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At the time of publication, author Songtao Shi was affiliated with the University of Southern California. Currently, he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

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Abstract

Some clinical case reports have shown that immature permanent teeth with periradicular periodontitis or abscess can undergo apexogenesis after conservative endodontic treatment. A call for a paradigm shift and new protocol for the clinical management of these cases has been brought to attention. Concomitantly, a new population of mesenchymal stem cells residing in the apical papilla of permanent immature teeth recently has been discovered and was termed stem cells from the apical papilla (SCAP). These stem cells appear to be the source of odontoblasts that are responsible for the formation of root dentin. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to completion. This article reviews current findings on the isolation and characterization of these stem cells. The potential role of these stem cells in the following respects will be discussed: (1) their contribution in continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and (2) their potential utilization for pulp/dentin regeneration and bioroot engineering.

Keywords

Apexogenesis, apical papilla, bioroot engineering, dental pulp stem cells, immature teeth, periodontal ligament stem cells, pulp regeneration, stem cells from human exfoliated deciduous teeth, stem cells from the apical papilla

Disciplines

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Comments

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The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and BioRoot Engineering

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Abstract

Some clinical case reports have shown that immature permanent teeth with periradicular periodontitis or abscess can undergo apexogenesis after conservative endodontic treatment. A call for a paradigm shift and new protocol for the clinical management of these cases has been brought to attention. Concomitantly, a new population of mesenchymal stem cells residing in the apical papilla of permanent immature teeth recently has been discovered and was termed stem cells from the apical papilla (SCAP). These stem cells appear to be the source of odontoblasts that are responsible for the formation of root dentin. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to completion. This article reviews current findings on the isolation and characterization of these stem cells. The potential role of these stem cells in the following respects will be discussed: (1) their contribution in continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and (2) their potential utilization for pulp/dentin regeneration and bioroot engineering.

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A number of recent clinical case reports have revealed the possibilities that many teeth that traditionally would receive apexification may be treated for apexogenesis. A call for a paradigm shift and new protocol for the clinical management of these cases has been made by the authors (1-3). A recent scientific finding, which may explain in part why apexogenesis can occur in these infected immature permanent teeth, is the discovery and isolation of a new population of mesenchymal stem cells (MSCs) residing in the apical papilla of incompletely developed teeth (4,5). These cells are termed stem cells from the apical papilla (SCAP), and they differentiate

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into odontoblast-like cells forming dentin when implanted into the subcutaneous space of immunocompromised mice using hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier vehicle (4). Although SCAP show similar characteristics to dental pulp stem cells (DPSCs) that were discovered earlier (6), they also behave differently in a number of aspects that were assessed by histologic, immunohistochemical, cellular, and molecular analyses. Evidence is accumulating to support the hypothesis that SCAP appear to be the source of primary odontoblasts that are responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement odontoblasts. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to its completion. The goal of this review is to introduce the background of these recently described stem cells, their isolation, and characterization. The potential role of these stem cells in the contribution of the continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and in autotransplanted teeth are discussed. The possibility of using SCAP and other types of pulp stem cells for pulp/dentin regeneration and the combination of SCAP and periodontal ligament stem cells (PDLSCs) for bioroot engineering shown by Sonoyama et al. (4) in a swine model as a potential future clinical approach to replace dental implants will be thoroughly described and analyzed.

Dental Papilla, Apical Papilla, and Pulp in Developing Teeth

It is well known that dental papilla is derived from the ectomesenchyme induced by the overlaying dental lamina during tooth development (7,8). This developing organ evolves into dental pulp after being encased by the dentin tissue produced by odontoblasts that come from this organ. The apical portion of the dental papilla during the stage of root development has not been described much in the literature. Most information regarding tooth development comes from studies using animal models. Recently, we described the physical and histologic characteristics of the dental papilla located at the apex of developing human permanent teeth and termed this tissue apical papilla (5). The tissue is loosely attached to the apex of the developing root and can be easily detached with a pair of tweezers (Fig. 1). Apical papilla is apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp. Importantly, there are stem/progenitor cells located in both dental pulp and the apical papilla, but they have somewhat different characteristics (4,5). Because of the apical location of the apical papilla, this tissue may be benefited by its collateral circulation, which enables it to survive during the process of pulp necrosis.

The Discovery of Multipotent SCAP

Stem-cell biology has become an important field for the understanding of tissue regeneration. In general, stem cells are defined by having two major properties. First, they are capable of self-renewal. Second, when they divide, some daughter cells give rise to cells that either maintain stem cell character or give rise to differentiated cells. Mesenchymal stem cells (MSCs) have been identified in many tissues and are capable of differentiating into many lineages of cells when grown in defined conditions including osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic lineages (9-11). MSCs were initially described as “fibroblast-like” in vitro as early as in the 1860s (12). Friedenstein et al. (13) in 1976 further identified MSCs as colony-forming unit fibroblasts (CFU-Fs), which were able to commit into osteogenic differentiation. Caplan (14) and Pittenger et al. (15) showed that MSCs underwent osteogenic, chondrogenic, and adipogenic differentiation in response to different biochemical signals. Bone marrow–derived mesenchymal stem cells (BMMSCs) are multipotent stem cells and have been the most studied MSCs. They undergo osteogenic differentiation when stimulated by osteogenic reagents in vitro such as dexamethasone, β-glycerophosphate, and bone morphogenetic proteins. More interestingly, when transplanted into immunocompromised mice, they are capable of forming bone and inducing recipient hematopoietic marrow

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components (6). So far there has been no evidence that BMMSCs have the capacity to undergo dentinogenic differentiation. The first type of human dental stem cells was isolated from dental pulp tissue of extracted third molars and characterized in comparison with BMMSCs (6). These dental pulp–derived cells were named DPSCs for their clonogenic properties (ie, exhibiting CFU-Fs in cultures) and, more importantly, their ability to differentiate into odontoblast-like cells and form dentin/pulp-like complex when implanted into the subcutaneous space of immunocompromised mice (6). Subsequently, a variety of dental MSCs have been isolated including stem cells from human exfoliated deciduous teeth (SHED) (16), periodontal ligament stem cells (PDLSCs) (17), and SCAP (4).

An MSC surface marker STRO-1 was found to be positive in apical papilla cells (4), which is the first piece of evidence suggesting the existence of stem cells in this tissue. Using collagenase/dispase digestion of detached apical papilla tissue, adherent clonogenic cell clusters (ie, CFU-Fs) are formed, similar to that observed for different MSC populations. Approximately 50 single colonies can be generated from 10^5 single cells cultured at low density (4). This colony-forming cell population showed a high uptake rate for bromodeoxyuridine (an indication of cell proliferation) and exhibited over 70 population doublings in vitro. Ex vivo–expanded SCAP were also found to express the cell-surface molecule STRO-1 (4).

Isolated SCAP grown in cultures can undergo dentinogenic differentiation when stimulated with dexamethasone supplemented with L-ascorbate-2-phosphate and inorganic phosphate. Alizarin Red (a dye that binds calcium salts)–positive nodules form in the SCAP cultures after 4 weeks of induction, indicating a significant calcium accumulation in vitro. Furthermore, cultured SCAP were found to express dentinogenic markers dentin sialophosphoprotein and CBFA1/Runx2 (4). In addition to their dentinogenic potential, SCAP also exhibit adipogenic and neurogenic differentiation capabilities when treated with respective stimuli (4,5). Human SCAP were also independently isolated and characterized by Abe et al. (18) who found similar properties of these cells to those observed in our studies.

SCAP Are a Unique Population of Postnatal Stem Cells

The capacity of SCAP to differentiate into functional dentinogenic cells in vivo has been verified using an implantation technique in animal models (4). Ex vivo–expanded SCAP were transplanted into immunocompromised mice using particles of HA/TCP as a carrier. A typical dentin structure was generated in which a layer of dentin tissue was formed on the surface of the HA/TCP along with connective tissue (4). Cells that are responsible for the dentin formation stained positively with a human-specific antimitochondria antibody (4). In order to examine whether SCAP are distinct from DPSCs, SCAP and DPSCs from the same tooth were isolated and grown in cultures under the same conditions. It was found that SCAP showed a significantly greater bromodeoxyuridine uptake rate, number of population doublings, tissue regeneration capacity, and number of STRO-1–positive cells when compared with DPSCs (4). In addition, SCAP express a higher level of survivin (anti-apoptotic protein) than DPSCs and are positive for hTERT (human telomerase reverse transcriptase that maintains the telomere length) activity (4), which is usually negative in MSCs. These lines of evidence suggest that SCAP derived from a developing tissue may represent a population of early stem/progenitor cells, which may be a superior cell source for tissue regeneration. Additionally, these cells also highlight an important fact that developing tissues may contain stem cells distinctive from that of mature tissues.

Despite SCAP expressing many dentinogenic markers after ex vivo expansion, they express lower levels of dentin sialophosphoprotein, matrix extracellular phosphoglycoprotein, transforming growth factor (TGF)βRII, FGFR3, Flt-1 (vascular endothelial growth factor [VEGF] receptor 1), Flg (FGFR1), and MUC18 (melanoma-associated glycoprotein) than do DPSCs (5). SCAP, as all ex vivo–expanded MSCs, are a heterogeneous population of cells.

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Subpopulations of STRO-1–positive SCAP are coexpressed with a variety of dentinogenic markers (5). In addition, SCAP show a positive staining for several neural markers including *β*III tubulin, glutamic acid decarboxylase, NeuN (neuronal nuclear antigen), nestin, neurofilament M, neuron-specific enolase, and CNPase (glial marker, 2′,3′-cyclic nucleotide 3′-phosphodiesterase) by immunocytochemical staining (5). The neurogenic potential of SCAP could be because of the fact that SCAP are derived from neural crest cells or at least associated with neural crest cells analogous to other dental stem cells such as DPSCs and SHED that have been shown previously to possess a neurogenic potential (16,19). Whether the neurogenicity of dental stem cells is more potent than that of BMMSCs has not been investigated. Evidence has shown that BMMSCs have the potential for neuronal differentiation but at a much lower scale compared with stem cells derived from neural tissues (20,21). If dental stem cells are more capable of neurogenic differentiation than BMMSCs, they may present an appropriate cell source for neural tissue regeneration.

The Potential Role of SCAP in Continued Root Formation

The role of apical papilla in root formation may be observed in clinical cases. A human immature incisor was injured and the crown fractured with pulp exposure. During the treatment, the apical papilla was retained while the pulp was extirpated. Continued root-tip formation was observed after root canal treatment (Fig. 2). Further investigation is needed to verify whether the radiographic evidence of continued apical development is because of dentin formation from the apical papilla or if it is merely cementum formation (3). Using minipigs as a model, a pilot experiment was conducted. Surgically removing the apical papilla at an early stage of the root development halted the root development despite the pulp tissue being intact. In contrast, other roots of the tooth containing apical papilla showed normal growth and development (Fig. 2*E*). Although the finding suggests that root apical papilla is likely to play a pivotal role in root formation, further research is needed to verify that this halted root development in the minipig was not due to damage of Hertwig's epithelial root sheath (HERS) during the removal of the apical papilla of that particular root apex.

The Potential Role of SCAP in Pulp Healing and Regeneration

Immature Teeth with Periradicular Periodontitis or Abscess Uudergo Apexogenesis

There have been sporadic case reports in the literature showing the potential of root maturation even with the presence of periradicular pathoses of endodontic origin (22-27). More recently, several clinical reports with careful documentation and follow-ups have further shown that immature permanent teeth diagnosed with nonvital pulp and periradicular periodontitis or abscess can undergo apexogenesis (1-3). These recent reports challenge the traditional approach in managing immature teeth by applying apexification treatment, where there is little to no expectation of continued root development. Instead, it is possible that alternative biologically based treatments may promote apexogenesis/maturogenesis. A common aspect of many of these reported cases is the preoperative presentation of apical periodontitis with sinus tract formation, a condition normally associated with total pulpal necrosis and infection that requires apexification. Although Iwaya et al. (1) and Banchs and Trope (2) applied the term "revascularization" to describe this phenomenon, what actually occurred was physiological tissue formation and regeneration.

The clinical presentation of a negative response to pulpal testing with the presence of apical periodontitis is generally interpreted to indicate pulpal necrosis and infection. Lin et al. (28, 29), however, found that vital tissues can even be present in pulp chambers in mature permanent teeth associated with periapical radiolucencies. The size of the lesions varies, and some may be described as moderately extensive. In the case of immature teeth, this situation could be more common, although there is a lack of histologic studies. The recent case reports of

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immature teeth that presented with radiolucent lesions and underwent remarkable apexogenesis after conservative treatment suggest that vital pulp tissue must have remained in the canals (1-3). Iwaya et al. (1) reported that during the treatment of an immature second mandibular premolar with a periradicular abscess, the patient felt the insertion of a smooth broach into the canal before reaching the apex suggesting a partial remaining vitality of the pulpal tissue. Thirty-five months after the treatment, the root formation was complete and the tooth responded to an electrical pulp test (1). Similarly, Chueh and Huang (3) reported four cases of immature teeth with periradicular lesions or abscess. In some of these cases, the patients either felt the entry of instruments into the pulp chamber or there was vital pulp tissue and hemorrhage observed. The open apex provided a good communication from the pulp space to the periapical tissues; therefore, it may be possible for periapical disease to occur while the pulp is only partially necrotic and infected. Along the same line of reasoning, stem cells in pulp tissue and in apical papilla may also have survived the infection and allowed regeneration of pulp and root maturation to occur. The infection could have spread through survived pulp tissue reaching the periapex (Fig. 3). Another possibility is that the bone resorption is the result of the released proinflammtory cytokines from the inflamed pulp. However, this situation is less likely to cause the formation of sinus tracts, an indication of abscess from severe infection. It should be noted that prolonged infection may eventually lead to a total necrosis of the pulp and apical papilla; under these conditions, apexogenesis or maturogenesis, a term that encompasses not just the completion of root-tip formation but also the dentin of the root (30), would then be unlikely.

The Potential Role of SCAP in Replantation and Transplantation

The fate of human pulp space after dental trauma has been observed in clinical radiographs. Andreasen et al. (31,32) and Kling et al. (33) showed excellent radiographic images of the ingrowth of bone and periodontal ligament (PDL) (next to the inner dentinal wall) into the canal space with arrested root formation after the replantation of avulsed maxillary incisors, suggesting a complete loss of the viability of pulp, apical papilla, and/or HERS. Some cases showed partial formation of the root accompanied with ingrowth of bone and PDL into the canal space, and in some cases the teeth continued to develop roots to their completion, suggesting that there was partial or total pulp survival after the replantation. It is noted, however, that a pronounced narrowing of canal space is usually associated with a surviving pulp. Skoglund et al. (34) observed revascularization of the pulp of replanted and autotransplanted teeth with incomplete root development in dogs. Ingrowth of new vessels occurs during the first few postoperative days. After 10 days, new vessels are formed in the apical half of the pulp and, after 30 days, in the whole pulp. In some instances, anastomoses seemed to form with preexisting vessels in the pulp. Although revascularization occurs, the pulp space is eventually filled with hard tissue.

Other animal studies focusing on the changes in pulp tissue after replantation showed that various hard tissues including dentin, cementum, and bone may form in the pulp space depending on the level of pulp recovery. If pulp and apical papilla are totally lost, then the root canal space may be occupied by cementum, PDL, and bone (35-40). By tracing the migration of periodontal cells after pulpectomy in immature teeth, Vojinovic and Vojinovic (41) found that periodontal cells migrate into the apical pulp space during the repair process. Therefore, one may assume that when there is a total loss of pulp tissue but the canal space remains in a sterile condition, the outcome is the ingrowth of periodontal tissues.

One of the clinical treatment options for missing teeth is autotransplantation. The process often involves extraction of a supernumerary tooth or third molar and implantation into a recipient site. With regard to the status of pulp survival and root formation of the transplanted immature teeth, the clinical observations nicely shown by Tsukiboshi (42,43) have been that (1) if the transplanted tooth has minimal root formation, there will be minimal, if any, further root

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development after transplantation; (2) if there is some root formation, it will continue to develop to some extent or to completion after transplantation; and (3) pulp tissue will be eventually replaced by hard tissue. It has been considered that as long as the HERS remains viable, it stimulates the undifferentiated mesenchymal cells in periradicular tissues to differentiate into odontoblasts that contribute to the formation of new dentin and root maturation. However, the current understanding is that pulp cells are different from periodontal cells. Based on current available information, it is likely that odontoblast lineages are derived from stem cells in pulp tissue or apical papilla. Both SCAP and HERS appear to be important for the continued root development after transplantation. SCAP are also highly probable to survive after transplantation because minimal vascularity is found in apical papilla based on preliminary findings (5). The reason that transplanting a tooth with little or no root formation results in almost no further root development is unclear. One may speculate that the integrity of the entire tooth organ at that stage is critical for the root development to continue. During the transplantation, any disruption of the structure such as the follicle, HERS, and apical papilla will prevent further root development.

Stem Cells for Pulp/Dentin Tissue Engineering and Regeneration

Dental pulp tissue engineering was first tested by Mooney's groups (44). Bohl et al. (45) reported that culturing pulp cells grown on polyglycolic acid (PGA) in vitro resulted in high cell density tissue similar to the native pulp. Buurma et al. (46) found that pulp cells seeded in PGA and implanted into the subcutaneous space of immunocompromised mice produced extracellular matrix. New blood vessels also penetrated the cells/PGA implants in vivo 3 weeks after the implantation. Since the isolation and characterization of DPSCs and SHED (6,16), using these stem cells for dentin/pulp tissue regeneration has drawn great interest (47-49).

Reparative dentin-like structure is deposited on the dentin surface if DPSCs are seeded onto a human dentin surface and implanted into immunocompromised mice, suggesting the possibility of forming additional new dentin on existing dentin (50). Nör has shown that by seeding SHED onto synthetic scaffolds seated in pulp chamber of a thin tooth slice, odontoblast-like cells can rise from the stem cells and localize against existing dentin surface after implanting the tooth slice construct into immunocompromised mice (49). These findings provide new light on the possibility of generating pulp and dentin in pulpless canals. However, when implanting cells/scaffolds into root canals that have blood supply only from the apical end, enhanced vascularization is needed in order to support the vitality of the implanted cells in the scaffolds. Recent efforts in developing scaffold systems for tissue engineering have been focusing on creating a system that promotes angiogenesis for the formation of a vascular network (51-56). These scaffolds are impregnated with growth factors such as VEGF and/or platelet-derived growth factor or, further, with the addition of endothelial cells. These approaches are particularly important for pulp tissue engineering for the aforementioned reason that blood supply is only from the apical end.

Collagen has been considered as a convenient pulp cell carrier and could conveniently be injected into canal space to regenerate pulp clinically, yet collagen as the matrix has been found to contract significantly when carrying pulp cells (47,57), which may considerably affect pulp tissue regeneration. Our in vivo experiments using mouse as a study model showed that when pulp cells were cast into collagen gel and placed into a canal space, the contraction interfered in the pulp regeneration. This observation was also noted in vitro, where cells/collagen gel filled the entire canal space right after casting but underwent contraction over time. Contraction-resistant scaffolds such as PLG (D,L-lactide and glycolide) appear to be a more suitable carrier for pulp cells. Seven weeks after seeding the DPSCs onto PLG scaffolds, no observable contraction was noted in vitro, and the cells attached well onto the scaffold surface (Fig. 4).

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Whether SCAP are a more suitable stem-cell source for pulp generation than DPSCs and SHED because of their natural role as a source for primary odontoblasts than the replacement odontoblasts requires further investigation (5). The utilization of stem cells and an optimal scaffold system may one day be used clinically as depicted in Figure 5 in which engineered constructs may be inserted into canals of immature teeth to allow regeneration of pulp and dentin. The assumption for the multistep engineered tissue installments is based on the concern that blood vessel ingrowth can only occur from the apical end. A single installment, although it is more ideal and will avoid chances of introducing infection, may lead to the cell death in the coronal third region because of a lack of nutrients. Whether impregnation of VEGF and other angiogenic factors would make single installment a possibility awaits experimentation. If the proposed clinical approach can be successful, it implies a fundamental breakthrough in clinical endodontic treatments using cell and tissue engineering therapy.

SCAP for Bioroot Engineering

Dental implants have recently gained momentum as a preferred option for replacing missing teeth instead of bridges or removable dentures. However, although dental implants have had great improvements over the past decades, the fundamental pitfall is the lack of a natural structural relationship with the alveolar bone (ie, the absence of PDL). In fact, it requires a direct integration with bone onto its surface as the prerequisite for success, an unnatural relation with bone as compared with a natural tooth. The lack of natural contours and its structural interaction with the alveolar bone make dental implants a temporary option until a better alternative is available. This alternative may be tooth regeneration. Using animal study models, cells isolated from tooth buds can be seeded onto scaffolds and form ectopic teeth in vivo (58-62). Nakao et al. (62) recently engineered teeth ectopically followed by transplantation into an othrotopic site in the mouse jaw. Tooth regeneration at orthotopic sites using larger animals such as dogs and swine has also been tested (63,64). The study in dogs failed to show root formation (63), whereas the swine model was able to show root formation with a 33.3% success rate (64).

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The other approach is to use SCAP and PDLSCs to form a bioroot (4). Using a minipig model, autologous SCAP and PDLSCs were loaded onto HA/TCP and gelfoam scaffolds, respectively, and implanted into sockets of the lower jaw. A post channel was precreated to leave space for post insertion. Three months later, the bioroot was exposed, and a porcelain crown was inserted. This approach is relatively a quick way of creating a root onto which an artificial crown can be installed. The bioroot is different from a natural root in that the root structure is developed by SCAP in a random manner. Nevertheless, the bioroot is encircled with periodontal ligament tissue and appears to have a natural relationship with the surrounding bone. What remains to be improved is the mechanical strength of the bioroot, which is approximately two thirds of a natural tooth. There are also many other challenges; for example, a common question that has been frequently raised in the field of tissue engineering and regenerative medicine is the source of stem cells. Where shall we obtain the stem cells? Autologous stem cells are the best source because allogenic or xenogenic are likely to have immunorejection issues. Banking teeth, tissues, or stem cells for autologous use is a viable option. The recent revelation of the immunosuppression properties of stem cells shed some light on the possibility of allogenic use of stem cells. Many in vitro and in vivo studies have confirmed the immunosuppressive effects of MSCs. The potential mechanisms underlying this immunosuppression can be explained by downregulation of T, dendritic, NK, and B cells (65).

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Conclusions

Understanding the biology of dental stem cells and the principle of tissue engineering/ regeneration provides us with a better knowledge base on which the clinical treatment plans

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can be established. As discussed in this review regarding the paradigm shift, a more conservative approach for treating immature teeth with endodontic infection entitles our attention. Although further research is needed to verify the role of SCAP in the continued root formation after treatment, the clinical observations of this great healing potential of immature teeth favors the possibility that SCAP in the apical papilla and in some cases perhaps along with DPSCs in the survived dental pulp are important in this healing process. With regard to the regeneration/engineering of pulp/dentin or bioroot engineering using SCAP, the issue of cell source needs to be resolved simultaneously. Banking teeth as an autologous cell source and the potential use of allogenic stem cells both require further research to determine the ultimate benefits to our patients.

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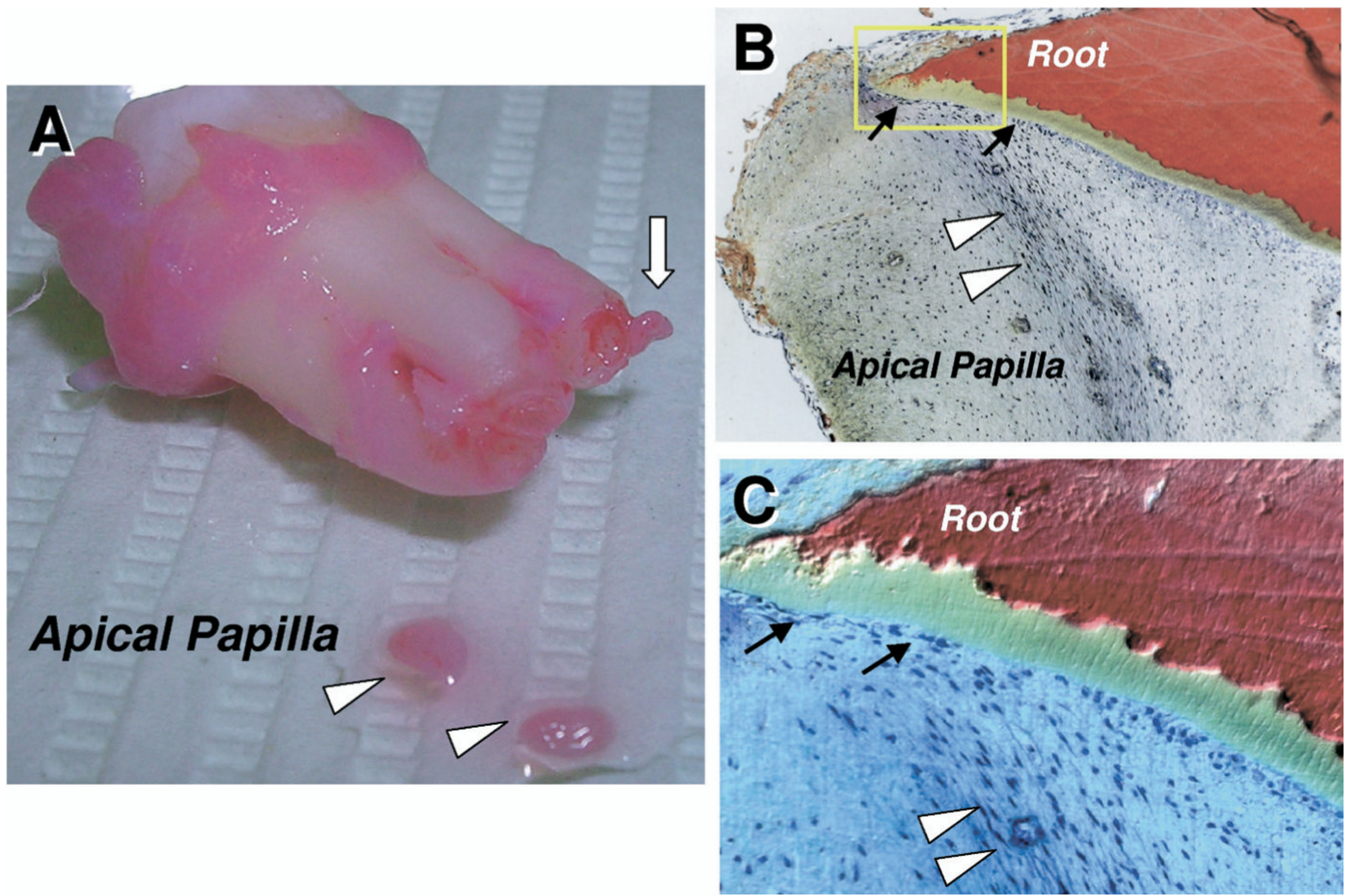

Figure 1.
Apical papilla. (*A*) An extracted human third molar depicting three immature roots with two pieces of apical papilla being removed from their apices (arrow heads) and one piece of apical papilla being peeled away from the root end but not completely detached (*arrow*). (*B*) A developing root tip with attached apical papilla was cultured in vitro for 3 days before being processed for hematoxylin and eosin (H&E) staining. Odontoblasts (*black arrows*), apical cell-rich zone (open arrowheads), and apical papilla tissue are indicated. (*C*) Magnified view of the area indicated by the yellow rectangle.

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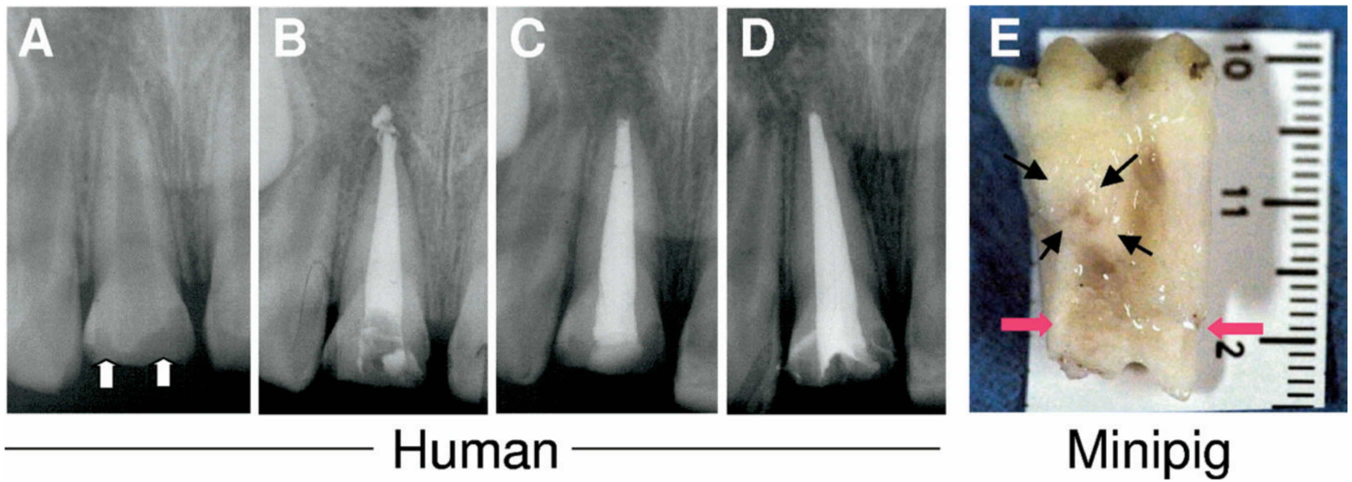

Figure 2.

The potential role of apical papilla in root maturation in human and minpig. (*A*) Human incisor crown fracture (arrows) occurred before the completion of root development. (*B*) Immediate root canal treatment was performed on the fractured tooth. Pulp tissue was completely removed and root canal was sealed with filling material. (*C*) Three months after endodontic treatment, the root tip continued to develop. (*D*) Seven months after treatment, a significant amount of root tip was formed. (*E*) When distal buccal root apical papilla of the lower first molar was surgically removed from a 9-month-old minipig, the distal buccal root stopped developing at the 3-month follow-up (*black arrows*), but other roots show a normal growth and development (*red arrows*).

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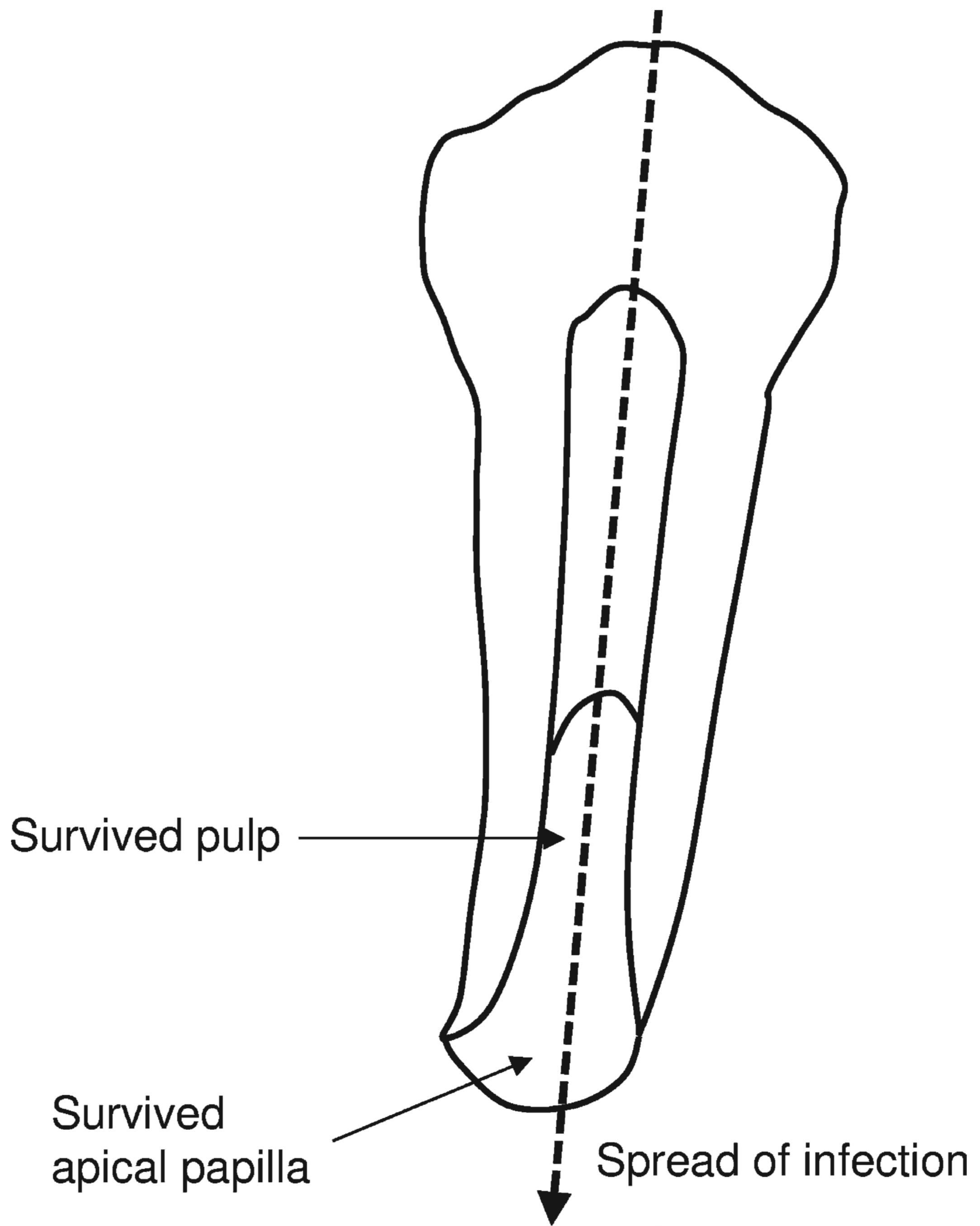

Figure 3.
The hypothetical pathway of infection of immature permanent teeth. The infection may pass through the survived pulp and apical papilla reaching the periradicular tissues and causing extensive bone resorption.

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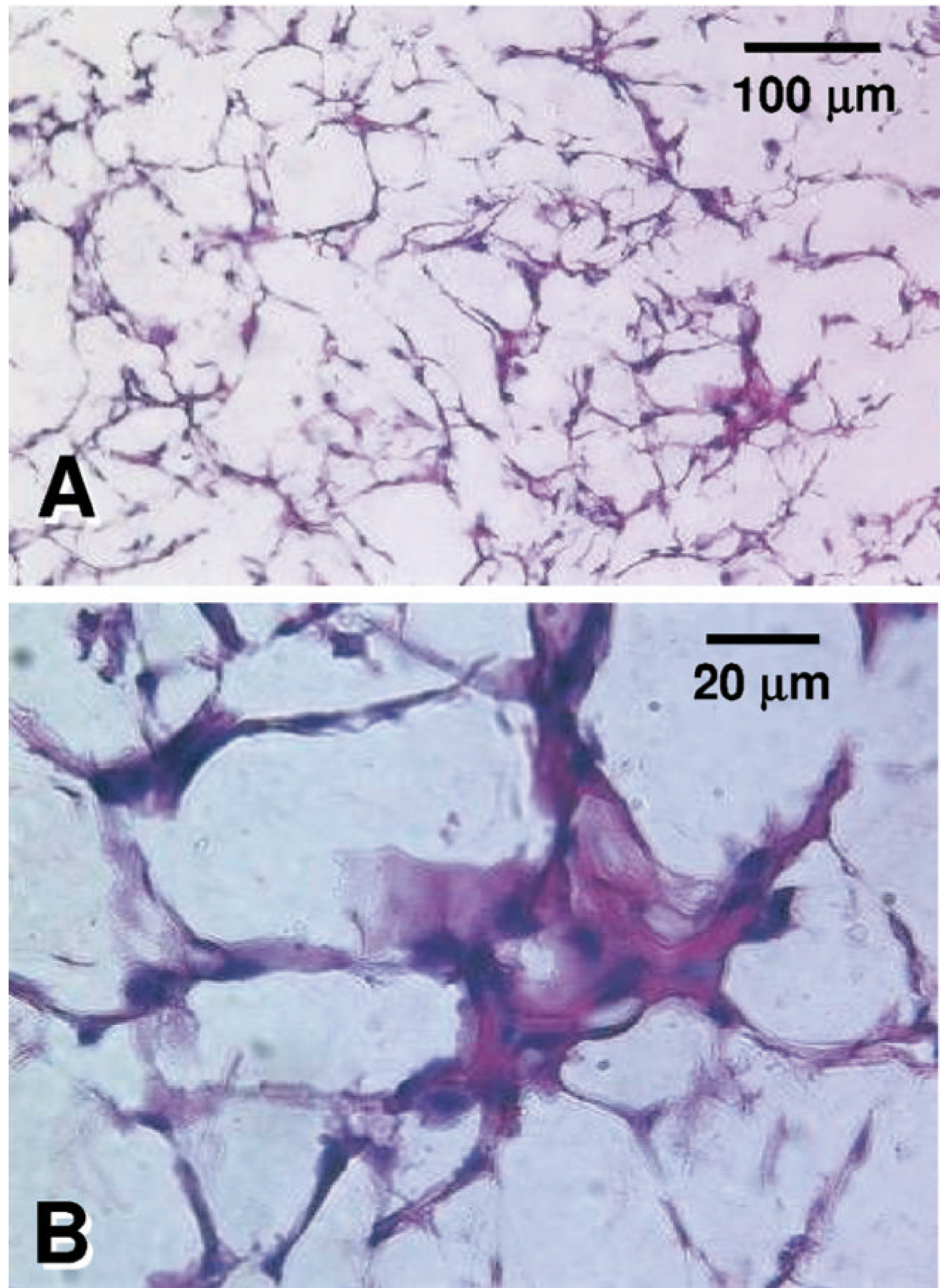

Figure 4.

DPSCs grown in PLG in vitro. DPSCs were seeded onto PLG scaffolds cylindrical shape, 5 (diameter) × 2 (height) mm with pore diameters between 250 and 425 μm kindly provided by Dr L.D. Shea (Northwestern University, Evanston, IL) and cultured in vitro for 7 weeks. Cells attached onto the inner surfaces of the scaffold (H&E). The scaffolds without cells (control, not shown) were also processed for H&E, and no visible staining was observed (original magnification: *A*, ×100; *B*, ×400).

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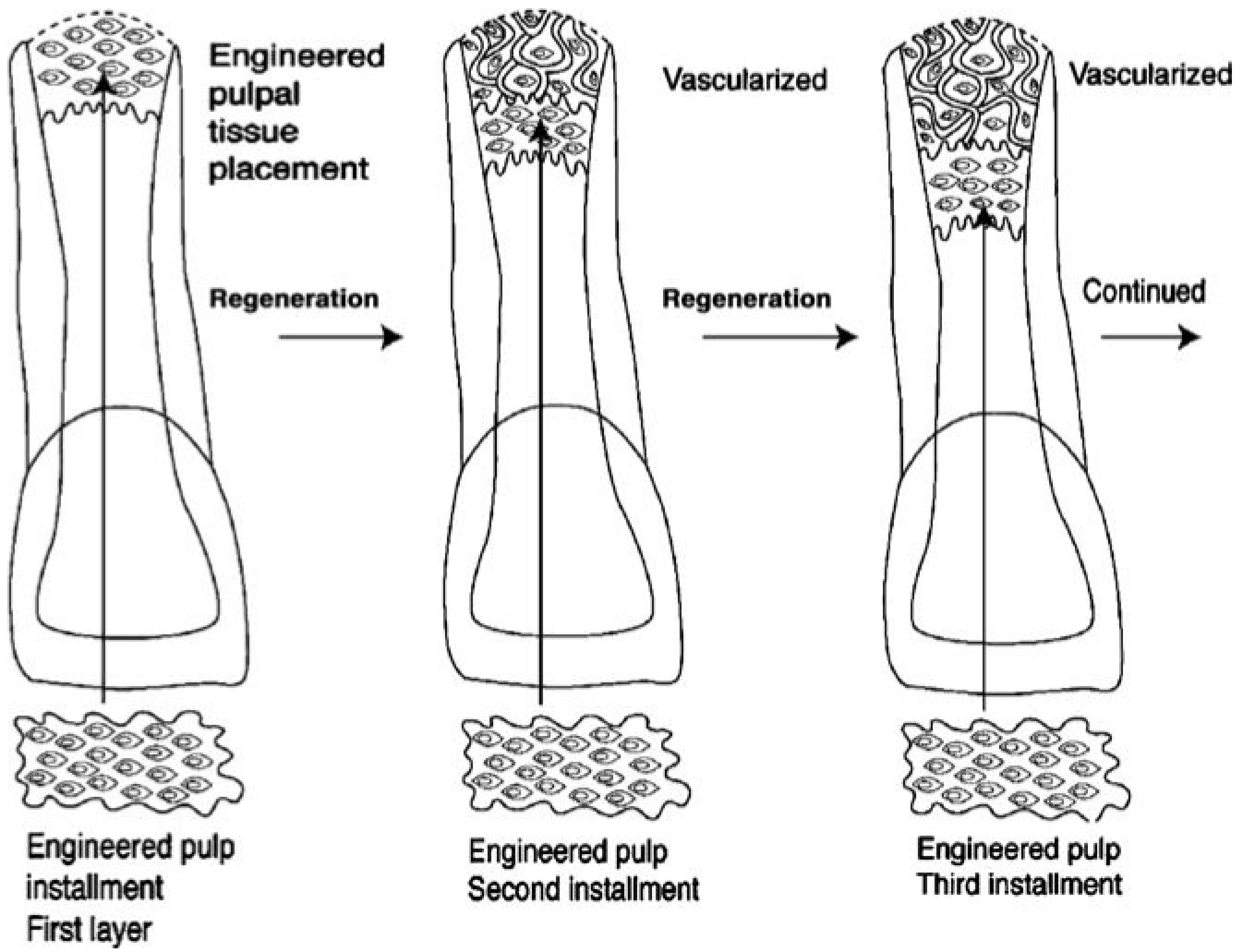

Figure 5.
A schematic illustration of proposed stepwise insertion of engineered pulp tissue in the clinical setting.