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REVIEW

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Tooth-forming potential in embryonic and postnatal tooth bud cells

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Abstract Humans are genetically programmed to replace their teeth once during childhood. Therefore, when adult teeth are lost or damaged, they cannot be regenerated or regrown. However, with the advancement of stem cell biology and tissue engineering, regenerating the whole tooth has become a realistic and attractive option to replace a lost or damaged tooth, and therefore has strongly attracted attention in the field of dental research. During the past several years, significant progress has been made in this research endeavor, providing greater understanding of the production of an entire biological tooth by tissue engineering using stem cells. There are several ways to reproduce an entire biological tooth. Approaches are categorized according to the cell sources that have the potential to produce teeth. One source is the embryonic tooth bud, and the other is the postnatal tooth bud. The results from embryonic and postnatal tooth buds differ considerably. In particular, the potential to regulate the shape of the tooth crown from embryonic tooth bud is higher than from postnatal tooth bud. This article describes the achievements to date in production of biological teeth, mostly from our laboratory. In particular, we describe the potential to produce teeth from embryonic and postnatal tooth buds.

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Introduction

Teeth are the hardest tissues that develop in the jaw and have several purposes including eating, pronunciation, defense, and esthetics. The loss of teeth because of caries, periodontal disease, or trauma is a relatively common problem among older people. Because adult teeth are not shed and do not regrow, several therapies such as artificial dentition, tooth transplantation, and dental implants are often necessary to recover lost masticatory function. However, at present, complete restoration therapy to compensate for complete tooth loss has not been achieved.

In the early 1990s, Langer and Vacanti conceived a new concept, tissue engineering to produce biological tissues or whole organs by seeding a prefabricated, biodegradable scaffold with specialized cells capable of generating the tissue or organ of interest.[1] Subsequent research has identified the principles for the possible design and fabrication of whole biological teeth.[2–6] In theory, producing biological teeth from the patient's own cells in situ would be an ideal tooth replacement therapy. We have previously reported several techniques for the production of biological teeth by tissue engineering using a cell–scaffold complex.[7–11] Complex tooth structures were reconstructed by seeding dissociated tooth bud cells onto biodegradable scaffolds, which were grown in situ for several weeks. Other approaches such as using cell–cell [12,13] or cell–tissue recombination[6] techniques with cells derived from embryonic tooth tissues are also successful in producing teeth. All studies to date on tissue engineering of teeth indicate that development of biological teeth occurs via a similar process to that of natural teeth. Both processes involve reciprocal interaction between epithelial and mesenchymal progenitor cells.[2] Interestingly, there were significant differences in the results from mouse embryonic tooth bud cells[7] and porcine postnatal tooth

bud cells,[2] although both approaches produced dentin and enamel after transplantation. The differences are predominantly in tooth-crown morphology.

This article mainly reviews the achievements to date in our laboratory on generating biological teeth by tissue engineering. In particular, we focus on the diverse abilities of two types of tooth bud cells obtained from embryonic and postnatal tooth buds. We also discuss the possible future steps required to produce functional biological teeth.

Tooth-crown morphogenesis

Ectodermal organs in vertebrates generally share developmental mechanisms that involve two adjacent tissue layers, epithelium and mesenchyme.[14–17] Tooth development is also regulated by reciprocal inductive signals between ectoderm-derived epithelium and neural crest-derived ectomesenchyme.[18,19] Teeth are initiated with an invagination of a thickening layer from the oral epithelium (Fig. 1A). The initial epithelium consists of two cell types, including a large stratum of mostly columnar cells in contact with the basement membrane and smaller cells in its center. The oral epithelium at this stage has been shown to have the potential to instruct tooth growth and development.[20,21] The transition from bud to cap stages is characterized by the initial development of the cervical loop (Fig. 1B).[22–24] The dental epithelium appears, and the horseshoe-shaped dental lamina generates the future dental arches at the cap stage.

During the cap and bell stages, transient signaling centers are generated in the epithelium, first in the placodes and later in the primary and secondary enamel knots.[14,25] By the early bell stage, the primary enamel knot disappears apoptotically, and secondary enamel knots are generated at the sites of future cusp tips and contribute to the shaping of the tooth crown and its cusps (Fig. 1C).[14] Therefore, it is thought that tooth-crown shape is determined by the number of the enamel knots and the location of enamel knot formation.

Teeth are also characterized by the shape of the tooth crown, for example, incisor or molar, and are determined early in development by spatially restricted expression of homeobox genes in the ectomesenchyme. Mice lacking functional Dlx1 and Dlx2 genes exhibit a phenotype of irregular tooth patterning wherein development of maxillary molar teeth is inhibited but development of all other teeth is normal.[26] Misexpression of Barx1 in presumptive incisor mesenchyme results in a transformation of incisors into molars.[27] Therefore, tooth-crown shapes are regulated

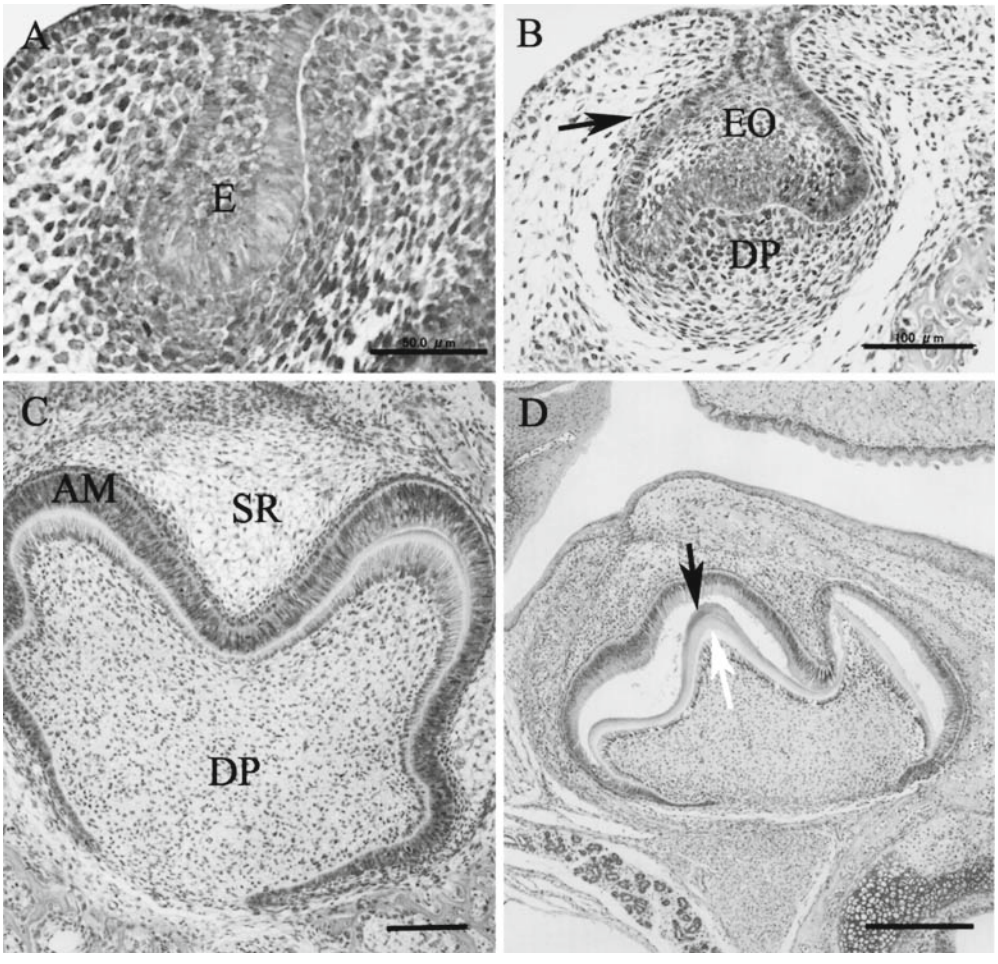

Fig. 1. **A** Tooth bud in the dental lamina stage at embryonic day 13. *E*, dental epithelium. **B** Tooth bud displaying the cap stage at embryonic day 14. The enamel organ (*EO*), dental papilla (*DP*), and dental follicle (*arrow*) together constitute the tooth bud. **C** Tooth bud displaying the bell stage at embryonic at postnatal day 0. During this stage, the tooth crown assumes its final shape. The center of the enamel organ is termed the stellate reticulum (*SR*). *AM*, ameloblasts; *DP*, dental papilla. **D** Tooth bud displaying the crown formation stage at postnatal day 3. Two principal hard tissues of the tooth: the dentin (*white arrow*) and the enamel (*black arrow*). *Bars* **A**, **C** 50 μm; **B**, **D** 100 μm

by specific gene expression patterns. At the crown formation stage, the enamel-secreting ameloblasts and dentin-secreting odontoblasts differentiate from inner enamel epithelium and dental pulp cells, which are derived from the dental papilla, respectively. Both ameloblasts and odontoblasts are subsequently involved in the formation of tooth crowns, which consist of the enamel–dentin complex (Fig. 1D).

Biological tooth engineering

According to a report from the Ministry of Health, Labour and Welfare in Japan in 2005 (http://www.mhlw.go.jp/houdou/2006/06/h0602-2.html), people aged 50–80 years have, on average, lost more than 3 and 18 teeth, respectively. In contrast to other hard tissues such as bone, which are capable of regeneration throughout postnatal life, the relatively static components of teeth do not readily undergo complete regeneration.

During the past two decades, tissue engineering technology has evolved from unrealistic expectations to clinical applications with sound scientific basis. Tissue engineering is based on the idea of placing a prefabricated biodegradable scaffold injected with progenitor/stem cells from the specific organ or tissue required to produce a new organ or tissue.[1] Stem cells are thought to have applications for regeneration of lost tissues and organs in addition to use in treatment of diseases.[28] In dental tissue, several stem cells are found, such as dental epithelial stem cells in rodent incisors,[29] dental pulp stem cells,[30,31] periodontal ligament stem cells,[32] and deciduous stem cells[5] in humans. However, it is unclear whether all stem cells can produre whole biological teeth.

Advances in stem cell biology and tissue engineering technology may allow the regeneration of missing teeth as an alternative to replacement. To date a number of studies have explained advances in the engineering of biological teeth, but these are still not complete procedures because of several unsolved issues. There has been no report on clinical application of these techniques.

The approaches for producing biological teeth so far reported in the literature fall into two main categories: identifying appropriate cell sources and application of scaffolds. In analyzing cell type, it has been reported that two sources of cells, either embryonic or postnatal tooth buds, can be utilized. A number of studies have suggested that both embryonic tooth bud and postnatal tooth bud cells have the potential to be utilized in regeneration of the whole tooth via-tissue engineering. However, very different results have been reported in the experimental use of these two cell types. The effectiveness of scaffolds as the carriers for these cells in the engineering of biological teeth has not yet been elucidated. Our laboratory uses scaffolds in combination with postnatal tooth progenitor cells to achieve biological tooth production. This approach is based on the concept that the scaffold is helpful in supporting the differentiation of progenitor cells with odontogenic induction signals.

Biological tooth engineering from embryonic tooth buds

It is thought that, in mice after day 11, an instruction for tooth initiation, which is capable of directing tooth morphogenesis, is transferred from the oral epithelium to the dental mesenchyme.[20,21,33–36] This evidence has contributed to the development of new techniques for biological tooth production.

Several groups have reported techniques for the production of biological teeth based on embryonic tooth cell–tissue or cell–cell recombination in the kidney capsule.[6,37,38] In 2002, Jung et al. reported the use of constructs from intact dental epithelium in combination with isolated and reaggregated dental mesenchymal cells from mouse teeth at the late bud stage.[37] Biological teeth were produced, and the shape of tooth crowns were similar in appearance to those of natural teeth.[6] Sharpe et al. demonstrated that mesenchymal stem cells isolated from adult bone marrow are capable of forming teeth with a regular tooth-crown shape.[6] This result suggests that embryonic dental mesenchyme can be effectively replaced by adult stem cells to produce teeth. Lesot et al. describe another possible technique.[39] The pellets from dissociated dental epithelial and mesenchymal cell populations at embryonic day 14 (E14) are combined in a culture dish. The constructs are shown to be capable of forming teeth with a similar appearance to natural teeth at the early crown formation stage. Surprisingly, the formation of the crown-cusp pattern was found to be comparable to naturally developed teeth even though the growth environment was in vitro.[39] However, tooth-root formation has never been seen in vitro or in cases of transplantation into a kidney capsule.

The methods of engineering biological teeth using embryonic tooth bud cells that have been described so far have not relied on the use of a scaffold.[6,12,37,39] It has been demonstrated that tissues such as bone and cartilage can be regenerated by transplanting cells seeded onto a scaffold.[40,41] However, it is unclear whether scaffolding enhances the potential of this material to produce teeth. To elucidate this issue, we used tooth buds at mice embryonic day 14.5 that were dissociated into single cells, seeded onto a biodegradable polymer scaffold, and were then transplanted into the kidney capsule of male adult mice for up to 14 days (Fig. 2).[7] Pieces of polyglycolic acid (PGA) fiber mesh (Albany International Research, Mansfield, MA, USA) approximately 1 cm^3 in size were prepared and collagen coated before seeding with 5×10^5 cells (from 20 tooth buds) per scaffold. Samples grown for 5, 7, 10, and 14 days were dissected and examined with hematoxylin and eosin staining and immunohistochemistry using an amelogenin antibody.

At 5 days posttransplantation, the epithelium outgrowth had started to encompass the dental mesenchyme in the implants. The epithelium formed exhibited the horseshoe shape similar to that seen at the cap stage of natural teeth (Fig. 3A). At high magnification, two types of dental epithelial cells could be identified, and the mesenchymal cells

seemed to have a polarity against the dental epithelium (Fig. 3B). At 7 days posttransplantation, the crown-cusp pattern of the biological teeth was established by folding of the inner enamel epithelium (Fig. 4A,C). The developing enamel organ tissues characterized the transition from cap to bell stage; the symmetrical epithelium morphology was similar to that at the bell stage of natural developing teeth. Interestingly, the biological teeth also exhibited two distinct crown cusp pattern formations, a phenomenon that is generally not observed in the development of natural teeth in mice: one is the one-tip type (Fig. 4A), and the other is the two-tip type (Fig. 4C). The biological tooth produced in the scaffold was connected to the keratinized oral epithelium-like structures (Fig. 4A,C). In the process of obtaining the teeth, we completely separate tooth buds from the oral epithelium. Therefore, the results indicated that the enamel organ epithelial cells may differentiate into keratinized tissues. High magnification revealed organized inner epithelial cells with long columnar morphology, stellate reticulum-like tissues, and odontoblast-like cells (Fig. 4B,D).

At 10 days posttransplantation, the biological tooth exhibited the morphology of the late bell stage of molar teeth (Fig. 5A). Higher magnification revealed the progression of inner enamel epithelium differentiation and the appearance of tall columnar cells (Fig. 5B). Then, we examined the amelogenin expression in the biological tooth at this stage. Amelogenin staining was detected in the tall columnar cells, which are well-polarized ameloblasts, and the matrix at the outer surface of the dentin matrix (Fig. 5C,D).

The newly grown biological teeth, which were surrounded by bone, show two patterns of tooth-crown shape; a flat cusp pattern (Fig. 6A), and a prominent cusp pattern (Fig. 6B), seen at day 14. The width of the enamel–dentin complex was greater than at 10 days posttransplantation at higher magnification. Dentin tubules were clearly displayed at that stage (Fig. 6C).

Enamel, the most highly mineralized dental tissue, is composed of crystalline calcium phosphate, and is approximately 96% mineral with the remaining 4% consisting of organic components and water. The basic microstructural unit of enamel is an enamel rod, which is tightly packed and adherent to other rods. The interwoven architecture of enamel crystals provides both strength and resistance to fracture.[42] The enamel-forming ameloblasts undergo apoptosis as they elaborate the enamel matrix, such that by the time the enamel is fully formed, no ameloblasts remain.[43] Enamel regeneration therefore is not possible in erupted teeth because the ameloblasts are no longer present. The fundamental organization units of mammalian enamel are the rods (prism) and interrod enamel (interprismatic substance). Enamel is built from closely packed and long, ribbon-like carbonatoapatite crystals measuring 60–70 nm in width and 25–30 nm in thickness.

Scanning electron microscopy (SEM) has added greatly to the understanding of tissue-engineered enamel structures. It is useful in determining whether enamel rods are formed. We used SEM (JSM 7000F; JEOL, Japan) to for ultrastructural studies of the enamel formation in our newly formed teeth (Fig. 7A). The normal enamel prism-like organization could be seen in the newly produced enamel (Fig. 7B). This is the first time that enamel rods and interrods have been produced by tissue engineering technology using mouse embryonic tooth bud cells.

All biological teeth produced by this method, in which E14 mice molar tooth buds are used, exhibited normal crown structures composed of enamel and dentin, covering

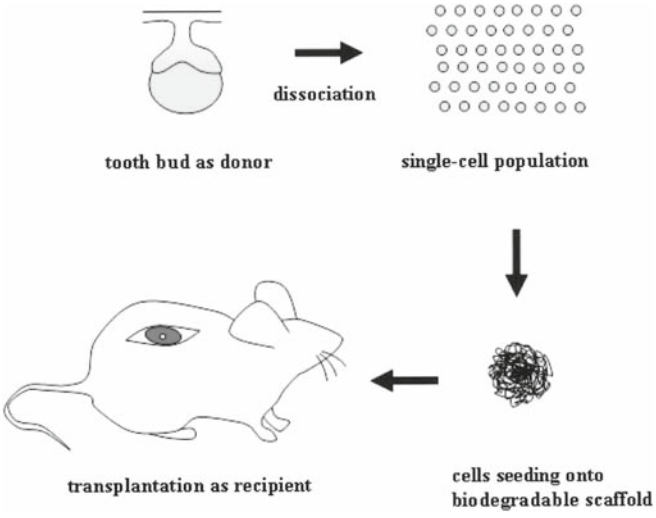

Fig. 2. Schematic diagram of the strategy for producing biological teeth using tissue engineering. Tooth buds were dissociated into single-cell populations and then seeded on the scaffolds and subsequently transplanted into the body

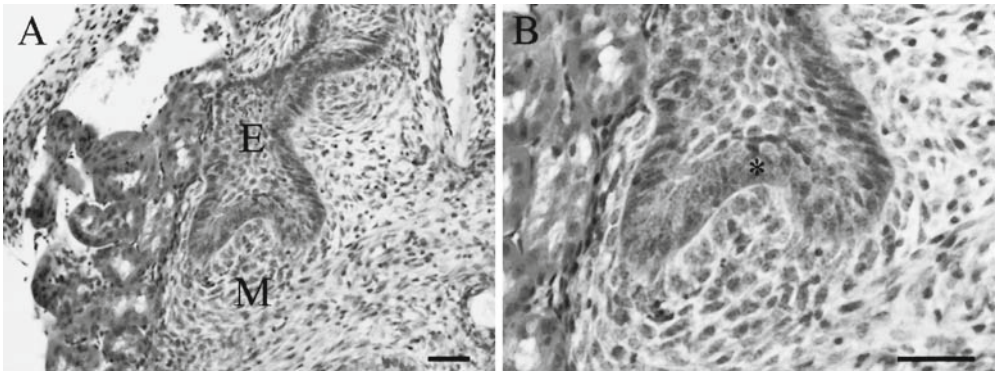

Fig. 3. **A** Biological tooth at 5 days post-transplantation; the horseshoe-shaped dental tissue is displayed in the implant. *E*, enamel; *M*, mesenchyme. **B** High magnification view demonstrates the presence of two distinct epithelial layers consisting mostly of columnar cells in contact with the basement membrane (asterisk) and smaller cells in the center. *Bar*s 20 μm

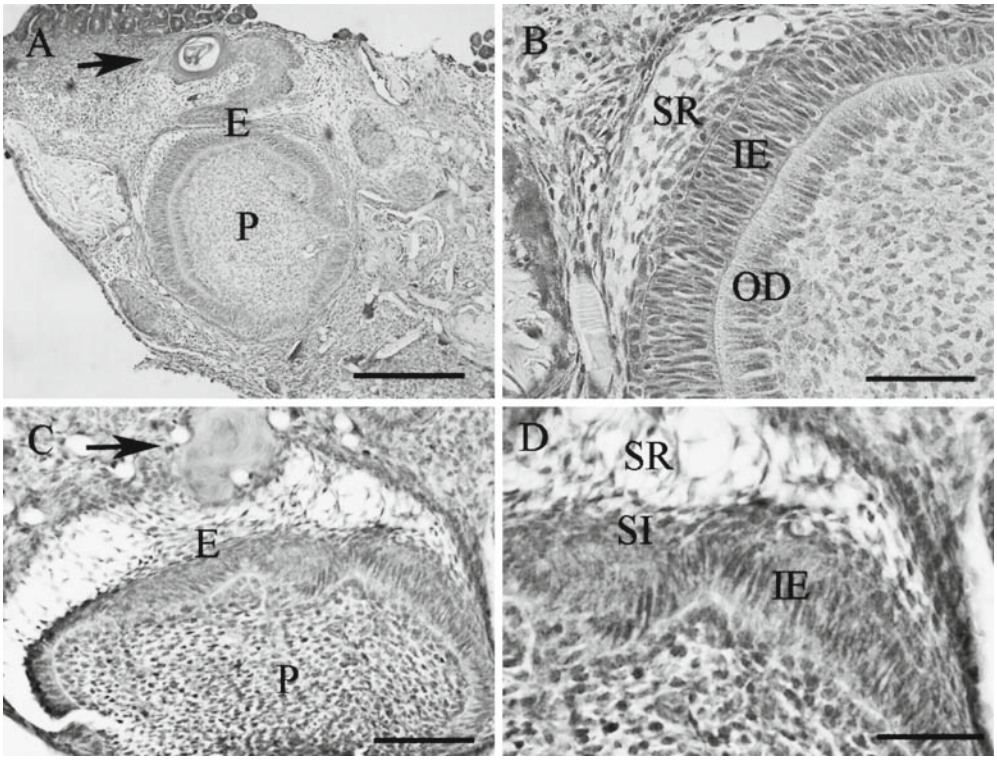

Fig. 4. **A** Histological view at 7 days posttransplantation is shown with keratinized tissue (*arrow*) and a biological tooth bud in the implant. *E*, epithelium; *M*, mesenchyme. The crown cusp shows the one-tip cusp pattern. **B** At higher magnification, stellate reticulum-like tissue, inner enamel epithelium-like tissue (*IE*), and odontoblast (*OD*), aligned with the inner enamel epithelium-like tissue, are clearly seen. **C** The biological tooth shows a prominent cusp morphology similar to that of a natural tooth at the bell stage. The top of a biological tooth is shown in the presence of keratinized tissue (*arrow*). *E*, epithelium; *P*, dental papilla. **D** At high magnification, stellate reticulum (*SR*), stratum intermedium (*SI*), inner enamel epithelium (*IE*), and odontoblasts are observed. *Bars* **A** 200 μm; **B**, **D** 20 μm; **C** 100 μm

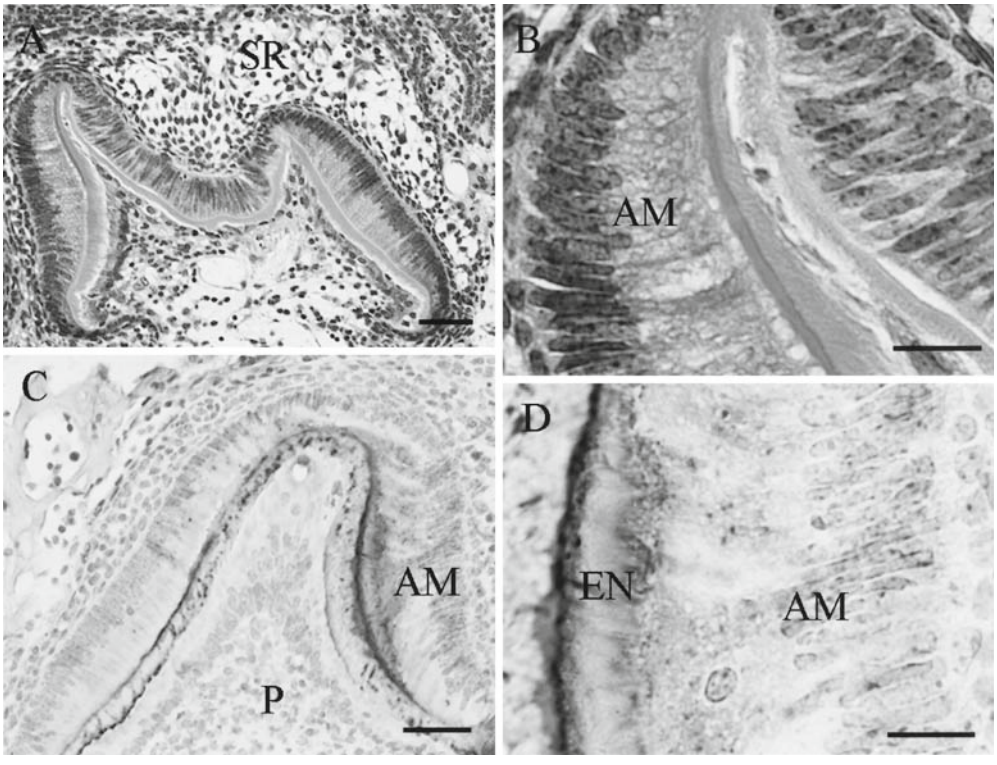

Fig. 5. **A** Histological examination shows the molar tooth with two cusps, the prominent cusp pattern formation. Wide stellate reticulum (*SR*) is observed on the top of ameloblast-like cells. **B** Typical ameloblast-like cells (*AM*) with tall columnar morphology and nuclei in the distal region of the cytoplasm were seen at the outer surface of the enamel under high magnification. **C** A strong immunoreaction to antiamelogenin immunoglobulin was visible in the enamel matrix and the proximal portion of ameloblasts (*AM*). *P*, dental papilla. **D** The immunoreaction of antiamelogenin was clearly observed in the enamel matrix (*EN*) and proximal region of the ameloblasts (*AM*). Dentin (*D*) on the surface of the enamel was also slightly positive to antiamelogenin. *Bars* **A**, **C** 20 μm; **B**, **D** 10 μm

pulp-like tissues. This study showed that completely dispersed tooth bud cells have the capacity to produce teeth even though scaffolds were used. However, many tooth buds are needed to obtain the large number of the cells required to produce a single tooth.

As to the observation of crown shape displaying two patterns, flat cusp and prominent cusp, we offer the following explanation. Until the cap stage, all biological teeth had a horseshoe-shaped appearance, consistent with the morphology of natural teeth at the same stage. Beyond the bell

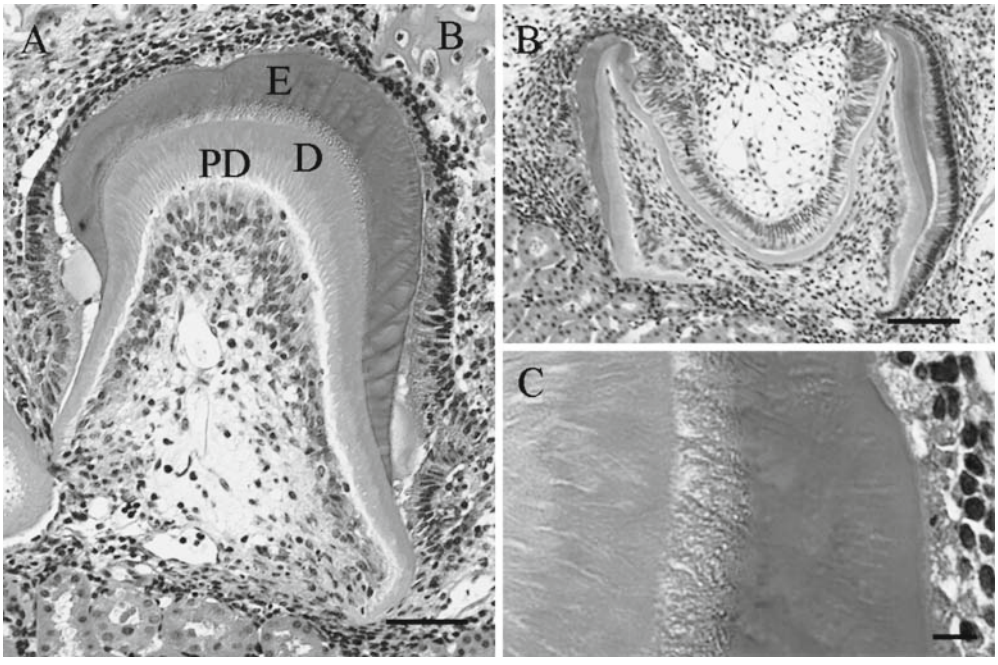

Fig. 6. **A** At 14 days posttransplantation, the thick enamel (*E*), dentin (*D*), and predentin (*PD*) were easily observed in the biological tooth with the flat cusp pattern. A reduced number of ameloblasts covered the top of the enamel produced. Tall columnar ameloblasts still existed inside the enamel. Bone (*B*) was also produced and surrounded the biological tooth in the implants. **B** Another type of biological tooth with prominent cusp pattern is observed in the implants at 14 days posttransplantation. **C** Dentin tubules and enamel structures were easily visible in the biological tooth. *Bars* **A** 50 μm; **B** 100 μm; **C** 10 μm

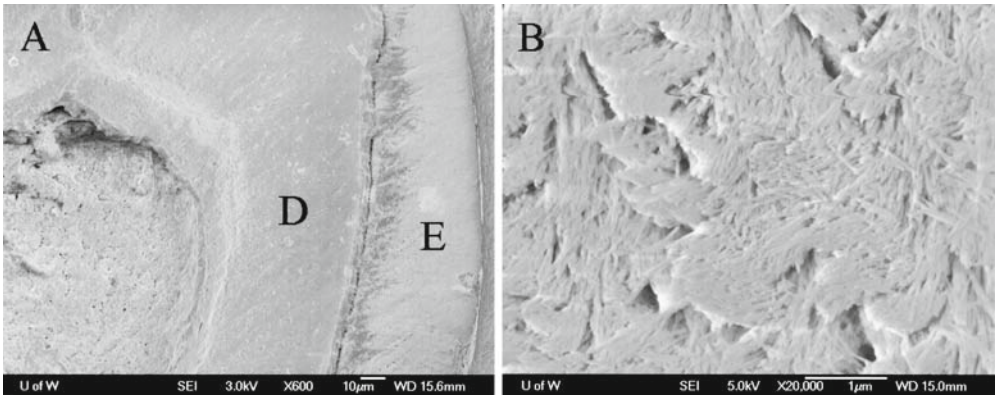

Fig. 7. **A** Scanning electron microscope (SEM) pictures show the enamel (*E*) and dentin (*D*) in the biological tooth at 14 days posttransplantation. Tissue-engineered enamel covered tissue-engineered dentin (*D*). **B** Enamel rods were clearly visible in tissue-engineered enamel. The arrangement of the enamel rods was slightly irregular

stage, however, the newly generated biological teeth developed into one of two morphological shapes, resulting in different crown shapes. We can only speculate as to the reason behind this variation in crown shape. The original tooth bud retains the information required to regulate the tooth-crown shape until the cap stage even when E14 tooth buds were dissociated into single-cell populations. However, tooth cusp number and shape are actually determined subsequent to the bell stage when the second enamel knot appears. This finding suggests that the signal required to determine the correct tooth cusp number does not come from the E14 tooth bud. Rather, it may be that this signaling information is derived from the oral epithelium, because in this procedure we cut off the oral epithelium from the tooth buds.

Interestingly the size of the new biological teeth, in particular, the width of the crowns was similar to the width of a natural tooth at this same developmental stage.[7] However, in this procedure 20 first molar tooth buds from embryonic mice were required to produce a single new biological tooth. In addition, similar to previous reports,[6,37,39] we never observed tooth-root formation in the biological teeth developed in the kidney capsule for 28 days.

We note that Nakao et al. have reported that the transplantation of pellets containing a combination of isolated dental epithelial and mesenchymal cells at E14.5, into the incisor tooth socket of mice, caused formation of teeth with regular shape including tooth crown and root.[12] Taken together, these results demonstrate that teeth produced from embryonic tooth bud tissue or cells using a recombination technique exhibit a regular tooth-crown shape not only in vitro but also in vivo. In addition, a tooth root with a periodontal ligament was formed when the pellet was transplanted into the incisor socket after extraction, but the tooth root did not appear when grown in the kidney capsule. The differences demonstrated that whether root development occurs may be the result of the environment of the transplantation site. These results suggest that the biological tooth with a tooth root may have the potential to erupt from the jaw into the oral cavity. Questions arise from our

study as well as previous reports: How much alveolar bone is required to support the biological tooth? What is the best method of securing the tooth to the jaw socket?

Biological tooth engineering from postnatal tooth bud cells

We have recently reported a number of studies explaining advances in the development of tooth tissue engineering technology using postnatal porcine tooth buds.[2,3,8,9,44,45] In these studies, porcine tooth buds harvested from the mandible at 6 months of age were dispersed into cell suspensions and then placed onto scaffolds. They were subsequently grown in the nude rat omentum. No organized tissue in the implants was observed in the initial 4 weeks.[2] Between 4 and 8 weeks, some cellular aggregates had formed an organized tissue in the presumptive tooth, resembling dental lamina at the initiation stage in tooth development (Fig. 8A). By immunohistochemistry, the organized tissue formation stained positive for cytokeratin 14 as the dental epithelial marker (Fig. 8B). Higher magnification revealed that the aggregated epithelial cells were strongly stained with cytokeratin 14 (Fig. 8C). The results suggested that first step in organized tissue formation in the implants was the aggregation of epithelial cells.

At 10 weeks posttransplantation, open circular-shaped structures were observed in the implants. The structures were composed of numerous low columnar cells in the periphery of the circle, stellate reticulum-like tissue encircled by the low columnar cells, and the aggregated cell population in the center of the circle (Fig. 8D).

After 20 weeks, recognizable complex tooth tissues are formed with structures containing dentin and enamel (Fig. 9A).[3] The feature structures of amelogenesis such as rods and interrods were seen through the light microscope, but the details were difficult to distinguish (Fig. 9B). Generally, when enamel formation begins, Tome's process is involved only in the proximal portion (Fig. 9C). Amelogenin was clearly stained with ameloblasts and enamel matrix (Fig. 9D). As the initial enamel layer is formed, ameloblasts migrate away from the dentin surface and develop the distal portion. The rod and interrod configurations of enamel crystals are determined by the ameloblasts and their Tome's process. The rod and interrod enamels differ only in the orientation of their crystallites. Ultrastructural studies of tissue-engineered enamel were conducted by SEM (JSM 7000F; JEOL, Japan) to see the details. Enamel rods in tissue-engineered enamel were observed, although the arrangement was slightly irregular (Fig. 10A,B). This is the first report showing enamel rods in tissue-engineered enamel/teeth produced from porcine postnatal tooth bud cells.

Most regenerated teeth produced using this strategy exhibit a disorganized heterogeneous morphology. However, although the tooth, including tooth crown and root, appeared in the implants, the size was considerably smaller than that of a natural tooth. These results suggested that stem cells that have the information to produce a whole biological tooth may exist in the tooth bud at crown formation. However, using tissue engineering techniques, all tooth tissues, with the exception of the periodontal ligament and nerve system, can be generated,[2,45] and the mechanism of development of these teeth closely parallels that of natural teeth.

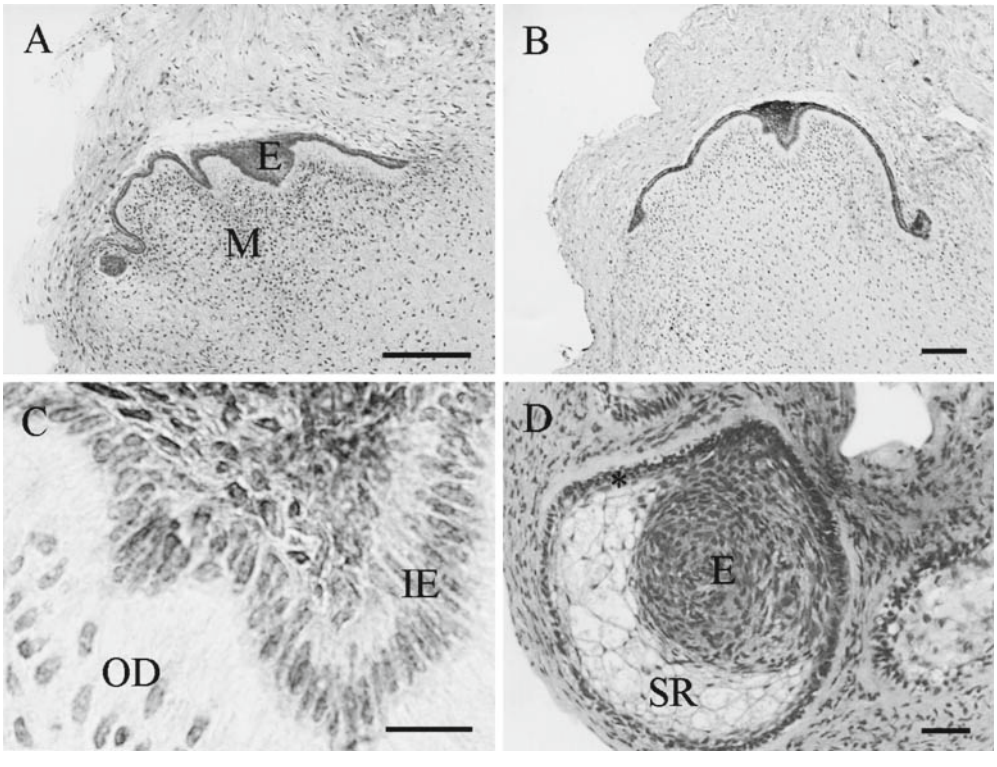

Fig. 8. **A** At 10 weeks posttransplantation, a dental lamina-like structure was observed in the implants derived from postnatal porcine tooth bud cells seeded on the scaffold. *E*, epithelium; *M*, mesenchyme. **B** Layers of epithelial cells stained with cytokeratin 14. **C** A higher magnification showed positive staining in most of the cells in the center of the epithelium. Tall columnar cells of the inner enamel epithelium (*IE*) were slightly positively stained with cytokeratin 14, but odontoblast-like cells were negative for cytokeratin 14. **D** The circular region increased in size and contained a stellate reticulum-like structure, and the aggregated cells (*E*) inside the low columnar cells formed the inner enamel epithelium (*asterisk*) at 12 weeks posttransplantation. *Bars* **A** 100 μm; **C** 20 μm; **B**, **D** 50 μm

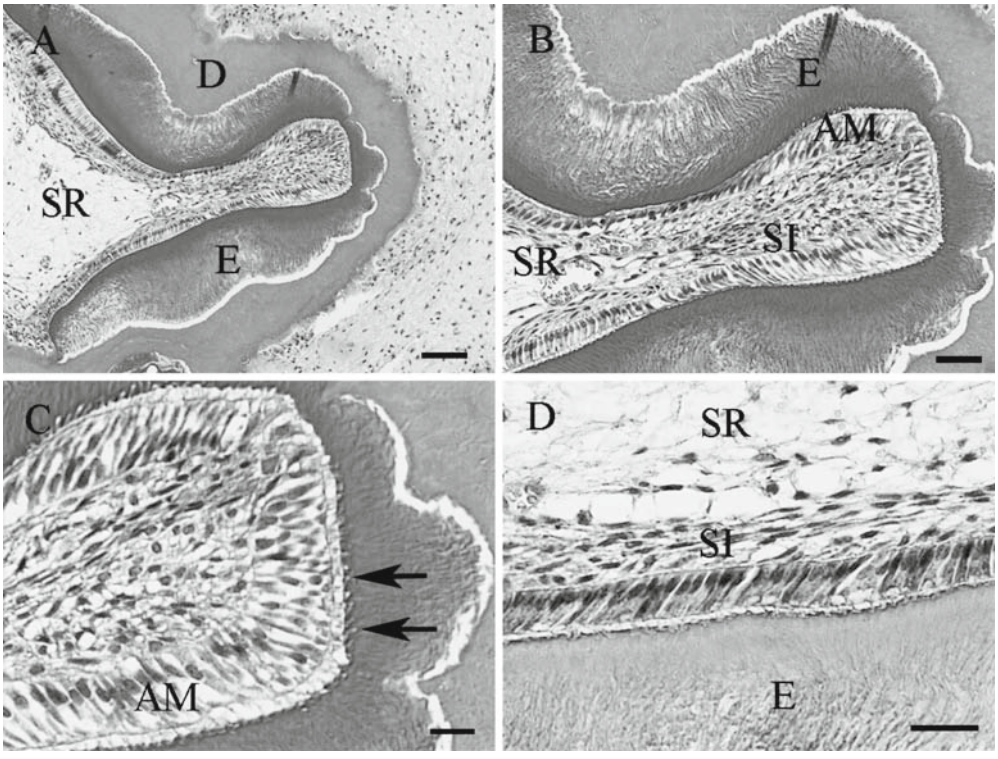

Fig. 9. **A** At 20 weeks posttransplantation, thick enamel (*E*) and dentin (*D*) were easily distinguished in the implants by histology. The shape was quite irregular in comparison with the appearance of the biological teeth derived from embryonic tooth bud cells. *SR*, stellate reticulum. **B** At high magnification, enamel organ-like tissues consisted of enamel, ameloblasts, stratum intermedium, and stellate reticulum in the usual order from the outside in the tissue-engineered tooth. **C** Tome's processes (*arrows*) were clearly observed in the proximal portion of the ameloblasts (*AM*) in the biological tooth. **D** Ameloblast and enamel (*E*) were strongly stained with amelogenin in the tissue-engineered tooth. *Bars* **A** 50 μm; **B**, **D** 20 μm; **C** 10 μm

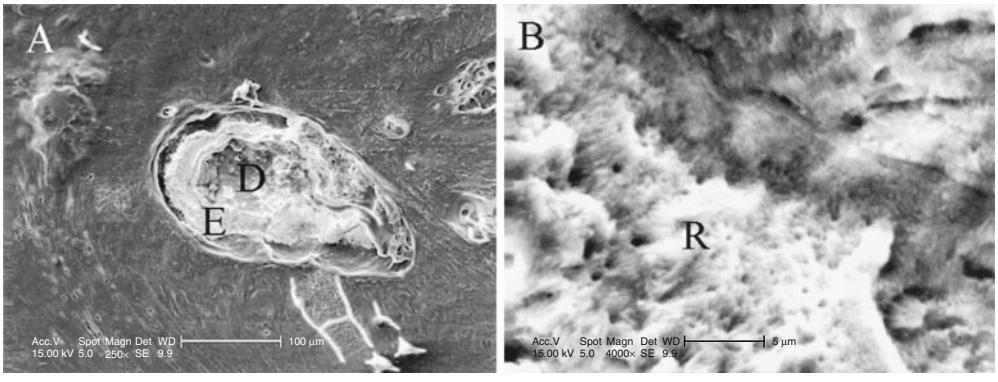

Fig. 10. **A** An SEM picture shows a tissue-engineered biological tooth derived from porcine postnatal tooth buds at 20 weeks posttransplantation. *E*, enamel; *D*, dentin. **B** Enamel rods were easily visible in the tissue-engineered enamel. Arrangement of the enamel rods was slightly irregular in comparison with natural tooth-enamel structures

A major shortcoming with this technique is that the tissue-engineered teeth do not reach the expected size of natural teeth. The mean dimension of the engineered structure was approximately 2 mm in length by 3 mm in width at 20–25 weeks after transplantation.[3] The natural length of the porcine mandibular third molar is approximately 30 mm, only one-tenth the length of that of the natural tooth. In this case, we obtained one tooth bud from a pig mandible to seed onto one scaffold. The procedure was completely different from that used in mice. The morphology of the tissue-engineered teeth was quite irregular in comparison with the appearance of teeth derived from embryonic tooth bud cells. Therefore, despite the use of this engineering strategy on postnatal tooth bud cells, the development of a complete tissue engineered tooth remains elusive.

Tooth-forming capacity of embryonic and postnatal tooth bud cells

As we described in the foregoing sections, the biological teeth derived from embryonic tooth bud cells developed regularly shaped crowns by both the recombination and the cell scaffold-based techniques.[2,45] These results are quite striking because teeth generated from porcine postnatal tooth bud cells do not develop the correct structure and shape, although clearly there is a difference in species. Moreover, rat molar tooth bud cells obtained from 4-day postnatal rats and seeded onto scaffolds can be used to produce tissue-engineered tooth crowns that have an asymmetrical tooth-crown shape.[46] The appearance was irregular in comparison with the morphology derived from embry-

onic mouse tooth bud cells. It may be that subculturing the cells for 6 days resulted in the irregular tooth-crown shape. According to our experiments, the differences between postnatal cell and embryonic cell studies did not seem to be related to whether scaffolds were used. Therefore, it is possible that the differences may be related to the developing stage of the donor tooth: embryonic or postnatal. In other words, postnatal tooth bud cells may have lost some essential factors required to regulate tooth-crown shape that are present in embryonic mouse tooth bud cells. A challenge of tooth tissue engineering will be to develop a method of producing teeth with regular tooth-crown shape using adult tooth bud cells.

It is thought that the aberrant cusp morphogenesis in the biological teeth could result from patterning and growth disturbances of the dental epithelium during cusp and early bell stages. It is well known in tooth development that an accurate control of enamel knot spacing must exist during morphogenesis as this process determines the correct tooth-crown shape.[14] Cusp patterning defects have been reported in a few mouse mutants. We have performed further studies to investigate genes such as growth factors and the super-repressor to determine the mechanism of irregular shape formation in biological teeth generated from adult tooth bud cells.

Prospects for growing new teeth

Regenerative techniques for producing new biological teeth are currently anticipated to improve the quality of life in people who are missing teeth. A frequent question I am asked is "Will you be able to grow new teeth for us?" I usually reply "yes" as there is such fantastic progress in the fields of tissue engineering and stem cell biology.

Adult stem cells, with the capacity of self-renewal and multilineage differentiation, play a crucial role in postnatal tissue development and provide an attractive progenitor cell source.[47,48] Our strategy to develop a future clinical application for human biological teeth generated from adult stem cells follows. Epithelial stem cells are harvested from the dental epithelium or oral epithelium, while dental pulp stem cells can be obtained from either dental pulp tissues or bone marrow stromal cells. Periodontal ligament stem cells can be obtained from either periodontal ligament tissues or dental follicle tissues. Isolated epithelial, dental pulp, and follicle stem cells are then multiplied in vitro using an appropriate supportive culture environment. After combining epithelial, dental pulp, or dental follicle stem cells, a tooth primordium begins to develop in vitro or in vivo with the support of appropriate signaling molecules; these are distributed with a precise spatial and temporal organization. Subsequently, the mimic tooth primordium is transplanted back into the jaw where there is a missing tooth. The best location for growing new teeth, such as inside bone or in vitro, needs further consideration.

Although dental pulp stem cells and mesenchymal stem cells in bone marrow have been discovered, their whole tooth-forming capacity is unknown. Moreover, dental epithelial stem cells in molar teeth have still not been discovered, although dental epithelial stem cells in continuously growing incisors in rodent animals are well characterized. However, it is not clear whether the epithelial stem cells in rodent incisors contribute to molar tooth production. Many factors need to be defined before we will achieve the technique of producing whole biological teeth from adult stem cells.

In addition to the issues discussed above, more research is needed to find ways to identify and harvest potential stem cells and to induce their differentiation in specific directions. Overcoming these obstacles will move us closer to bringing tooth regeneration to dental practice.

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