Test 229
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Disclosure: Dr. Robert Gregg II is president of Millennium Dental Technologies, Inc.

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Disclosure: Dr. Dawn Gregg is vice president of operations of Millennium Dental Technologies, Inc.

Laser-Assisted Periodontal Regeneration and Human Histology

This article presents 2 case studies that demonstrate the human histological evidence required to make the claim of true periodontal regeneration using the laser-assisted regeneration (LAR) protocol.

Scientific evidence to support a treatment modality is the hallmark of the ethical practicing clinician. Human histology is the highest form of scientific analysis for proving the truth of a complex healing event such as periodontal regeneration. To date, hundreds of positive patient outcomes using the laser-assisted regeneration (LAR) protocol have been published by various authors in numerous peer-reviewed and non-peer-reviewed publications and in 2 International Association for Dental Research abstracts.[1-11]

Periodontal regeneration on a previously diseased tooth root surface is a unique, challenging, and elusive healing event to obtain in humans.[FN-1] True periodontal regeneration requires the 3 original components of the periodontal apparatus to arise anew and eventually form into new cementum, a new periodontal ligament (Sharpey's fibers), and new alveolar bone.[FN-2] The health of the attachment apparatus can be assessed by measuring the clinical attachment level (CAL) (the distance from the cementoenamel junction to the tip of a periodontal probe during periodontal diagnostic probing).

Typically, the realization of complete regeneration of the 3 tissues of the periodontal attachment apparatus has required extensive surgical site preparation to allow for instrumentation sufficient for the addition of exogenous materials such as growth factors, bone grafts, and biologics. Proving a claim for true periodontal regeneration in humans *requires* that histologic, microscopic analysis be conducted in living human beings.

In this article, 2 examples from 2 different human histology studies are presented—one for a single-rooted tooth[5] and the other for a molar tooth.[8] The studies were conducted by 2 separate principal investigators that were included in 2 separate submissions to the US Food and Drug Administration (FDA) over a period of 12 years. As a result of these submissions, the FDA issued a clearance (March 2016) for the claim of "true periodontal regeneration" using the PerioLase MVP-7 pulsed Nd:YAG Dental Laser System (Millennium Dental Technologies) with the LAR protocol. These cases included clinical assessments, radiographic evidence, microcomputed tomography (microCT), light microscopy, and backscatter scanning electron microscopy together with a human histological evaluation of 3 months duration in one study[5] and 9 months duration in the more recent study.[8]

The objective of this article is to demonstrate the histological evidence required to make the claim of true periodontal regeneration, based on the FDA clearance noted above.

Evidence-Based Dentistry

Evidence-based dentistry (EBD) has become a buzzword in certain arenas of academia and organized dentistry. Not all who use the term are defining EBD the same way. Some define EBD in ways that fit their personal perspectives, such as only what the literature states in a systematic review, but that is not EBD. EBD integrates 3 important components[12,13]:

1. the most current, clinically relevant scientific evidence;
2. a dentist's clinical expertise; and

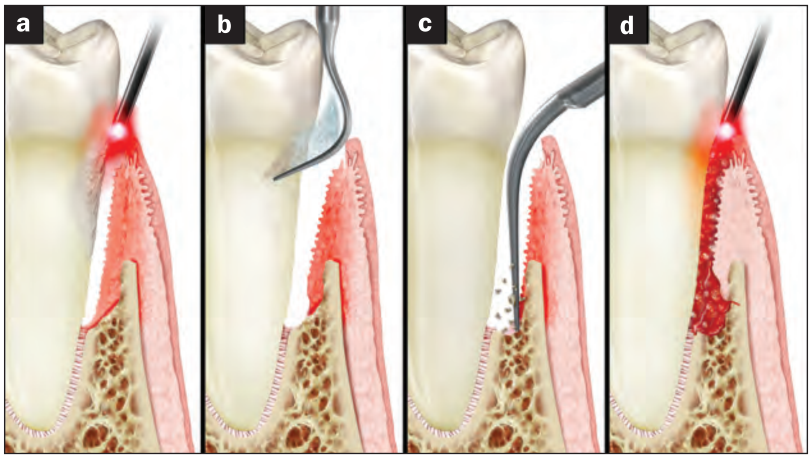


Figure 1. Essential components of the laser-assisted regeneration (LAR) protocol. **(a)** The LAR protocol's pulsed Nd:YAG laser selectively vaporizes bacteria, endotoxins, and diseased epithelial lining of the pocket; denatures pathologic proteins; and alerts the practitioner to the presence of calculus. **(b)** An ultrasonic scaler and specialized tips are used to remove root surface calculus. **(c)** Bone is modified to reshape; to decorticate; and to stimulate the release of fresh blood, stem cells, and growth factors. Angiogenesis is initiated. **(d)** The same laser is then used to form a stable fibrin clot, activate growth factors, and upregulate gene expression.

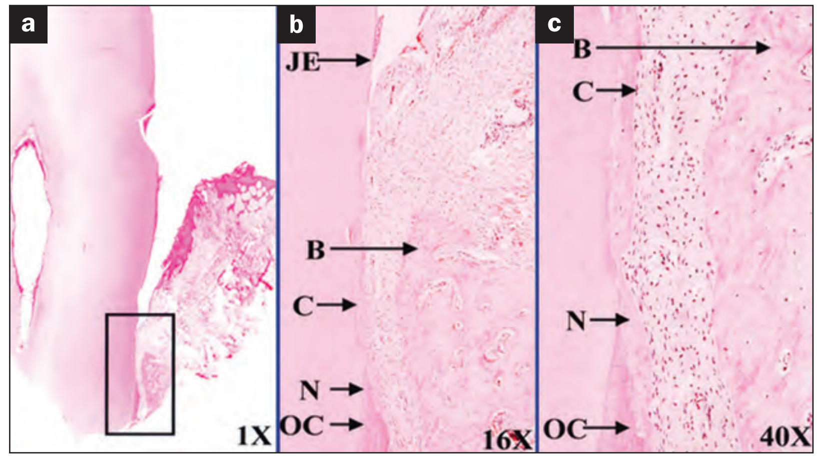


Figure 2. A periodontal regeneration of tooth No. 6: **(a)** Histologic sections, stained with hematoxylin and eosin, of the maxillary right canine with a deep infrabony defect 3 months after laser treatment (inset box around area of interest). **(b)** A medium-power (16×) view, demonstrating the apical extent of the junctional epithelium (JE), new bone (B) adjacent to the calculus notch (N) and new cementum (C) in and coronal to the notch, and old cementum (OC) apical to the notch. **(c)** A higher-magnification (40×) view, showing new alveolar bone (B), a new periodontal ligament and gingival fibers attached to the tooth, new cementum (C) filling the notch (N) and extending coronally, and old cementum (OC) apical to the notch covered by new cementum.

3. each individual patient's needs and preferences.

EBD is actually being practiced only when all 3 components are given due consideration in individual patient care. The clinical, human histological evidence,[5,8] as well as an American Academy of Periodontology (AAP) systematic review,[14] provide substantial evidence of compliance with the essential EBD components.

Regeneration Background

Periodontitis is an infectious disease that progressively destroys the alveolar bone, periodontal ligament (PDL), and root cementum that attach the teeth to the bone. Destruction of this attachment apparatus results in the loss of teeth.

Quest for the Holy Grail:

"...the Holy Grail of periodontics: the restoration of the destroyed periodontium by new cementum, periodontal ligament, and bone, as well as their *re-creation in normal anatomic relationships and function.*"[15]

Kao et al[14] point out that various approaches have been developed in attempts to achieve periodontal regeneration, among them the use of a demineralized freeze-dried bone allograft, guided tissue regeneration with the use of barrier membranes, the application of biomimetic agents such as enamel matrix derivative, and the LAR technique. Of these, the laser protocol is the least invasive.[FN-3]

The AAP's Position Paper on Periodontal Regeneration (2005)[16] defines terms relevant to this discussion:

- *New attachment* is defined as the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be an epithelial adhesion and/or a connective tissue adaptation or attachment and may include new cementum to which periodontal ligament fibers are attached.
- *Bone fill* is defined as the clinical restoration of bone tissue in a treated periodontal defect. Bone fill does not address the presence or absence of histologic evidence of a new connective tissue attachment or the formation of a new periodontal ligament.
- *Regeneration* refers to the reproduction or reconstitution of a lost or injured part, in contrast to *repair*, which describes the healing of a wound by tissue that does not fully restore the architecture or function of the part.
- *Periodontal regeneration* is defined *histologically* as regeneration of the tooth's supporting tissues, including (1) alveolar bone, (2) the periodontal ligament, and (3) cementum over a previously diseased root surface.

Figure 3. A maxillary left first molar (tooth No. 14) presented with an initial clinical attachment level (CAL) of 11 mm.

Figure 4. A grade III furcation, and a mesial intrabony defect.

Figure 5. The 9-month post-treatment radiograph suggests bone fill.

The ultimate aim of periodontal regeneration techniques is to induce or guide healing to regenerate the morphology back to its original configuration. In order to evaluate a regeneration technique experimentally, a notch is made on the root surface at the bottom of a periodontal pocket to provide a histological landmark for the apical extent of the destruction and determination of subsequent coronal periodontal wound healing. True periodontal regeneration is then defined by histological evidence of new bone, a periodontal ligament, and cementum appearing above the notch on a previously diseased root surface.[17]

Yukna et al[5] wrote in their peer-reviewed and published manuscript:

The 1996 World Workshop in Periodontics established specific histologic criteria for proof of regeneration. Experimental teeth must have loss of connective tissue attachment (CTA) and alveolar bone

(AB) associated with periodontitis. In addition, subgingival and/or subcrestal calculus must be present at the time of surgery so that a [bur] notch can be made into the root at the apical extent of calculus (into the calculus and beyond its apical boundaries). Proof of new attachment is demonstrated by new cementum (CEM) and CTA; and regeneration is evidenced by the presence of new CEM, PDL, and AB coronal to the apical extent of the notch.

According to Yukna et al[5], "The three most central 200-µm serial step sections were *blindly* and *randomly evaluated* for the nature of the healed tissues—specifically the presence and length of new CEM, new CTA, new AB, and healed junctional epithelium relative to the apical extent of the calculus notch."

LAR Protocol

A near-infrared, variable pulsed dental laser specifically designed and optimized for the LAR protocol was utilized in both studies (PerioLase MVP-7 pulsed Nd:YAG Dental Laser System). In the LAR protocol, no exogenous materials of any sort are used. The true periodontal regeneration achieved with LAR is accomplished by using the patient's own blood proteins that contain the stem cells, fibroblasts, native growth factors, and blood constituents. The blood is thermally affected in LAR in such a manner that the regenerative factors are trapped within a red thrombus that is formed using scientifically determined algorithms of optimal laser operating parameters (Figure 1).

In LAR, there is a lack of any wide surgical access outside of the bony housing, a lack of deep dissection into the vestibule or across the palate, and a lack of extensive vertical releasing incisions. The protocol uses a minimally invasive periodontal flap to enable access *under the periosteum* and directly *into bone for ostectomy and/or osteotomy*. The soft tissues are thereby easily approximated and stabilized without the need to suture with tension to adapt the margins of the flaps together. It is essential to provide a sealed system of unviolated and unmolested external periodontal tissues for the regenerative growth factors to be contained within for protection of the regenerative cells and process.

CASE 1
Human Canine Histological Study

Figure 2 demonstrates the histological evidence of periodontal regeneration for tooth No. 6 using the LAR protocol.[5]

CASE 2
Human Molar Histological Study

Figures 3 to 18 demonstrate the histological evidence of periodontal regeneration for tooth No. 14 using the LAR protocol.[8]

DISCUSSION

In the study demonstrated in Case 2, Nevins et al[8] noted, "*The supracrestal environment regenerated to its natural state* with supracrestal inserting collagen [Sharpey's] fibers into the new cementum just apical to the junctional epithelium [emphasis added]...."

This is a very rare and exceptional healing event. Not only is there regeneration of the lost attachment apparatus, but of the supracrestal environment as well. This is an extraordinary healing event to document, and it is the pinnacle of periodontal regenerative responses, achieving the maximum height of possible regeneration with new supracrestal fibers just apical to the CEJ.

From the histological data, clinicians can extrapolate from scientific research on the LAR protocol to clinical expectations when following the strict LAR protocol and treatment algorithms.

SUMMARY

The restoration of destroyed periodontium and re-creation of its normal anatomic relationship and function is the ultimate aim of periodontal regeneration methodologies. One particular well-defined LAR technique has been shown to regenerate the attachment apparatus (new cementum, periodontal ligament, and alveolar bone) on a previously diseased root surface. Published histological evidence from 2 human studies provides the scientific authentication of true periodontal regeneration achieved through the minimally invasive surgical LAR protocol. These investigations confirm the clinical findings and radiographic evidence reported by clinicians who

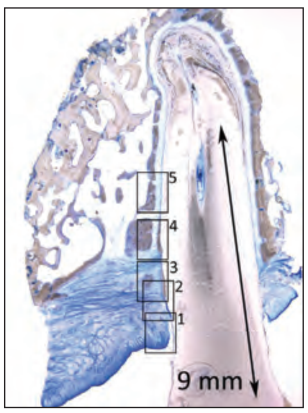


Figure 6. A panoramic view, showing bone fill and *periodontal regeneration*. The clinical attachment level (CAL) gain at 9 months was 5 mm.

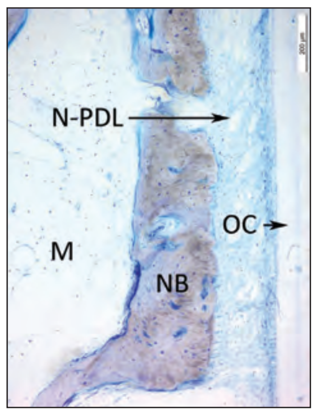


Figure 7. This close-up of inset 5 from **Figure 6** demonstrates new alveolar bone (NB) and a new periodontal ligament (N-PDL). (M is bone marrow; OC is old cementum.)

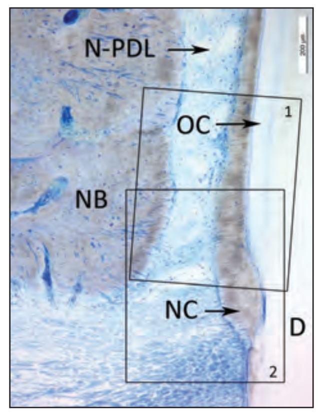


Figure 8. In this close-up of inset 4 (from **Figure 6**), a distinct layer of new cementum (NC) is several millimeters coronal to the calculus notch. (D is dentin.)

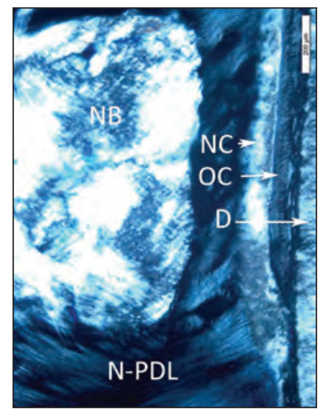
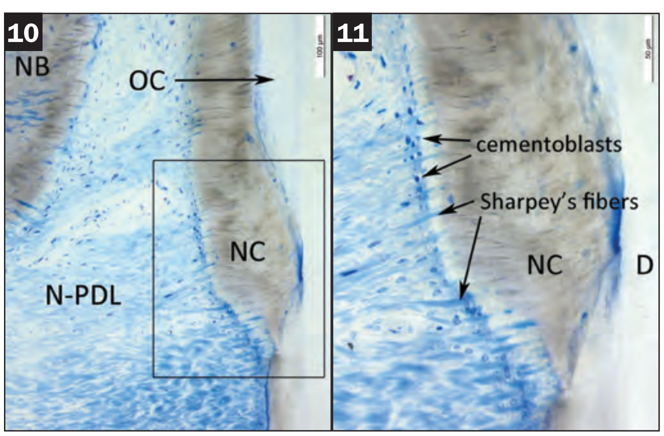


Figure 9. Polarized light microscopy enables the visualization of bundles of collagen fibers connecting the new bone to the new cementum.



Figures 10 and 11. Higher magnifications of box 2 from inset 4 (**Figure 8**), showing Sharpey's fibers and cementoblasts on the surface of the new cementum.

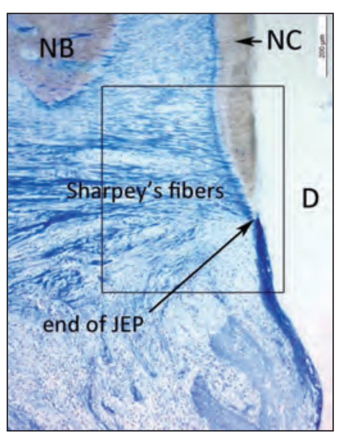


Figure 12. This close-up of inset 2 (from **Figure 6**) demonstrates that the supracrestal environment has regenerated to its natural state with Sharpey's fibers inserting into the new cementum just apical to the junctional epithelium (JEP).

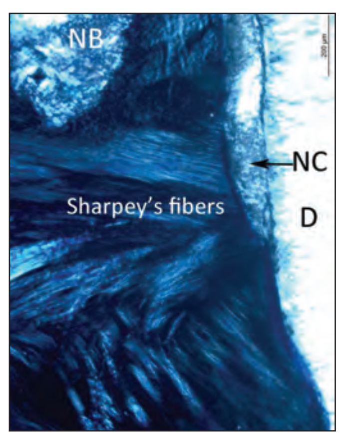


Figure 13. Polarized light reveals fascicles of birefringent collagen fibers.

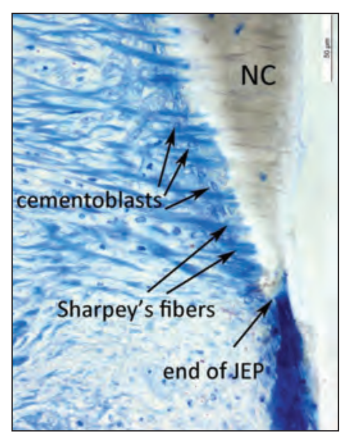


Figure 14. A high-magnification view of the box from inset 2 (**Figure 12**), showing cementoblasts and Sharpey's fibers at the junction with the JEP.

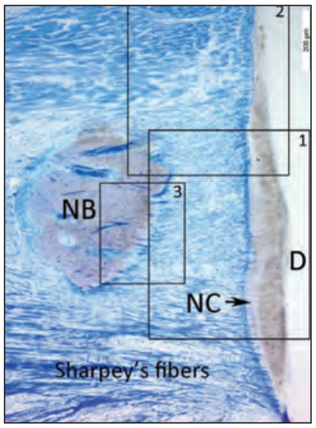
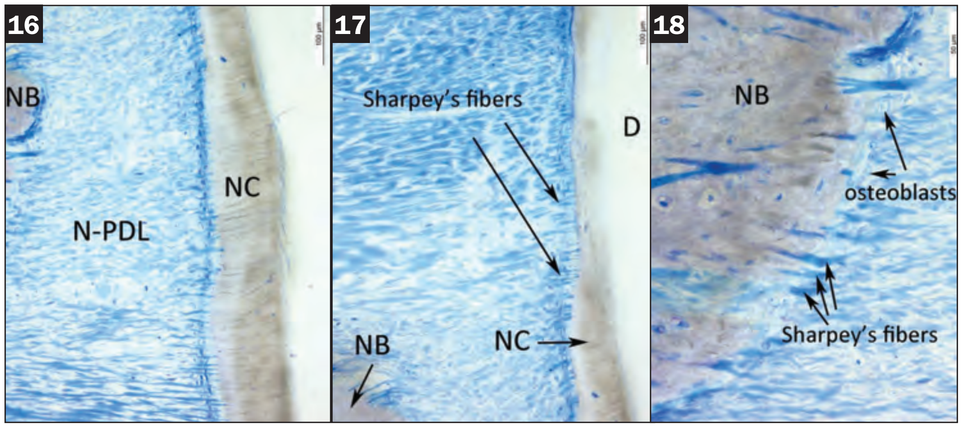


Figure 15. Inset 3 (from **Figure 6**) shows continued regeneration of the supracrestal environment 9 months following treatment. There is an island of new bone (NB), and a thick layer of new cementum (NC) is adjacent to the root dentin (D), with interconnecting fibers of the PDL Sharpey's fibers perpendicular to the new cementum, establishing a new level of attachment and presenting a barrier to JEP migration.



Figures 16 to 18. Higher-magnification of boxes 1 to 3 from inset 3 (**Figure 15**) shows the orientation of Sharpey's fibers.

have utilized this technique over the last 20 years to achieve true periodontal regeneration in patients with moderate-to-severe periodontal disease.✦

Acknowledgments:

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Figure 2 was reprinted from Yukna RA, Carr RL, Evans GH. Histologic evaluation of an Nd:YAG laser-assisted new attachment procedure in humans. *Int J Periodontics Restorative Dent.* 2007;27:577-587. Figures 3, 4, 5, 6, 8, 11, and 12 were reprinted from Nevins ML, Camelo M, Schupbach P, et al. Human clinical and histologic evaluation of laser-assisted new attachment procedure. *Int J Periodontics Restorative Dent.* 2012;32:497-507. By permission of Quintessence Publishing Co, Inc, Chicago.

Footnotes

1. "Currently, osseous grafting and guided tissue regeneration (GTR) are the two techniques with the most histologic documentation of periodontal regeneration. Other regenerative therapies have also provided a promising potential for significantly improving clinical parameters and demonstrating substantial 'fill' of treated defects. However, only limited histologic evidence of true regeneration has been demonstrated with the majority of these therapies."[16]

2. "Regeneration refers to the reproduction or reconstitution [restoration] of a lost or injured part, in contrast to repair, which describes healing of a wound by tissue that does not fully restore the architecture or the function of the original part. Periodontal regeneration is defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum over a previously diseased root surface. New attachment is defined as the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/or connective tissue adaptation or attachment and may include new cementum. It is to be distinguished from reattachment, which describes the reunion of epithelial and connective tissue with a root surface. Bone fill is defined as the clinical restoration of bone tissue in a treated periodontal defect. Bone fill does not address the presence or absence of histologic evidence of new connective tissue attachment or the formation of new periodontal ligament."[16]

3. Kao et al[14] is the most recent of a long series of systematic reviews of published methods to achieve periodontal regeneration.[18-27] Current approaches include a demineralized freeze-dried bone allograft (DFDBA), guided tissue regeneration (GTR), bone fill with enamel matrix derivative (EMD), recombinant human platelet-derived growth factor BB (rhPDGF-BB), and open flap debridement (OFD). Kao et al[14] have included a new category of regenerative approaches, termed laser-assisted regeneration (LAR), which is specifically identified by the authors as the Millennium Dental Technologies' LANAP protocol that uses the PerioLase MVP-7 pulsed Nd:YAG Dental Laser System.[FN-4] Based on the review of 2 peer-reviewed studies of human histology,[5,8] following LAR, Kao et al[14] conclude, "Using the Nd:YAG laser with this procedure, periodontal regeneration is achievable on a previously diseased root surface."[FN-5]

4. "...this technique is intriguing in that it is another approach to minimally invasive surgical therapies as reviewed by Cortellini.[28] A minimally invasive surgical approach may offer advantages in regeneration of defects in the esthetic zone in which minimal soft tissue change is required. Additionally, because of the minimally invasive nature and expendable surgical materials required, this approach may be appropriate for multiple defects as a first line of management."[14]

5. Regarding other laser devices, one cannot extrapolate regenerative findings to other laser devices or treatments that have no defined protocols, operating parameters, or treatment algorithms and have no documentation of healing events from human histological studies.

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TEST 229 Expiration date of this CE article is January 1, 2022

To submit Continuing Education (CE) answers, use the answer sheet below. Or, use our easy online option at dentalcetoday.com. This article is available for 2 hours of CE credit. The following 10 questions were derived from "Laser-Assisted Periodontal Regeneration and Human Histology" by Robert H. Gregg II, DDS, and Dawn M. Gregg, DDS, on pages 70 to 73.

Learning Objectives: After reading this article, the individual will learn: (1) the essential components of the laser-assisted regeneration (LAR) protocol, and (2) the histological evidence necessary to support the claim of true periodontal regeneration. **Subject Code:** 490.

1. What are the components of the periodontal attachment apparatus?
- **a.** Fibroblasts, junctional epithelium, and lamina propria.
- **b.** Cementum, periodontal ligament, and alveolar bone.
- **c.** Sharpey's fibers, bone marrow, and dentin.
- **d.** Odontoblasts, gingival epithelium, and enamel.

2. What is the main function of cementum?
- **a.** To provide anchorage for periodontal ligament fibers.
- **b.** To protect the predentin from periodontopathogens.
- **c.** To generate fibroblasts for collagen bundles.
- **d.** To remodel alveolar bone through an osteoclastic process.

3. Which of the following fiber types insert into alveolar bone and cementum?
- **a.** Von Korff fibers.
- **b.** Alveologingival fibers.
- **c.** Circular fibers.
- **d.** Sharpey's fibers.

4. Which of the following regenerative procedures is the least invasive?
- **a.** A demineralized freeze-dried bone allograft.
- **b.** A guided tissue regeneration via membranes.
- **c.** A laser-assisted new attachment procedure.
- **d.** The application of an enamel matrix derivative.

5. What is the definition of "clinical attachment level"?
- **a.** The measurement from the crest of the gingival margin to the base of the pocket.
- **b.** The distance between the apical extent of the calculus notch and the basement membrane.
- **c.** The measurement from the dentoenamel junction to the marginal ridge.
- **d.** The distance from the cementoenamel junction to the tip of the periodontal probe.

6. What is the rationale for performing decortication to treat intrabony defects?
- **a.** To enhance the bone-healing process by increasing revascularization.
- **b.** To facilitate bone remodeling by conventional instrumentation.
- **c.** To establish histological landmarks to evaluate new attachments.
- **d.** To remove overlying necrotic osseous tissue and lipopolysaccharides.

7. What is the primary justification for performing occlusal adjustment during a laser-assisted regeneration procedure?
- **a.** To minimize patient discomfort during the healing event.
- **b.** To ensure an optimum aesthetic result post-healing.
- **c.** To reduce traumatic forces that cause the breakdown of supporting structures.
- **d.** To provide unobstructed excursive glides of the mandible.

8. Rete ridges strengthen the attachment of epithelium to which structure(s)?
- **a.** Tooth cementum.
- **b.** Connective tissue.
- **c.** Salivary glands.
- **d.** The trigeminal nerve.

9. In a human histological study, what is the purpose of placing a calculus notch on a root surface?
- **a.** It inhibits the apical migration of long junctional epithelium.
- **b.** It enables the assessment of subsequent periodontal wound healing coronally.
- **c.** It releases the tension of circumferential periodontal fibers for greater access.
- **d.** It increases the surface area for a stronger retention of splints in mobile teeth.

10. In periodontal tissues, how is regeneration differentiated from repair?
- **a.** Regeneration re-adapts tissue to periodontal defects.
- **b.** Repair re-establishes the function of the injured tissue.
- **c.** Regeneration restores lost or diminished periodontal tissue.
- **d.** Repair restores the architecture of original tissue.

ANSWER SHEET Test 229, beginning on page 70

Complete the paper answer sheet and submit your answers for this issue's CE examination. Answer at least 7 of the 10 questions correctly to earn 2 CE credits. Please check the correct box for each question below.

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PROGRAM EVAUATION FORM

Please complete the following activity evaluation questions.

Rating Scale: Excellent = 5 and Poor = 0

Course objectives were achieved. ________

Content was useful and benefited your clinical practice. ________

Review questions were clear and relevant to the editorial. ________

Illustrations and photographs were clear and relevant. ________

Written presentation was informative and concise. ________

How much time did you spend reading the activity and completing the test? ________

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