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The Outlook for Implants and Endodontics: A Review of the Tissue Engineering Strategies to Create Replacement Teeth for Patients

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Pulpal regeneration after tooth injury is not easy to accomplish, which is why the infected pulp requires tooth extraction or root canal therapy. Otherwise, more serious complications, such as periapical lesions, can occur [1]. In a few cases, partial pulpotomy, sometimes called Cvek pulpotomy [2], is the treatment of choice for injured permanent incisor teeth with exposed vital pulp tissue and immature apices [3]. This treatment preserves pulpal function and allows continued root development.

The healing of severely damaged teeth is difficult to accomplish [4]. The major problem seems to be the lack of ability of the dental pulp to regenerate its cell populations and mineralized structures after injury or infection. The loss or fracture of tooth dentin may expose the pulp tissue during operative dental treatment, because of accidental injury, or as a result of caries decay. The placement of a dental material in contact with exposed pulp tissue to restore the tooth structure is called direct pulp capping, and the placement of a dental material without any pulp contact is called indirect pulp capping. The prognosis of direct pulp capped teeth is much reduced in comparison with indirect pulp capped teeth. The success of direct pulp capping treatment is 37% after 5 years and 13% after 10 years [5], which compares with an 86% rate of success for indirect pulp capped teeth over 10 years [6]. Because of this low rate of treatment success, most dentists immediately

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refer patients for pulpotomy of part or all of the pulp tissue or extraction of the tooth [7]. This approach is one reason for the 21 million teeth that develop symptoms requiring endodontic therapy and the uncounted millions of teeth that are extracted every year in the United States [8]. One fourth of all Americans aged 65 to 74 have had all their teeth extracted. There seems to be a large variation between socioeconomic groups and states. Hawaii has the lowest rate of tooth extraction (13.9%) and West Virginia has the highest (47%) [9]. After tooth extraction, patients have the option of wearing artificial tooth implants or dentures. Currently, 45 million Americans wear dentures; one fourth are dissatisfied with these artificial teeth [9]. Each year, more than $60 billion are spent on professional dental treatments in the United States. The numbers and types of dental treatments are shown in Table 1.

Many aspects of endodontic therapy have proved to be controversial. A recent meta-analysis of the success rate for single versus multiple visit endodontic treatment found no significant difference in the success rate of teeth healing after apical periodontitis [10]. A survey of 350 endodontic treated teeth over 5 years reported that specialist endodontists can accomplish a treatment success rate of 98.1% compared with general dentists, who can accomplish a success rate of 89.7% [11]. The success rate of more than 85% for endodontically treated teeth seems to be supported by the dental literature [12–14]. It should be noted, however, that there is a general lack of information on long-term treatment success and that variations in the preoperative periapical status and the apical limit of the obturation may influence strongly the longevity of treatment [15].

During the last two decades, dental implants have become increasingly used as an alternative to conventional removable dentures. Some clinical studies have indicated that implant therapy has a favorable long-term prognosis [16]. The success rate of dental implants is approximately 90% [17,18]. The high clinical survival rate even in partially edentulous patients has led to a widespread acceptance and use of oral implants [19]. Problems do occur, however. Factors such as bone quality, surgical trauma, and bacterial contamination during implant surgery have been associated with early failures [20]. Overload, defined as a situation in which the functional load applied to the implants exceeds the capacity of the bone implant interface to withstand

Table 1
Numbers of dental treatments in the United States in millions per year

Dental treatments	Millions/y
Dental restorative treatments	300
Periodontal therapies	80
Endodontics (root canal therapies)	21
Cleft palate and birth defect surgery	0.25
Craniofacial trauma victims who require surgery	0.15
Cancer	0.03
Implants	0.1+

Stem cell therapies may impact all these dental treatments in the future.

it, is another possible cause of implant failure once the prosthesis is installed. Factors associated with late failures of implants are less well understood and seem to be related to the peri-implant environment and host parameters [21].

The 90% treatment success rates reported in the literature are a direct result of the dental profession continuing to improve the standard and quality of care provided to patients. To replace existing dental treatments, any new therapy must be proved to be more successful. The high success rates of existing dental therapies must be matched or exceeded by tissue engineering therapies if they are to be used successfully by the dental profession. This presents a formidable goal and barrier to the introduction of dental therapies that use tissue engineering approaches to regenerate diseased, lost, and missing teeth.

Odontoblasts and dentinogenesis

The successful resolution of restorative treatments depends on harnessing and using the natural repair responses of the pulpal cell populations, especially odontoblasts [22]. Odontoblasts are highly differentiated postmitotic cells that regulate dentin synthesis, secretion, and mineralization throughout life [23]. The other pulpal cell populations that occupy the subodontoblast layer and the pulp core are important in supporting dentinogenesis but do not seem to play a direct role in the secretion of dentin matrix [24]. The maintenance and repair of dentin are accomplished by the secretory activity of the odontoblast cells [25]. The odontoblasts are located peripherally around the pulp with their cellular process transversing dentin, and these cells have been demonstrated to detect and respond to dentin injury after caries and restorative dental procedures [26–31]. The rate of dentin secretion by odontoblasts has been observed to vary according to the chronology and circumstances of its secretion. Dentin can be classified as primary, secondary, or tertiary in origin. In humans, primary dentin is secreted at a rate of approximately 4 μm/d during tooth development until the completion of root formation [32]. Thereafter, physiologic secondary dentin is laid down at a reducing rate of approximately 0.5 μm/d along the pulp-dentin border throughout life [33,34]. Essentially, the odontoblasts go into a resting state after primary dentinogenesis, and the limited rate of secondary dentin formation over several decades represents a basal level of activity. If the dentin should become damaged, however, the synthesis and secretion of dentin are upregulated by the underlying odontoblasts to provide pulp protection. The increase in dentin secretion is called tertiary dentinogenesis because it is a dentin regenerative response by the odontoblasts. The histology of the pulp-dentin complex of teeth is shown in Fig. 1.

Tertiary dentinogenesis

The process of tertiary dentin secretion can be classified as reactionary in origin, depending on the severity of the initiating response and the

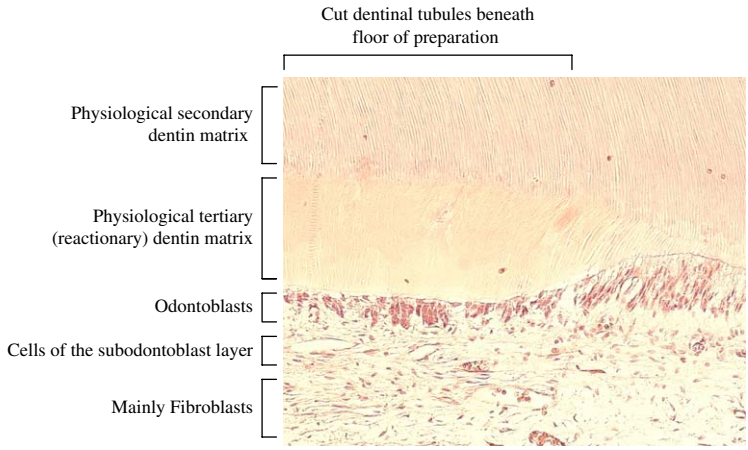

Fig. 1. Histology of the pulp-dentin complex of teeth.

conditions under which the dentin matrix was secreted [35–37]. The secretion of reactionary dentin is the main postoperative odontoblast repair response to an unexposed cavity restoration that has been cut carefully into the dentin of a tooth [38]. The rate at which reactionary dentin has been estimated to be laid down is 1.5 μm/d [39]. This rate is approximately three times the rate of normal secondary dentin mineralization. The secretion of reactionary dentin is initiated in response to environmental and accidental events, such as caries, attrition, abrasion, erosion, accidental trauma, and restorative dentistry. Sources of injury associated with restorative procedures have included acid etching of the cavity wall [40], presence of bacteria [41–43], method of placement of the restorative material [35,44], desiccation [45], pulpal inflammation relating to the remaining dentin thickness [46,47], chemical irritants [48], restoration material toxicity [49], and restoration material temperature [50,51]. Odontoblast survival is a multifactorial process and these factors may play a role individually or cumulatively in the degree of injury to the odontoblasts. If odontoblasts are not injured or are injured slightly, then no increase in dentinogenesis is likely to be observed in teeth. After more severe injury, some reactionary dentin deposition may be observed to repair lost or injured secondary dentin matrix. The secretion of reactionary dentin is proportional to the degree of injury to the pulp dentin tissue and the number of surviving functional odontoblasts [52].

If the odontoblasts are destroyed because of dentin injury or pulp exposure, then no reactionary dentin repair can be accomplished because no functional secretory odontoblast cells remain. Instead, pulp dentin repair is accomplished by an entirely different form of tertiary dentin called reparative dentinogenesis [53]. The reparative form of tertiary dentinogenesis is a much more complex biologic process than the reactionary type, because it involves the differentiation, proliferation, and migration of a new cell

population to replace the odontoblasts that were destroyed. An important factor that influences the severity of pulp dentin injury, the survival of odontoblasts, and their secretion of tertiary dentin is the remaining dentin thickness of dental preparations [54]. A schematic diagram of the relationship between remaining dentin thickness as the source of injury and the pulp healing response is shown in Fig. 2.

Reparative dentinogenesis

The dental pulp has a well-documented ability to form hard tissue barriers called reparative dentin after direct pulp capping or pulpotomy. If the reparative dentinogenesis forms across the wound site of a pulp exposure, it is called a dentin bridge (henceforth called a reparative dentin bridge) [55,56]. Ideally, a reparative dentin bridge establishes complete dentinal closure of the pulpal tissue; its presence after capping of the pulp exposure is observed in 90% of human pulps and is considered to be a sign of successful healing [4]. The structure of reparative dentin bridge can resemble tubular physiologic secondary dentin matrix, attubular osteodentin, or

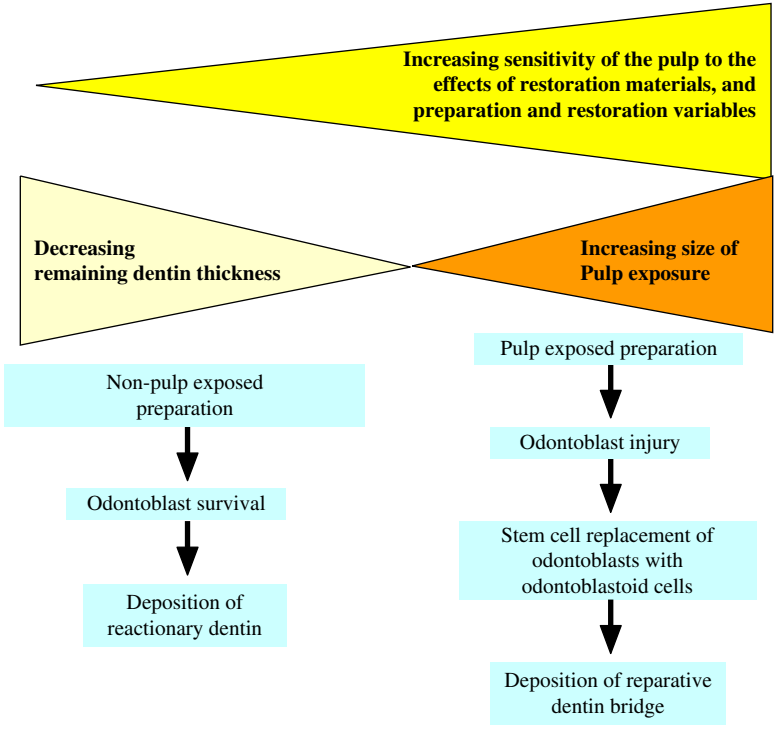

Fig. 2. Model of human pulp healing activity in response to the remaining dentin thickness of dental preparations.

globular fibrodentin. Fibrodentin formed as an intermediate matrix zone during the wound-healing process has been suggested for the initiation of reparative dentin bridge formation [26]. The observations of different types of reparative dentin bridge suggest that it is the culmination of a multistage regenerative process. The variable quality in reparative structure can influence dentin permeability in the wound region. Dentin permeability is the most important factor that determines pulp reactions to caries, operative procedures, and localized lesions [57]. A high permeability allows toxic substances from materials and bacteria leakage to reach the pulp easily [58], and they can act as irritants if their concentration reaches an inflammatory threshold [59]. The barrier properties of the reparative dentin bridge are advantageous for preserving the vitality of the pulp tissues, which explains the widespread interest in the process of reparative dentinogenesis and the number of investigations that assess the role of pulp dentin injury and restorative materials. Tooth dentin and reparative dentin bridge provide better protection for the pulp tissue than any restorative material [60]. The ability of the reparative dentin bridge to seal the site of tooth injury depends on the activity of the dental pulp stem cells (Fig. 3).

Source of pulp stem cells

Much attention has focused on the origin of the odontoblastoid cells that secrete the matrix for reparative dentin bridge. After severe pulp damage or mechanical or caries exposure, the odontoblasts are often irreversibly injured beneath the wound site [54]. Odontoblasts are postmitotic terminally differentiated cells and cannot proliferate to replace subjacent irreversibly injured odontoblasts [61]. The source of the odontoblastoid cells that replace

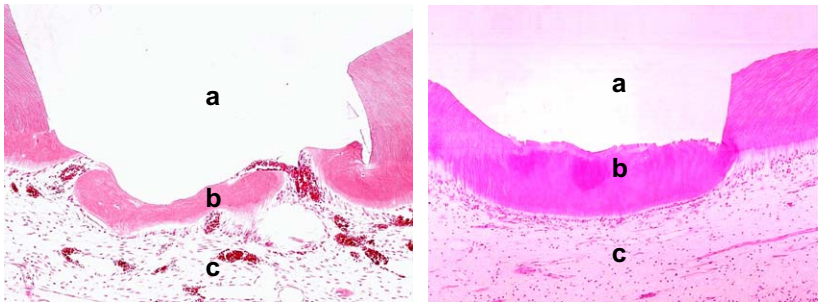

Fig. 3. Reparative dentin bridge formation by pulp stem cells. The histology of reparative dentin bridging is shown in two different teeth after direct pulp exposure. The dentin bridge formed on the right side is complete. Note the site of pulp exposure (*a*), the dentin bridge (*b*), and pulp tissue (*c*). (*Left*) The gaps in the dentin bridge probably were caused by a failure of the pulp stem cells to reach some parts of the site of injury and secrete tertiary dentin. In this case, blood clots may have been responsible. (*Right*) The complete formation of the dentin bridge suggests that the pulp stem cells were able to align across the site of pulp exposure. The addition of pulp stem cells may improve the quality of pulp repair.

the odontoblasts and secrete reparative dentin bridges has proved to be controversial. Initially, the replacement of irreversibly injured odontoblasts by predetermined odontoblastoid cells that do not replicate their DNA after induction was suggested. Researchers proposed that the cells within the subodontoblast cell-rich layer or zone of Hohl adjacent to the odontoblasts differentiate into odontoblastoids [62]. The purpose of these cells seems to be limited to an odontoblast supporting role, however, because the survival of these cells was linked to the survival of the odontoblasts, and no proliferative or regenerative activity was observed. The use of tritiated thymidine to study cellular division in the pulp by autoradiography after damage [63] revealed a peak in fibroblast activity close to the exposure site approximately 4 days after successful pulp capping of monkey teeth [64].

An additional autoradiographic study of dentin bridge formation in monkey teeth after calcium hydroxide direct pulp capping for up to 12 days revealed differences in the cellular labeling depending on the location of the wound site [65]. Labeling of specific cells among the fibroblasts and perivascular cells shifted from low to high over time if the exposure was limited to the odontoblastic layer and the cell-free zone, whereas labeling changed from high to low if the exposure was deep into the pulpal tissue. More cells were labeled close to the reparative dentin bridge than in the pulp core. The autoradiographic findings did not show any labeling in the existing odontoblast layer or in a specific pulp location. The findings provided support for the theory that the progenitor stem cells for the odontoblastoid cells are resident undifferentiated mesenchymal cells [63]. The origins of these cells may be related to the primary odontoblasts, because during tooth development, only the neural crest–derived cell population of the dental papilla is able to respond specifically to the basement membrane–mediated inductive signal for odontoblast differentiation [66,67]. The ability of young and old teeth to respond to injury by induction of reparative dentinogenesis suggests that a similar population of competent progenitor cells still may exist within the dental pulp, which can later differentiate into odontoblastoid cells. The debate on the nature of the precursor stem cells giving rise to the odontoblastoids and questions concerning the heterogeneity of the dental pulp population in adult teeth remain to be resolved [68,69].

Stem cells are defined by their capacity for asymmetric division, wherein a single cell division can give rise to one cell identical to the mother cell, a new stem cell, and another more differentiated cell [70]. The latter can be predetermined if it arises from a committed stem cell, whereas pluripotential stem cells can give rise to diverse progeny. Committed stem cells arise from these pluripotential stem cells, such as the adult mesenchymal stem cell or the embryonic stem cell. Although in adults, mesenchymal stem cells reside in hematopoietic tissue, committed stem cells often are located in the deep layers of tissues, where they are positioned to restore continuously the outer tissue cells lost during aging or mobilize rapidly to restore function

after disease or trauma [71]. Mesenchymal stem cells may have the potential to regenerate diseased, lost, and missing human dental structures [72]. A schematic representation of the lifecycle and activity of dental pulp stem cells is shown in Fig. 4.

Molecular regulation of dental pulp stem cell migration

Directional migration of stem cells is necessary for embryonic development and homeostatic maintenance and repair of injured organs and tissues in adults [73]. In the absence of migration, the contribution of stem cells to the development of functional organs and tissues would not be possible, because all stem cells must migrate to sites at which they are required to function. We have studied the molecular regulation of dental pulp stem cells and have found some similarities to the mechanisms of migration in other cell types. The Rho family of GTPases constitutes a family of intracellular messengers that are regulated by their location and state of activation. They seem to exert important influences in almost all functions of the stem cell, including adherence and migration [74]. Rho seems to exert important effects on cellular contraction and detachment, whereas Rac exerts effects needed for directed migration of polarized cells. Cdc42 activates many of the same receptors as Rac, but its effects seem limited to those involving cellular morphology and lamillopodia development. Preliminary studies have demonstrated Rac as being at the leading edge of migrating cells where Rho is either inactivated or disintegrated. Conversely, at the tail edge of migrating stem cells, activated Rho associates with its effector Rho kinase. The

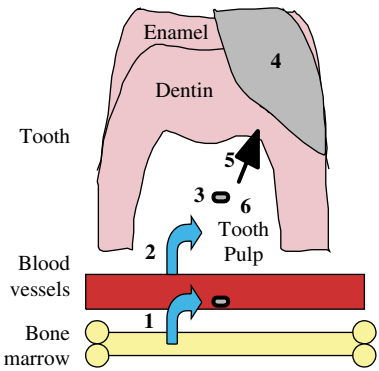

Fig. 4. Adult stem cell life cycle and activity. The sequence of the life cycle of adult stem activity is as follows: (*1*) Pluripotential mesenchymal stem cells reside in bone marrow. (*2*) These cells are mobilized to the circulation in response to specific factors generated during injury or infection. They localize to the site where needed by chemotactic migration through local tissue and differentiate into committed stem cells (*3*). In teeth they are known as pulp stem cells. (*4*) Tooth damage stimulates pulp stem cell migration and differentiation (*5*), which results in restoration of tooth structure, whereas asymmetric cell division maintains the pulp stem cell population (*6*).

effector or Rac that leads to actin development and migration is not clear but seems to be a unique enzyme, Pak-1 [75].

The kinase activity of Pak-1 is enhanced when it engages Rac in its GTP "activated" form. The targets for the enzyme that are essential for mobility are not clear, but myosin kinases activated by P13 kinase products, the stress kinase p38, and cell contractibility have been implicated. In the nucleus, the tumor suppressor protein p27 kip binds with its amino terminal region (N) to complexes of cyclins and cyclin-dependent kinases (CDKs) to inhibit cell proliferation. When phosphorylated, p27 kip1 might move into the cytoplasm, where, as shown by Besson and colleagues [76], it binds through its carboxy terminus to RhoA and interfaces with RhoA activation by guanine-nucleotide-exchange factors. RhoA, Cdc42, and Rac regulate the cytoskeletal changes required for cell migration. Cdc42 and Rac work mainly at the front of polarized cells and regulate the actin-driven protrusion and the formation of new adhesions required for forward movement. RhoA, through the Rho-associated kinase (ROCK) protein, works mainly at the rear and determines (among other processes) the turnover of adhesive sites, known as focal adhesions and rear retraction. By interfering with RhoA activation, focal adhesion kinase (FAK) inhibits or promotes cell migration, depending on the cell type. The migration of dental pulp stem cells seems to be controlled by a balance in Rac/Rho-kinase activation. When Rac is activated the cell migrates forward; when Rho-kinase is activated the cell remains fixed in position. Our hypotheses for the signaling pathways between the proteins involved in cell migration are summarized in Fig. 5. These proteins are useful targets for developing drugs to control stem cell migration as part of tissue engineering therapies.

Stem cell therapy

Stem cell therapy is one of the most promising areas of tissue engineering because the transplantation of materials that contain pulp stem cells grown in the laboratory provides an excellent inductive means to regenerate new tooth tissues. The transplantation of odontoblastoid stem cells into teeth to accomplish regeneration removes the problems of delivering growth factors and genes into host target cells and waiting for the target cells to differentiate, proliferate, and migrate to sites of injury before the reparative activity can commence. These considerations are important because pulp tissue replacement must be almost immediate to begin the regeneration of tooth tissues before the restorative treatment irreversibly fails. The use of cultured cells derived from adult human pulp tissue to regenerate lost cell populations eliminates the ethical drawbacks associated with fetal stem cell therapy. Because of ethical and legal restrictions associated with fetal human pulp stem cells, attention has focused on the xenotransplantation of human tissues into animals. Recently researchers called for a moratorium on research using xenographs because of the health hazards that this therapy presents to humans [77].

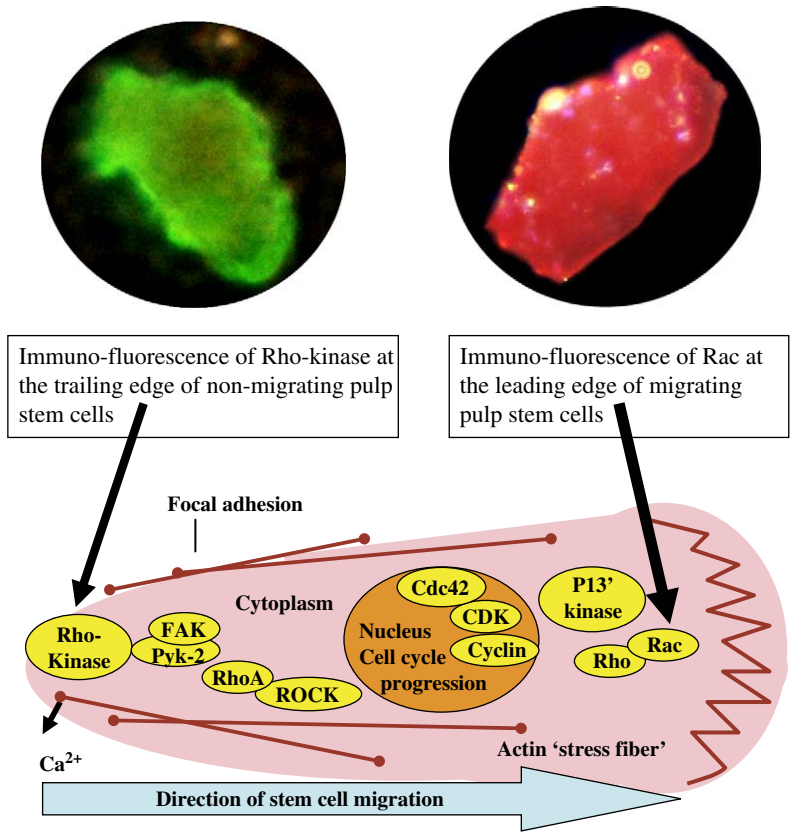

Fig. 5. The molecular regulation of pulp stem cell migration. The migration of dental pulp stem cells seems to be controlled by a balance in the Rac/Rho-kinase activation. When Rac is activated, the cell migrates forward. When Rho-kinase is activated, the cell remains fixed in position. The signaling pathways between Rac and Rho-kinase are shown here.

Recent studies focused on evaluating the use of human odontoblastoid stem cell transplantation for regenerating oral tissues in conjunction with in vitro tissue engineering to produce regenerative biomimetic materials.

Stem cell engineering of biomimetic materials

The regeneration of decayed or lost tooth tissue is problematic. No restorative material exists that can be placed into a tooth provides better protection for pulp tissue than dentin [78]. Any injury to the dentin pulp complex can trigger inflammatory cell activity [79]. These inflammatory reactions can injure the pulpal cell populations and lead to complications [80]. If unchecked, severe inflammatory activity often can progress to total pulp necrosis and lesion development followed by local bone destruction [81].

Observations of the low success rates of some types of restorative materials and how they contribute to postoperative complications, such as hypersensitivity, pulp inflammation [82], and treatment failure, have stimulated interest in the transplantation of natural tooth substance, which has been grown using in vitro culture. Few restorative materials share the same physical or chemical characteristics of the natural tooth, which may explain why a high proportion of cavity restorations fail mechanically. Resin-modified glass ionomer is a common restorative material [83]; however, surveys show that more than 50% of these restorations require replacement because of mechanical failure [84,85]. Few restorative materials share the appearance of tooth aesthetics, although resin composite materials can be color matched [86]. These results contrast with the use of tooth tissue, which shares the same chemical, physical, and aesthetic properties of natural teeth. These properties are ideal for restoring damaged tooth surfaces because abrasion, erosion, attrition, and tooth wear remain prevalent [87]. Porcelain veneers are often placed for aesthetic purposes, but the low success rates and longevity of this treatment would make the immediate replacement of lost tooth surfaces with synthetically mineralized tooth tissues a welcome advance in restorative dentistry [88].

Harvesting teeth created by tissue engineering

The ability to create in the laboratory teeth that can be harvested and implanted into patients to restore extracted or lost teeth long has been a goal for dental research [89,90]. The characterization of dental mesenchymal differentiation into pulp, dentin, and enamel has highlighted some developmental processes that may be used by tissue engineering therapies to create synthetic teeth [35,91–94]. The growth and formation of new teeth has been partially successful, because only small parts of tooth structures have been created and all of the approaches used existing developmental tooth structures as a template [95–97]. The development of tissue engineering science recently allowed new tissues, such as small intestine tissue, to be created after seeding stem cells onto biodegradable polymer scaffolds [98]. The use of this tissue engineering technique has allowed the formation of tooth crowns from stem cells taken from porcine third molars [99]. After the cell/polymer constructs have been formed using in vitro tissue culture, they are implanted into a suitable host animal to provide a vascular blood supply to support the growth and development of higher ordered tissue structures [100].

Adult human pulp stem cells have not yet used these techniques to create human teeth by tissue engineering, however. We propose that the future creation of replacement teeth for patients involve a chair-side technology with the following process: The first step is to create a computer-aided biomodel of the oral cavity and analyze the aesthetics of existing teeth. The second step is to use a database of tooth sizes, shapes, and aesthetics as a blueprint

for designing a replacement tooth. The third step is to biomanufacture the tooth using a scaffold and three-dimensional cell pattern printing and deposition methods. Slabs of biosynthetic enamel and dentin are cut into the shape of the tooth. The forth step is to implant the tooth surgically into the patient and reconnect blood flow, nerves, and periodontal ligaments. This process for the tissue engineering of a replacement tooth is shown in Fig. 6. Much of this technology already exists or is close to development, and the goal for dental researchers is to put the technology together and make it work reliably.

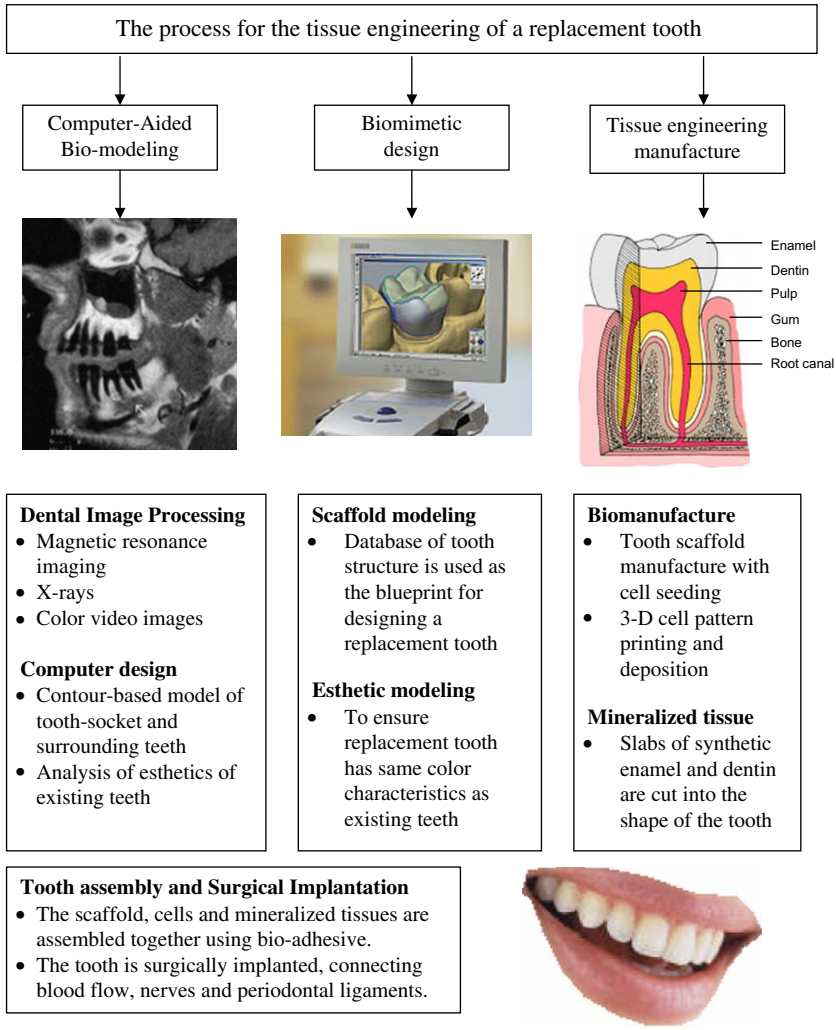

Fig. 6. The tissue engineering of a replacement tooth.

Significance of tissue engineering therapy

An increase in the success of restorative dental treatment will transform completely the economics of oral health in the United States. Every year it is estimated that $60 billion are spent on dental expenditure [101]; $5 billion per year has been saved, mostly because of preventive dentistry [102]. Total expenditure continues to increase every year, however. Because two thirds of all restorative dentistry cases involve the replacement of failed treatments, a tremendous potential remains for improvement [81,82]. Even a small percentage of increase in the ability to preserve severely injured teeth could cut dental expenditure dramatically by reducing the number of patients who require dentures or tooth implants. Forty-five million Americans wear dentures, and at least one fourth are dissatisfied with these artificial teeth [9]. Although there always has been a need to minimize the number of teeth that are extracted, the limited ability of conventional restorative treatments to regenerate teeth has prevented dentists from saving many diseased and damaged teeth from extraction. The introduction of stem cell–based therapies means that more teeth may be saved, which could reduce dramatically the numbers of Americans who must wear dentures or care for their artificial tooth implants. This progress will improve the quality of life for millions of ordinary people.

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