* hybridization. Dotted lines indicate the interface between the epithelial and mesenchymal cells and the inside of the lined areas indicate the epithelial cell aggregates. Scale bar, 200 μm. (a) Expression analysis of enamel knot and mesenchymal cell markers. Shh, Wnt10b, Msx1, Activin βA and Fgf3 transcripts are detectable in sequential sections. (b) *In situ* hybridization analysis of genes associated with the induction of the enamel knot in bioengineered tooth germ. mRNAs for Shh, Ectodin, p21 and Edar were analyzed in sequential sections of the explant. (c) Analyses of Shh-signaling related genes that function in the regionalization of odontogenic and non-odontogenic mesenchyme. Ptc1 and Gas1 transcripts are detectable in sequential sections of reconstituted tooth germ.



Supplementary Fig. 4 Generation of a reconstituted whisker *in vivo* from a bioengineered follicle. (a) Schematic representation of the bioengineering method employed for the reconstitution of a whisker follicle *in vitro*. Scale bar, 250 μm. (b) Whole mount analysis of a bioengineered whisker following a subrenal capsule transplant for 14 days. Scale bar, 250 μm. (c) Histological analysis of a bioengineered whisker following a subrenal capsule transplant for 14 days. Abbreviations: ws, whisker shaft; irs, inner root sheath; ors, outer root sheath. Scale bar, 100 μm.



Supplementary Fig. 5 Development and transplantation of individual primordia. (a) *In vivo* and (b) *in vitro* development of individual primordia, regenerated either in a subrenal capsule or by *in vitro* organ culture, both for 14 days. Boxes indicate the area shown by higher magnification in the right hand panels. Scale bar, 100 μm. (c) Bioengineered single incisor developed from a dissected single primordium via *in vitro* organ culturing for 2 days were sectioned and stained with haematoxylin-eosin. The sections were then observed by an Axio Imager A1 (Carl Zeiss, Jena, Germany) with an AxioCAM MRc5 (Carl Zeiss) and processed with AxioVision software (Carl Zeiss). Serial images were compiled and aligned using an LSM 5 Image Browser (Carl Zeiss) and AutoAligner (Bitplane AG, Zurich, Switzerland), and analyzed using Imaris 4 software (Carl Zeiss). A three-dimensional overview, x-y projection, y-z projection and x-z projection are demonstrated. (d) Transplantation of bioengineered incisor tooth germ reconstituted by a combination of normal and GFP-transgenic mouse epithelial cells and mesenchymal cells. GFP fluorescent and DIC images were merged. Scale bar, 200 μm.

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PRESS RELEASE BY NATURE

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Katherine Anderson, Nature London

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Within this release, a few papers of particular newsworthiness are highlighted, with author contact details, and the rest are listed. Journalists are also given online access to the papers, so they can follow up stories of interest.

A method to regrow teeth

DOI: 10.1038/nmeth1012

Scientists have for the first time successfully replaced natural teeth in mice with teeth that were created in a Petri dish from single cells. The experiment is described online this week in Nature Methods. Takashi Tsuji and colleagues started with the two cell types that develop into a tooth -mesenchymal and epithelial cells. First they grew each cell type separately to get larger quantities of cells and then injected them into a drop of collagen - a substance which 'glues' cells together in an organism. The cells developed into a budding tooth with high efficiency, and when transplanted into the cavity of an extracted tooth in a mouse developed normally and showed the same composition and structure as natural teeth. The authors provide further evidence that this method can be applied to any organ that develops from these cell types by regrowing a follicle that eventually forms a whisker in a mouse.

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ORIGINAL ARTICLE

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Wnt10a regulates dentin sialophosphoprotein mRNA expression and possibly links odontoblast differentiation and tooth morphogenesis

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Abstract We have explored the role of Wnt signaling in dentinogenesis of mouse molar teeth. We found that Wnt10a was specifically associated with the differentiation of odontoblasts and that it showed striking colocalization with dentin sialophosphoprotein (Dspp) expression in secretory odontoblasts. Dspp is a tooth specific non-collagenous matrix protein and regulates dentin mineralization. Transient overexpression of Wnt10 in C3H10T1/2, a pluripotent fibroblast cell line induced Dspp mRNA. Interestingly, this induction occurred only when transfected cells were cultured on Matrigel basement membrane extracts. These findings indicated that Wnt10a is an upstream regulatory molecule for Dspp expression, and that cell–matrix interaction is essential for induction of Dspp expression. Furthermore, Wnt10a was specifically expressed in the epithelial signaling centers regulating tooth development, the primary and secondary enamel knots. The spatial and temporal distribution of Wnt10a mRNA demonstrated that the expression shifts from the secondary enamel knots, to the underlying preodontoblasts in the tips of future cusps. The expression patterns and overexpression studies together indicate that Wnt10a is a key molecule for dentinogenesis and that it is associated with the cell–matrix interactions regulating odontoblast differentiation. We conclude that Wnt10a may link the differentiation of odontoblasts and cusp morphogenesis.

Key words odontoblast · Dspp · Wnt10a · dentinogenesis · tooth

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Introduction

Dentin is one of the three mineralized tissues of the tooth, and it is produced by odontoblasts differentiating from dental papilla mesenchymal cells. Dentin is very similar to bone in its matrix protein composition. However, whereas bone remodels throughout postnatal life and participates in calcium homeostasis, dentin, once formed, does not undergo remodeling. On the other hand, it can respond to injury or stimulation by forming reparative dentin to protect the dental pulp (Linde and Goldberg, 1993). Unlike osteoblast differentiation, the differentiation of odontoblasts is regulated by epithelial–mesenchymal interactions, which instruct both tooth morphogenesis and cell differentiation (Thesleff et al., 1989, 1991; Thesleff and Aberg, 1999). Recombination experiments of the dissociated developing dental