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Contemporary approaches to biofilm management in the 21st century's oral health crisis

A PEER-REVIEWED ARTICLE | by Lisa Dowst-Mayo, MHA, BSDH, RDH

ABSTRACT
Annual gross domestic product dollars spent on oral care in the United States has decreased each year since 2013, while the prevalence rates of periodontal disease and the percentage of dentally uninsured adult Americans continues to rise. This public health crisis puts added pressure on oral health-care providers to treat patients in oral dysbiosis. Manufacturers are responding with new and innovative technology that raises the standard of care and provides the tools necessary to treat this millennium's dental patient. It is now up to practitioners to incorporate this evidence-based technology into their care plans and office protocols. This course will discuss the oral microbiome and the contributing factors to symbiosis and dysbiosis. Research trends in biofilm management that utilize the most current technology available will be presented to assist providers in treating the 21st century dental patient.

EDUCATIONAL OBJECTIVES
At the conclusion of this course, the oral health-care provider will be able to:
1. Develop a deeper understanding of health statistics in the United States as they pertain to oral health.
2. Differentiate between symbiosis and dysbiosis and relate those concepts to the etiology of periodontal and peri-implant diseases and conditions.
3. Identify the potential role genetics plays in the predisposition and management of patients' periodontal disease status.
4. Utilize the most current dental technology available for biofilm management to promote oral symbiosis.

Periodontal disease prevalence rates are at an all-time high with 47.2% of adult Americans age 30-plus years and 70% age 65-plus years.[1,2] When untreated and uncontrolled, periodontal disease adds inflammatory burden to the human body, which contributes to systemic dysbiosis. The etiology of periodontal disease is complex, but one statistical anomaly that should be considered is the percentage of Americans without dental insurance. When uninsured, financial barriers may prevent many from seeking treatment for their oral disease. The Centers for Disease Control and Prevention (CDC) reports 50.2% of dentate American adults (age 18–64) have dental insurance and less than one-fourth (21.1%) of those individuals did not see a dentist in the past 12 months.[1] When one in two adult Americans has dental insurance and almost one in two Americans has active periodontal disease, one cannot help but see a possible link between the two.

In 2021, the United States spent 18.3% of its gross domestic product (GDP) dollars on health-care expenditures.[3] Dental services are now categorized as "other" expenses, only accounting for 3% of total expenditures as compared to the 10% spent on prescription drugs and 20% on physician and clinical services.[3] In 2013, dental services accounted for 7% of GDP dollars.[4] It is unequivocally apparent that Americans are facing an oral public health crisis with patients spending less on oral health services each year, demonstrating a growing need for outcry from the dental community to protect the public's oral health and wellness.

Symbiosis and dysbiosis

When Americans lack dental insurance, their access to routine oral care becomes limited, and then disease rates inevitably rise. When the oral cavity trends toward disease, an individual's systemic health can be adversely affected. Systemically, the human microbiome is composed of genetic material, environmental influences, and microbiota (bacteria, fungi, protozoa, and viruses).[5] The composition of the microbiota varies throughout the body. The most diverse microbiomes reside in the gut and oral cavity.[5] A symbiotic environment exists when those microorganisms are in balance and in homeostasis with one another. A dysbiotic environment occurs when the microorganisms are in disharmony with one another and results in disease.

The relationship between the host (human) and the oral microbiome is dynamic. A divergence from oral symbiosis into dysbiosis causes profound physiological effects throughout the body. Oral dysbiosis (caries, periodontal disease, peri-implant disease, cancer, immunological diseases) can occur due to many factors, such as tobacco use, poor oral hygiene, microbiological composition (biofilm), tooth- or prosthesis-related factors, stress, saliva flow or composition change, alteration in diet, systemic diseases, host-immune responses, genetics, or medication use.[6]

Periodontal disease etiology

Periodontal disease represents a state of dysbiosis, where homeostasis of the oral ecosystem is disrupted. Periodontal disease causes destruction to the epithelium, connective tissues, cementum, and underlying bony structures of the periodontium.[7] The disease occurs when the immune system no longer regulates inflammatory responses efficiently and allows disease-promoting microbiota to populate the mouth in response to the presence of oral biofilm.

Oral biofilm in periodontal health remains stable over time and exists in a state of biological equilibrium and homeostasis.[7] When oral biofilm grows uncontrolled, an imbalance of organisms can occur where pathogenic bacteria dominate while less-pathogenic bacteria are suppressed.[7] The new biofilm complex favors dysbiosis in which a host-immune response may occur in a susceptible individual who has one or more identified risk factors.[6,8,9]

Dysbiosis in the oral cavity can lead to dysbiosis systemically. When a patient has active periodontal disease, the provider knows two things: (1) the oral microbiome is dysbiotic, and (2) biofilm is present in quantities that have led to the dysbiotic state. The clinical interventions required must be able to reduce biofilm concentrations and inflammatory burden to a level where the body can reestablish oral symbiosis and homeostasis.

Dental calculus is not the direct etiological agent of periodontal disease, but neither is it an innocuous substrate in the mouth. It provides a roughened surface for biofilm adherence and its nonmineralized portions appear microscopically as channels that contain bacteria and other debris.[7,10] As dental calculus matures, it will contain both organic components and inorganic components.[11] Effectively removing and reducing both dental calculus and biofilm concentrations in the mouth will promote oral symbiosis.

Genetic predisposition to periodontal disease

Exploration into the genetic origins of periodontal disease has been going on for decades. There is evidence to support that genes and gene polymorphisms play a role in the predisposition, susceptibility, and progression of periodontal diseases.[12] Discovering genetic links to any disease leads to improved therapies and intervention strategies.

When appraising the literature, keep in mind the verbiage used for periodontal diseases reflects the diagnostic language of that time. A paradigm shift occurred in 2018 with the release of the new *Classification of Periodontal and Peri-implant Disease and Conditions* that changed diagnostic language. Literature published prior to 2018 will use dated terms.

- A mutation of the SOS1 gene has been identified in individuals with hereditary gingival fibromatosis.[13] The SOS1 gene determines whether cells grow, divide, or differentiate, and the only clinical manifestation of mutated SOS1 appears in the periodontium.[13]
- A landmark study by Boughman et al. identified an autosomal dominant form of localized aggressive periodontitis in one family. This study showed statistically significant results that this form of periodontal disease had a link to a single gene locus on chromosome 4.[14]
- Studies have shown a significant association between generalized

aggressive periodontitis and alleles associated with rheumatoid arthritis (DRB1).[12]

- Investigation into the genes that encode for interleukin-1 (IL-1) has shown an ability to detect a person's susceptibility to chronic periodontitis; however, this genotype has limitations in specific racial and ethnic groups where it is not present.[12] IL-1 is a pro-inflammatory cytokine that acts on leukocytes and is associated with immune and healing responses commonly found in periodontal diseases.[7,12,15]

Genetic factors influence the way a human body responds to antigens and inflammatory reactions.[12] For example, people with asthma respond to an allergen with bronchial swelling and airway obstruction, while those without asthma do not.[16] The same theory is true for a patient's response to biofilm insults in the mouth. Some may initiate an inflammatory response while others do not. Periodontal disease is a multifactorial disease process, and a patient's genetic composition may alter their response to traditional oral therapeutic interventions, requiring providers to incorporate more advanced techniques.

Contemporary technology for biofilm management

Twenty-first-century research has uncovered astonishing information about the relationship between the oral microbiome and systemic/genetic status. Dental manufacturers have kept up with this evidence-based research and developed technology designed to respond to the needs of the contemporary dental patient. Ultrasonic devices with varied insert/tip designs for natural and implant teeth, air-polishing devices that debride both supragingival and subgingival surfaces, dental lasers, and ozone are examples of technology advancement designed to promote oral symbiosis through biofilm management. These technologies are becoming widely used in the private practice sector and are taught in many dental and dental hygiene programs throughout the globe.

Ultrasonic technology

The field of ultrasonics in dentistry has grown tremendously in the past 30 years. Manufacturers have developed a wide range of shank designs to allow for safe, efficient, and effective debridement of complex and noncomplex anatomy of natural teeth and implant architecture. The devices themselves have evolved to produce finely tuned ultrasonic waves that deliver phenomenal fluid-dynamic therapeutic effects such as acoustic cavitation, acoustic microstreaming, and liquid jets while irrigating soft tissues.

During ultrasonic instrumentation, acoustic cavitation vapor gas bubbles are released from the shank of a magnetostrictive insert or piezoelectric tip and are swept into an acoustic microstreaming current created in the mouth and carried throughout the oral cavity. Note the bubbles seen in **figure 1.** When they reach a temperature in the mouth below that of vapor gas, the acoustic cavitation bubble will implode and release a powerful shock wave into the oral environment that will damage the cell walls of bacteria, producing lysis (cell death), detach adherent biofilm, and alter the oral flora bacterial composition by reducing motile rods, filaments, and spirochetes and increasing cocci-shaped bacteria.[17-24] These profound microbiological changes promote oral symbiosis.

Liquid jets are created inside acoustic cavitation vapor gas bubbles and are ejected from the bubble when nearing a solid surface (bacterial cell wall). Liquid jets and acoustic microstreaming cause hydrodynamic shear stress, which is a force applied

FIGURE 1: During ultrasonic instrumentation, acoustic cavitation vapor gas bubbles are released from the shank of a magnetostrictive insert or piezoelectric tip.

FIGURE 2: Straight ultrasonic shanks are useful to debride noncomplex anatomy.

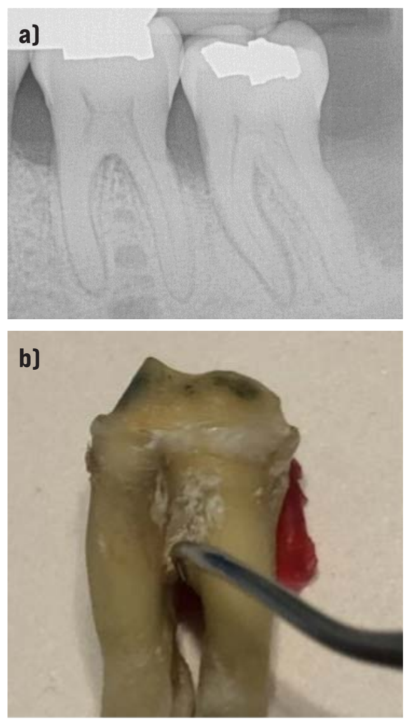

FIGURE 3: a) Complex root anatomy due to significant bone loss on the mandibular left terminal molar; b) Curved shank debriding the furcation on extracted mandibular left second molar

per unit area in a fluid medium and results from the friction between fluid particles.[20,25] Hydrodynamic shear stress causes deformation of nearby substances such as bacterial cell walls and membranes and causes a decreased ability for bacteria to store elastic energy, leading to detachment from an adherent biofilm.[20,26,27]

Ultrasonic manufacturers have released shank designs that are thick, thin, and ultrathin in diameter to access any type of tissue consistency and deposit level. Straight shanks are useful to debride noncomplex anatomy **(figure 2)**, and curved shanks will conform and contour to complex root anatomy such as furcations, root concavities and convexities, as well as interproximal surfaces **(figure 3)**.[20] The American Academy of Periodontology has been recommending the use of thin-diameter curved shanks for the debridement of furcations since 2000, owing to their thinner diameter and ability to conserve cementum when compared to hand-activated instrumentation.[28]

Decades of evidence-based research have reported two constant and consistent outcomes when comparing hand-activated to ultrasonic instrumentation of cemental root surfaces:

- Ultrasonic instrumentation of the cemental surface produces on average 50% less removal, alteration, and injury than hand-activated instrumentation during oral deposit removal.[20,28-35]
- Hand and ultrasonic instrumentation will remove both oral deposits and diseased cementum, but hand-activated instruments produce a more glassy-smooth cemental finish.[20,28-35] Contemporary periodontal debridement does not support the removal of cementum to a glassy-smooth finish during procedures and advocates for providers to remove deposits while conserving healthy cemental structures.[7,20]

Air polishing

Contemporary air-polishing devices (APDs) have a variety of clinical applications. New powders continue to be developed and released to the market, providing both preventive and therapeutic functionality. APDs release a slurry of pressurized air, powder, and water designed to remove extrinsic stain, biofilm, and immature dental calculus both coronal and apical to the CEJ and are therapeutically used in the management and maintenance of periodontal and peri-implant diseases.[20] APDs will not remove firmly established mature dental calculus.[20]

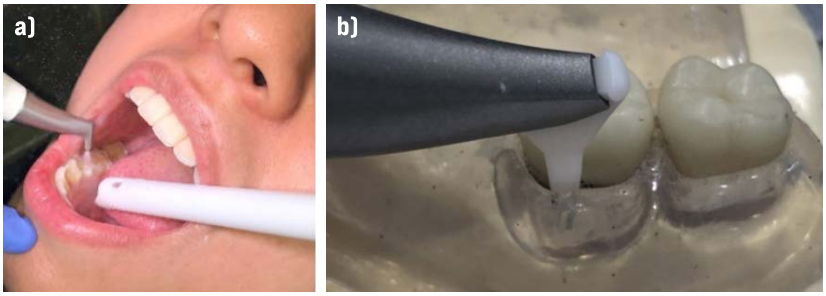

FIGURE 4: a) Supragingival APD delivery; b) Subgingival APD delivery

There are six powders that vary in chemical composition, particle size, and Mohs hardness, which dictates their clinical and therapeutic applications. Sodium bicarbonate, aluminum trihydroxide, and calcium carbonate are used coronal to the CEJ, while glycine and erythritol are used both coronal and apical to the CEJ.[7,20,36] Specialized nozzles are used for supragingival and subgingival delivery **(figure 4)**. There is no universal particle size or hardness of powders as manufacturers vary in design and composition.

Sodium bicarbonate powder is made of sodium and bicarbonate with or without added tricalcium phosphate or silicon oxide to improve its flow characteristics.[20,37,38] The particle size range is 40–250 μm with a Mohs hardness of 2–3.[20,37,39] Sodium bicarbonate powder can be used in the management of dentinal hypersensitivity because it will occlude dentinal tubules.[40] Aluminum trihydroxide powder is made of aluminum and hydroxide with a particle size range of 80–325 μm and a Mohs hardness rating of 3–4.[20,36] Calcium carbonate powder has a particle range of 44–55 μm and a Mohs hardness of 3.[20]

Glycine powder is composed of glycine and silicic acids, which are added to improve powder flow characteristics.[37] The particle size is 20–63 μm and a Mohs hardness rating of 2.[20,41] Glycine is a useful adjunctive aid in the management of subgingival biofilm and gingival inflammation around natural teeth and dental implants, because it has anti-inflammatory effects through inhibiting inflammatory cell activation and immunomodulatory effects through decreasing cytokines and toxic mediators such as free radicals.[20,42] Glycine powder is also destructive to bacteria through inhibiting the synthesis of a peptidoglycan component necessary to maintain cell wall integrity of bacteria.[43]

Erythritol powder is composed of erythritol and 0.3% chlorhexidine, with the lowest particle size of 14 μm and a Mohs rating of 2.[20] Like glycine, erythritol powder is a useful adjunctive in the management of subgingival biofilm and gingival inflammation around natural teeth and dental implants because of its antimicrobial action, inhibitory effects on bacterial replication by decreasing nucleic acid synthesis and amino acids, and its ability to slow down the extracellular matrix biosynthesis of bacteria, thus disrupting the structural integrity of biofilms.[20,44,45]

When compared to traditional rubber cup polishing, air polishing is more effective in biofilm removal with less abrasion to hard tissues (enamel, dentin, cementum) due to the following reasons:

- Powder structure: APD powders have a smaller particle size, lower Mohs hardness, and more uniform shape than rubber cup polishing agents (calcium carbonate, flour of pumice).[20] The Mohs hardness rating of polishing agents is 5–7.[46,47] In general, the higher the particle size and Mohs hardness, the higher the abrasive capability.[36,48,49] Higher particle sizes will remove biofilm and stain with less contact time than smaller particle sizes; however, they may not be well suited for less mineralized hard tissues such as cementum and dentin and will cause overabrasion.[20]
- Fluid dynamics of delivery: The water expelled from an APD nozzle causes the release of kinetic energy, which fragments the powder particles, reducing their size before they contact the tooth surface, and dampens the impact of the abrasive powder particle that strikes the tooth surface.[39]

The American College of Prosthodontists recommends air-power polishing with glycine or erythritol for the debridement and maintenance of dental implants, abutments, and restorations owing to their dental material compatibility and decreased risk for surface changes.[20,50] Glycine and erythritol also offer the benefits of antimicrobial and antibiofilm activity that can be useful in the treatment of peri-implant diseases.

- A study by Mensi et al. found erythritol with 0.3% chlorhexidine produced superior results for inhibiting the regrowth of biofilm after treatment when compared to sodium bicarbonate.[51]
- A study by Drago et al. tested the antimicrobial effects of glycine and erythritol around implants with peri-implantitis.[52] Erythritol displayed better bactericidal effects and reduced microbes tested two to three times more than glycine. Both powders reduced microbes and removed biofilm, but erythritol had stronger antimicrobial and antibiofilm effects than glycine.

Lasers

Dental lasers have been on the market since the 1980s but have gained popularity in dentistry in the last two decades for their cutting and noncutting abilities.[53] Dental lasers of varying wavelengths have the ability to alter the microbial concentrations in the oral microbiome. Lasers produce photothermal effects in the mouth by converting their light energy into heat, which allows them to optimize various photobiological effects in the mouth.[20]

- Lasers have a bactericidal effect and can decontaminate infected periodontal pockets. Many periodontal pathogens are readily deactivated at temperatures of 50°C, and dental lasers produce thermal effects well beyond that threshold.[53,54]
- Lasers increase osteoblastic and fibroblastic activity to accelerate bone and tissue healing.[55-57]
- Lasers decrease inflammation through changes in inflammatory and immune mediators.[55-57]
- Lasers provide analgesia postprocedurally or in the management of painful conditions such as TMD, burning mouth, ulcers, oral candidiasis, or stomatitis.[55-57]

Dental lasers are utilized for many other clinical applications in dentistry, but this course is specifically focused on the oral microbiome and biofilm alterations.

A systematic review and meta-analysis, published in 2015 by the *Journal of the American Dental Association*, evaluated scaling and root planing (SRP) results compared to SRP with adjunctive aids, including the diode laser (wavelength 660–980).[58] The authors were a panel of experts chosen by the American Dental Association Council on Scientific Affairs, and they concluded with a moderate level of certainty that the diode laser using photodynamic therapy improved clinical attachment levels when compared to SRP alone.[58]

Photodynamic therapy (PDT) is a laser procedure used in medicine and dentistry that utilizes a wavelength energy in the infrared or near-infrared of the light spectrum in a noncutting mode. It was first introduced for the treatment of cancer in medicine as it uses autography, a method of cell catabolism, and leads to the destruction of abnormal cells.[59] PDT in dentistry is used for its wound-healing, therapeutic, and antibacterial effects, which are great benefits when treating patients with oral dysbiosis.[20,59]

Ozone

Ozone (O_3) has been used in medicine since the 1930s.[60-62] Ozone is an allotropic modification of the oxygen molecule mixing pure oxygen with pure ozone to make a chain of three atoms of oxygen that is delivered in dentistry as a gas or a liquid (ozonated water, oil, or saline).[20,60-64] Ozone has multiple mechanisms of action in the mouth such as:

- Decreases viable bacteria and inhibits the accumulation of biofilm.[65-67] Human cells are not harmed by ozone because they have protective enzymes, proteins, and nutrients that neutralize the oxidative effects.[68]

- Disinfectant and wound-healing properties owing to antioxidant, anti-inflammatory, analgesic, and immune-stimulating effects[60-62]
- Promotes fibroblastic activity; reduces cytokines, interleukins, and prostaglandins[61,63]
- Alters the cytoplasm, cell wall, and enzymatic function of bacterial cells[61,63]
- Alters the by-products produced by microorganisms as well as necrotic debris[69]

Ozonated water, oil, or saline is used in dentistry to irrigate periodontal pockets, irrigate intracanals during endodontic procedures, in caries risk reduction by oxidizing pyruvic acid produced by cariogenic bacteria, as an oral wound healer (herpes, aphthous ulcers, candidiasis, lichen planus, stomatitis), and is used alongside dental lasers for postsurgical pain control in oral surgery procedures and in the management of TMD pain.[61-63]

Conclusion

As evidence-based science continues to discover more about the oral and systemic links between health and disease through the interplay of the human microbiome, oral healthcare providers have a call to action to stay abreast of changing recommendations, technology, and treatment modalities. The goal of treatment is to maintain symbiotic relationships that promote homeostasis to ward off and/or control dysbiotic diseases. The public health crisis of periodontal disease prevalence, coupled with the lack of dentate adults with dental insurance and the decreasing GDP dollars spent on oral services, adds a new dimension to the dental field and places a significant burden on providers charged with maintaining the public's health. This changing climate forces providers to think outside the box of traditional approaches to biofilm management and incorporate 21st century technology.

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1. What percentage of adult Americans ages 18–64 have dental insurance?
   A. 25%
   B. 35%
   C. 2%
   D. 75%

2. What percentage of adult Americans ages 30-plus have periodontal disease?
   A. 10%
   B. 20%
   C. 30%
   D. 2%

3. What percentage of gross domestic product (GDP) dollars was spent on dental services in 2021?
   A. 2%
   B. 4%
   C. 10%
   D. 15%

4. What percentage of GDP dollars was spent on prescription drug services in 2021?
   A. 1%
   B. 9%
   C. 12%
   D. 15%

5. What percentage of GDP dollars was spent on dental services in 2013?
   A. 2%
   B. 4%
   C. 7%
   D. 10%

6. Which of the following is a component of the human microbiome?
   A. Genetic material
   B. Fungi
   C. Bacteria
   D. All of the above

7. What term is used to describe the human microbiome and microorganisms living in harmony with one another, leading to homeostasis?
   A. Symbiosis
   B. Dysbiosis
   C. Microbiome
   D. Genetics

8. What term is used to describe the human microbiome and microorganisms not living in harmony with one another, causing a disruption in homeostasis?
   A. Symbiosis
   B. Dysbiosis
   C. Microbiome
   D. Genetics

9. Which of the following is an example of oral dysbiosis?
   A. Dental caries
   B. Periodontal disease
   C. Peri-implant disease
   D. All of the above

10. Which of the following can contribute to dysbiosis in the oral cavity?
    A. Tobacco use
    B. Poor oral hygiene
    C. Change in salivary flow or composition
    D. All of the above

11. Periodontal disease causes destruction to which of the following structures?
    A. Epithelium
    B. Connective tissues
    C. Cementum
    D. All of the above

12. Which gene can mutate in individuals with hereditary gingival fibromatosis and result in the clinical manifestation of periodontal disease?
    A. SOS1
    B. ASO2
    C. IL-1
    D. DRB1

13. Which cells release the cytokine interleukin-1?
    A. Erythrocytes
    B. Leukocytes
    C. T-cells
    D. Platelets

14. Which technology produces acoustic cavitation, acoustic microstreaming, and liquid jets that have profound therapeutic effects in the mouth?
    A. Ultrasonic
    B. Air polisher
    C. Laser
    D. Ozone

15. When an acoustic cavitation vapor gas bubble implodes, which of the following occurs in the mouth?
    A. Cell walls of bacteria are damaged leading to lysis (cell death)
    B. Adherent biofilm is detached
    C. Reduction of motile rods, filaments, and spirochetes
    D. All of the above

16. Which of the following is created inside an acoustic cavitation vapor gas bubble and when ejected pierces a bacterial cell wall and causes hydrodynamic shear stress?
    A. Liquid jet
    B. Acoustic microstreaming
    C. Irrigation
    D. Lavage

17. Which of the following ultrasonic shanks are designed to conform and contour to complex root anatomy and are useful for debriding furcations?
    A. Straight thick shank
    B. Straight thin shank
    C. Straight ultrathin shank
    D. Curved shank

18. Which of the following technologies releases a slurry of pressurized air, powder, and water designed to disrupt biofilm and remove tooth stains?
    A. Ultrasonic
    B. Air polishing
    C. Laser
    D. Ozone

19. Which of the following cannot be removed by an APD?
    A. Extrinsic stain
    B. Biofilm
    C. Immature dental calculus
    D. Mature dental calculus

20. Which APD powder is not used apical to the CEJ?
   A. Sodium bicarbonate
   B. Glycine
   C. Erythritol
   D. None of the above

21. Which APD powder has the lowest particle size of 14 µm?
   A. Erythritol
   B. Sodium bicarbonate
   C. Aluminum trihydroxide
   D. Calcium carbonate

22. Which APD powder has anti-inflammatory effects through inhibiting inflammatory cell activation and immunomodulatory effects through decreasing cytokines and toxic mediators such as free radicals?
   A. Sodium bicarbonate
   B. Aluminum trihydroxide
   C. Calcium carbonate
   D. Glycine

23. Which APD powder has antimicrobial action, inhibitory effects on bacterial replication, and can slow down the extracellular matrix biosynthesis of bacteria, thus disrupting the structural integrity of biofilms?
   A. Sodium bicarbonate
   B. Aluminum trihydroxide
   C. Calcium carbonate
   D. Erythritol

24. Which of the following statements is true?
   A. APD powders have a higher Mohs hardness rating than polishing agents.
   B. An APD causes the release of kinetic energy, which fragments the powder particles, reducing their size before they contact the tooth surface.
   C. APD powders cause more abrasion than rubber cup with a polishing agent.
   D. Lower particle size agents remove biofilm and stain with less contact time than higher particle sizes.

25. Which of the following APD powder(s) does the American College of Prosthodontics recommend for the debridement and maintenance of dental implants, abutments, and restorations?
   A. Glycine
   B. Erythritol
   C. Aluminum trihydroxide
   D. Both A & B

26. In an APD study by Drago et al., which APD powder displayed better bactericidal effects and reduced microbes tested two to three times more?
   A. Sodium bicarbonate
   B. Aluminum trihydroxide
   C. Glycine
   D. Erythritol

27. Which technology uses varying wavelengths to produce photothermal effects in the mouth by converting their light energy into heat, which allows them to optimize various photobiological effects in the mouth?
   A. Ultrasonic
   B. Air polishing
   C. Laser
   D. Ozone

28. At what temperature are periodontal pathogens readily deactivated?
   A. 20°C
   B. 40°C
   C. 50°C
   D. 90°C

29. Which effect can a dental laser have on the mouth?
   A. Bactericidal effects
   B. Increased osteoblastic and fibroblastic activity
   C. Decreased inflammation through changes in inflammatory and immune mediators
   D. All of the above

30. Which effect can ozone have on the mouth?
   A. Decreases viable bacteria and inhibits the accumulation of biofilm
   B. Anti-inflammatory effects
   C. Analgesic effects
   D. All of the above

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1. Develop a deeper understanding of health statistics in the United States as they pertain to oral health.
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3. Identify the potential role genetics plays in the predisposition and management of patients' periodontal disease status.
4. Utilize the most current dental technology available for biofilm management to promote oral symbiosis.

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6.	Ⓐ	Ⓑ	Ⓒ	Ⓓ	21.	Ⓐ	Ⓑ	Ⓒ	Ⓓ
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9.	Ⓐ	Ⓑ	Ⓒ	Ⓓ	24.	Ⓐ	Ⓑ	Ⓒ	Ⓓ
10.	Ⓐ	Ⓑ	Ⓒ	Ⓓ	25.	Ⓐ	Ⓑ	Ⓒ	Ⓓ
11.	Ⓐ	Ⓑ	Ⓒ	Ⓓ	26.	Ⓐ	Ⓑ	Ⓒ	Ⓓ
12.	Ⓐ	Ⓑ	Ⓒ	Ⓓ	27.	Ⓐ	Ⓑ	Ⓒ	Ⓓ
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