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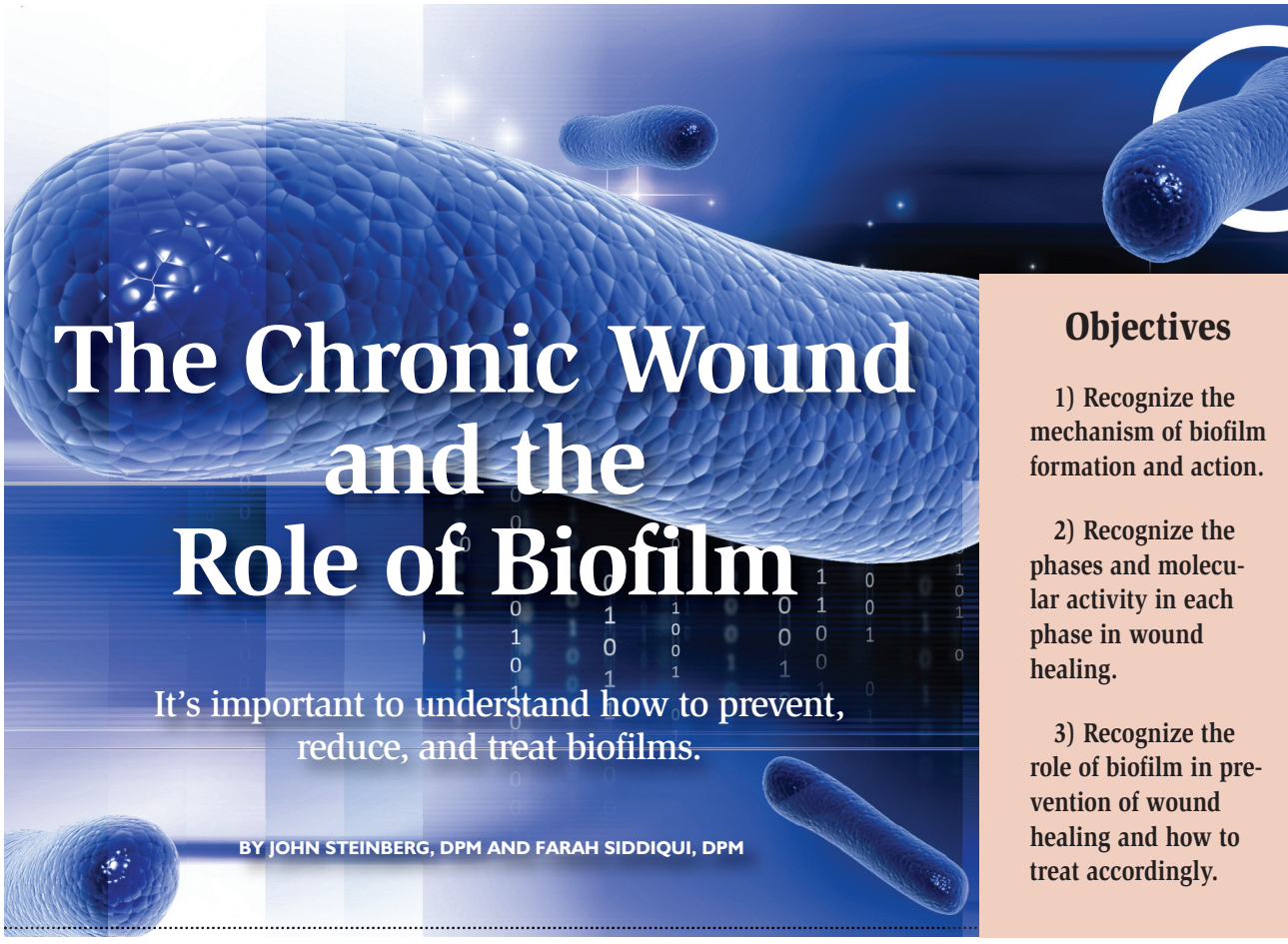

The Chronic Wound and the Role of Biofilm

It's important to understand how to prevent, reduce, and treat biofilms.

BY JOHN STEINBERG, DPM AND FARAH SIDDIQUI, DPM

Objectives

1) Recognize the mechanism of biofilm formation and action.

2) Recognize the phases and molecular activity in each phase in wound healing.

3) Recognize the role of biofilm in prevention of wound healing and how to treat accordingly.

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*Following this article, an answer sheet and full set of instructions are provided (pg. 188).—**Editor***

Chronic wounds are a worldwide epidemic. These wounds can develop due to diabetes, venous disease, or pressure ulcerations. Diabetic patients develop ulcerations due to neuropathic impairment of musculoskeletal balance as well as immune compromise from leukocyte dysfunction and peripheral vascular disease. It has been estimated that 15% of individuals with diabetes mellitus will develop lower extremity ulcers,[1] and 14-24% of diabetic patients with foot ulcers will eventually undergo amputation.[2] Approximately 100,000 limb amputations are performed on diabetic patients each year in the United States. A study by Pecoraro, et al. found that 84% of amputa-

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tions were due to lower extremity ulcers.[3]

Pressure ulcerations result from ischemia due to prolonged pressure over a bony prominence. Bed-bound patients or patients with paralysis are at high risk for developing such ulcerations. Also a costly epidemic, patients often require stays in skilled nursing facilities. These wounds commonly are at risk of becoming chronic as continued off-loading may be difficult.

Venous stasis ulcerations result from hypoxia in areas of venous congestion in the lower extremity. Three hypotheses have been postulated for the cause of venous ulcerations: 1) The thick perivascular fibrin cuff impedes oxygen diffusion into surrounding tissues; 2) Macromolecule leaking into perivascular tissue may trap growth factors needed for maintenance of skin integrity; and 3) Leukocytes are migrating through capillaries more slowly than usual, possibly even occluding them, becoming activated, and damaging the vascular endothelium.[4]

Many chronic wounds are susceptible to acute infections as a result of biofilm growth. Additionally, many studies have postulated the role of biofilm as an inhibitor of wound healing. While many therapies are directed towards eradicating biofilm, there is still not a true understanding of its mechanism of growth and its actual deleterious effects on wounds, namely in the prevention of healing.

The fully mature biofilm structure is comprised of bacterial cells, the polymer matrix, and interstitial channels that facilitate the exchange of nutrients and wastes in and out of the biofilm into the surrounding environment.

Biofilm

Biofilm is a complex species, either single or multiple, that are encased within the extracellular polymeric matrix produced by that same complex.[5] Biofilm is associated with persistent infections as it greatly resists body immune systems and antimicrobials.[6] Knowledge about biofilm structure and physiology is important in understanding the effects of biofilm and to gain effective control.

The main stages of biofilm formation are reversible attachment, irreversible attachment, maturation-1, maturation-2, and dispersion. These stages of biofilm formation are initiated when the planktonic organism transforms to the sessile form.[7] Each stage is regulated by quorum-sensing molecules. Quorum-sensing is a communication process by which bacteria 'sense' molecules produced by other bacteria in the vicinity and can lead to altered gene expression and changes in phenotypic growth patterns.

In the attachment phase, planktonic bacteria transform to the sessile form prior to biofilm formation as they adhere to a favorable surface, such as the host tissue.[8] There are two stages of attachment. Reversible attachment occurs when the organisms are able to revert back to the planktonic form and move away from the surface of attachment, whereas in the irreversible stage, the organisms are attached and biofilm formation is initiated.

During the maturation phase, as the cells adhere to a surface, they divide and multiply outward and upward from the point of attachment. The dividing cells produce quorum-sensing molecules and extracellular polymeric substances or polymer matrix. The matrix attaches the biofilm to the surface on which it is formed.[9,10] As the number of organisms increase, so does the quantity of extracellular polymeric substances. The fully mature biofilm structure is comprised of bacterial cells, the polymer matrix, and interstitial channels that facilitate the exchange of nutrients and wastes in and out of the biofilm into the surrounding environment.[11,12]

Activation of complement and platelets, and initiation of molecular cascade of processes usually result in infiltration of the wound with granulocytes or polymorphonuclear monocytes (PMNs) within 24 to 48 hours of injury.

Studies have shown that detachment occurs when the organisms respond to chemical substances secreted as signaling molecules.[11] Some studies have shown detachment of biofilm is due to nutrient starvation,[13] while others show it is due to an optimal amount of nutrients.[14] Once detached, biofilm disperses to other locations to recommence biofilm formation.[15] This process aids the spread of biofilm infections within a host.

Factors That Influence Biofilm Formation

Formation of biofilm initially begins with the attachment of free-floating microorganisms to a surface. If the biofilm is not initially disrupted, it uses cell adhesion structures to permanently anchor onto the surface. The ability of the organisms to adhere to surfaces, as well as the rate of adherence, will influence biofilm formation.[8] Structures such as flagella and pili are important at this initial stage of biofilm formation as they are re-

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quired for adherence.[16]

Biofilm formation also depends on availability of nutrients. It has been demonstrated that increased amounts of nutrients enhance the production of quorum-sensing molecules, enzymes, and other essential amino acids necessary for the formation and growth of biofilm.[14] However, it also has been shown that a lack of nutrients causes biofilm to detach and then disperse more efficiently.[13] Bacteria monitor and respond to the types and amounts of nutrients in their environment. The largest role in biofilm formation belongs to quorum-sensing. It allows bacteria to coordinate gene expression and help the biofilm transition along during the formation process.

The Chronic Wound

The process by which the body repairs the damaged tissue involves a series of stages, including vascular response (hemostatic), inflammatory, proliferative, and remodeling phases. The vascular response phase is the body's immediate response to the wound via a clotting cascade to limit blood loss as well as to restore the integrity of the damaged tissue.[17] Three main events occur during this phase, including vasoconstriction, platelet plug, and formation of a clot that seals the gap until the tissue is repaired, thus providing a temporary matrix for cellular and fibroblast migration.[18]

The most important phase in regard to chronic wounds is the inflammatory phase. At this phase, the white blood cells remove the foreign bodies while fibroblast cells produce collagen and the extracellular matrix. Activation of complement and platelets, and initiation of molecular cascade of processes usually result in infiltration of the wound with granulocytes or polymorphonuclear monocytes (PMNs) within 24 to 48 hours of injury. This activation serves to eliminate any microorganisms from the wound.[19,20] Following this, neutrophils and macrophages control bacteria, release growth factors, and induce proliferative signals on human keratinocytes and fibroblasts.[21] This phase lasts for three to five days and is characterized by edema, erythema and pain; however, this should not be confused with signs of infection. These signs are a result of increased vascular permeability secondary to the initial vasoconstriction. This phase is the most important because it is the key stimulant to the subsequent phases.

The Proliferative Phase

The proliferative phase is characterized by fibroplasia, matrix deposition, angiogenesis, and re-epithelialization and may last up to four weeks in an uninfected wound.[22] Fibroblasts migrate into the wound with new collagen being placed over the wound. Fibroblasts are also responsible for initiating angiogenesis, which will form granulation tissue and eventually lead to epithelialization.[23] Keratinocytes and endothelial cells also proliferate at this time, eventually producing autocrine growth factors that maintain their growth. Endothelial expansion further contributes to angiogenesis.

Degradation of the fibrin clot and temporary matrix is accompanied by the deposition of granulation tissue which continues until the wound is covered. Decreasing hyaluronic acid levels and increasing chondroitin sulfate levels slow fibroblast migration and proliferation while inducing fibroblast differentiation, which then leads to the maturation phase of healing.

For the first six weeks, new collagen production dominates the wound-healing process. As the wound matures, the collagen is remodeled into a more organized structure with increased tensile strength. Gradually, type I collagen replaces type III. At this juncture, the wound gains strength which helps the wound contract, and scar formation begins. This phase can last from one month to one year.

A chronic wound is a wound that does not heal in an orderly set of stages and/or within a predictable time frame as most wounds do.[20,24] Such a wound usually fails to heal despite all efforts to aid healing, and becomes persistent or reoccurs after a period of time when the wound has healed.[25,26] In general, any wound that fails to heal within three months is often considered a chronic wound, while many studies are able to predict non-healing after just three to four weeks. Many hypotheses have been formed as to why or how a wound becomes chronic, including lack of keratinocyte migration or fibroblast formation.[27,28]

Fibroblasts isolated from chronic wounds show impairment of synthesis, migration, and proliferation. Endothelial cells become deficient in production of enzymes and growth factors and formation of new capillaries. Keratinocytes are also impaired and have decreased ability to synthesize cytokines, the temporary wound matrix, and basement membrane. Furthermore, matrix metalloproteases are up-regulated up to 30-fold in chronic wounds. The most important inhibitor of metalloproteases—TIMP, tissue inhibitor metalloprotease 1—is significantly down-regulated in chronic wounds.[27,28]

Direct evidence of the presence of biofilms in wounds was proven in 2008 when biopsies of acute and chronic wounds were taken and examined by both light and scanning electron microscopy.

When a wound forms, the subcutaneous tissue is exposed and is vulnerable to microbial contamination. The presence of microorganisms within the wound without any host reaction is referred to as contamination.[29] Sources of contamination can either be endogenous or exogenous. Endogenous sources include normal body flora of the patient such as the common organism *Staphylococcus Epidermidus*. In immune-competent

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individuals, the body's immune system is able to eliminate the organisms and eradicate the formation of biofilm. In immune-compromised individuals, the perpetuation of organisms in the wound leads to colonization and, in some cases, wound infection.

Within the wound, the presence of microorganisms that are either multiplying or associated with host reactions is referred to as colonization.[29,30] The wound environment provides warmth, moisture, and nutrition, all of which are favorable for colonization.[31] If the microbial proliferation rate increases, colonization could eventually lead to infection.[32]

Wound infection occurs when the microbial load and virulence factors overwhelm the host's immune system.[33,34,35] In individuals with compromised immunity, microbial growth may increase optimally within a short period especially in devitalized tissues.[36] Major signs of infection are redness, swelling, heat, and pain. Wound infections, especially in diabetics, are mostly polymicrobial, are usually associated with the synergistic effect of the microbial virulence, and can eventually lead to septicemia and death if not treated appropriately.[33,34]

Role of Biofilm

Direct evidence of the presence of biofilms in wounds was proven in 2008 when biopsies of acute and chronic wounds were taken and examined by both light and scanning electron microscopy. 60% of the chronic wounds showed biofilm in comparison with 6% of acute wounds.[37] Biofilms have been associated with chronic infections in wounds because these organisms often resist host mechanisms and antimicrobial interventions.[37,38] The biofilm organisms are encased in extracellular polymeric matrix and are able to resist phagocytic action and impede the action of the host immune system and antimicrobials.[38,39] Quorum-sensing molecules are required for biofilm formation and increase production of virulence factors such as cytotoxic enzymes. Increased production of toxins further drains the immune mechanism of the patient and reduces the healing process. In 2007, Loryman and Mansbridge observed the effect of quorum-sensing molecules on inhibition of keratinocyte migration.[40] All of these factors explain the inability of wounds with biofilms to heal.

Resistance starts at the 'attachment' phase and increases as biofilm develops.

Furthermore, biofilms frequently show resistance towards antimicrobials. Organisms within biofilm are able to resist antimicrobials through various mechanisms due to the architecture and composition of biofilm. While we know biofilm forms and proliferates rapidly, the turnover rate is quite slow. A period of 24 to 48 hours is required for biofilm formation. This is a factor that enhances its ability to resist host immune mechanisms and antimicrobial interventions. Resistance starts at the 'attachment' phase and increases as biofilm develops. The components of extracellular polysaccharides (EPS) of biofilm act as a barrier and physically restrict diffusion of antimicrobial agents into the biofilm, thus protecting the organisms from the effect of antimicrobials.[39]

Another reason for antimicrobial resistance can be due to close cell-to-cell contact that permits bacteria to transfer plasmids to one another more effectively than in the planktonic state. These plasmids can then encode for resistance to several different antimicrobial agents.[41] Also, the heterogenous environments within biofilm such as pH, oxygen tension, and other chemical substances have been shown to reduce the activities of antimicrobials.[42]

Ultrasound therapy has been suggested to not only remove tissue, but also to disrupt the quorum-sensing and lead to decreased coordinated virulence.

The biofilm also provides a physical protection to bacteria because antimicrobial agents are also ineffective at penetrating the biofilm, decreasing the concentration acting on the bacterial cells within the biofilm and therefore their efficacy.[41] In addition to resistance to antimicrobials, biofilms also appear to have an antiphagocytic property which makes the leukocytes within the matrix ineffective.[43]

Treatment Intervention for Biofilm

There are five main ways to possibly prevent, reduce, or treat biofilms. These include the following: 1) preventing the bacterial attachment; 2) preventing biofilm formation; 3) disrupting the biofilm to allow penetration of topical antimicrobial agents; 4) interfering with quorum-sensing; and 5) enhancing dispersion of bacteria from biofilms so the planktonic bacteria could be more easily destroyed.[44]

The effect of lactoferrin, a glycoprotein with antimicrobial and anti-inflammatory properties, has been investigated as a possible treatment for preventing biofilm formation. The mechanism by which it inhibits biofilm formation is by chelating iron, resulting in a low iron state that causes bacteria to 'twitch' and therefore inhibits bacterial adhesion. While this has been shown to prevent formation, it has less impressive effects on already-formed biofilms.[45]

The most effective way to disrupt biofilm that has already formed is via physical methods such as debridement, electrical stimulation, or ultra-

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sound. Removal of bacterial biofilm by debridement and prevention of reformation by killing bacteria before they regenerate biofilm is necessary to reverse the molecular imbalances and initiate healing.[46] Sharp debridement is the fastest method of removal of any necrotic tissue, thus decreasing the bacterial burden and stimulating wound healing. Bacteria tend to thrive in devitalized tissue and, therefore, removal of such tissue is imperative.

Maggot therapy is another form of debridement. Maggot secretions and excretions have been found to not only inhibit the formation of *Staphylococcus epidermidus* biofilm, but to also disrupt other pre-formed biofilms. More specifically, maggots reduce the degrading factors that are involved in biofilm accumulation and thus weaken the biofilm, increasing bacterial susceptibility to antibiotics.

Ultrasound therapy has been suggested to not only remove tissue, but also to disrupt the quorum-sensing and lead to decreased coordinated virulence.[47] Electrical stimulation has been used over the years to assist penetration of various topical agents.[48] The use of enzymatic debriding agents is another method by which biofilms could be penetrated and disrupted as they digest the extracellular proteins.

Treatment of wounds with topical antimicrobial dressings, such as silver compounds, has proved successful.[49] Silver has a lethal effect on biofilm organisms, but at high concentrations, which might be up to 10-100 times the concentration required to kill the planktonic organisms.[50] The rate at which the silver is released from the dressings determines the effectiveness of the antimicrobial activity of the silver dressing.[49] Antimicrobials such as metronidazole have been reported to hinder the synergistic action of bacteria, and thus aid the healing of chronic wounds.[51]

Cadexomer iodine is an effective antiseptic agent for chronically exudative wounds. It works against glycocalyx production and directly destroys biofilm structures. Therapeutic honey has also been advocated by many clinicians as another option for topical wound care. Honey was found to be bactericidal against all strains of bacteria and contains bactericidal substances that penetrate the biofilms.

Summary

Wound healing is a complex process by which the tissue restores its integrity after injury at the earliest possible time. It is inevitable that wounds will be colonized with microorganisms, but the innate immune system of the body usually eliminates the microbial load in the course of wound healing. However, when the immune system is compromised, the microbial burden overwhelms the immune response and may lead to infection. This is when biofilm takes advantage of the less-than-optimal wound condition and proliferates. Biofilm formation on these wounds may be responsible for the chronicity of these wounds and for their common infectious complications.

Quorum-sensing molecules and biofilm formation are a great burden to the host environment and are difficult to treat. The aim for treatment intervention should be reducing the quantity of organisms and the virulent expressions of the organism. This can best be accomplished by attacking the wounds at different stages of biofilm formation. Sharp debridement with the conjunctive use of topical antimicrobials is the first line of treatment. While adjunctive therapies such as ultrasound therapy may need to be employed, it is most important that continuous treatment is engaged to initially reduce and eventually eradicate biofilm. **PM**

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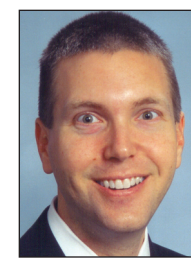

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SEE ANSWER SHEET ON PAGE 189.

1) Which of the following is NOT true about quorum-sensing?
- A) It regulates each stage of biofilm formation
- B) It can lead to altered gene expression and changes in phenotypic growth
- C) It is produced by the dividing cells in the maturation phase
- D) It causes detachment of biofilm

2) The fully mature biofilm structure comprises all of the following except:
- A) Polymer matrix
- B) macrophages
- C) Interstitial channels
- D) Bacterial cells

3) Detachment of biofilm can occur
- A) When organisms respond to chemical substances secreted by them
- B) Due to nutrient starvation
- C) Due to optimal amount of nutrients
- D) All of the above

4) What is the role of the extracellular polymeric matrix?
- A) Resist phagocytic actions
- B) Impede host immune actions
- C) Physically restrict diffusion of antimicrobial agents
- D) All of the above

5) Infiltration of the wound with PMNs during the inflammatory phase occurs
- A) Within the first 24 hours
- B) Within 24 to 48 hours
- C) 3 to 5 days after injury
- D) 4 weeks later

6) Neutrophils and macrophages
- A) Release growth factors
- B) Induce quorum-sensing
- C) Increase bacterial counts
- D) Initiate angiogenesis

7) The phase that is a key stimulant to the subsequent phases is
- A) Hemostatic
- B) Inflammatory
- C) Proliferative
- D) Remodeling

8) Which of these events does NOT occur during the hemostatic phase?
- A) Infiltration of wound with granulocytes or PMNs
- B) Platelet plug formation
- C) Activation of clotting cascade
- D) Formation of temporary matrix

9) The proliferative phase is characterized by which of the following?
- A) Matrix deposition
- B) Angiogenesis
- C) A and B
- D) None of the above

10) The inflammatory phase is characterized by all of the following except:
- A) Erythema
- B) Edema
- C) Pain
- D) Purulent drainage

11) Quorum-sensing inhibits the healing process by
- A) Inhibiting keratinocyte migration
- B) Increasing production of cytotoxic enzymes
- C) A and B
- D) None of the above

12) Biofilm develops 'resistance' at what phase?
- A) Maturation
- B) Attachment
- C) Dispersion
- D) None of the above

13) During the maturation phase
- A) Organisms revert back to the planktonic form
- B) Cells adhere to a surface and divide and multiply
- C) Biofilm disperses to other locations to re-initiate formation and growth
- D) All of the above

14) What does NOT contribute to the reduction of antimicrobial activities in biofilm?
- A) pH
- B) extracellular polysaccharides
- C) glycoproteins
- D) oxygen tension

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CME EXAMINATION

15) Which of the following about lactoferrin is false?

A) It induces a low-iron state
B) It works on already-formed biofilm
C) It has antimicrobial and anti-inflammatory properties
D) It inhibits bacterial adhesion

16) Sharp debridement

A) Helps reverse molecular imbalances
B) Stimulates wound healing
C) Decreases bacterial burden
D) All of the above

17) Ultrasound therapy helps to

A) Disrupt quorum-sensing
B) Enhance dispersion
C) Allow penetration of antimicrobials
D) None of the above

18) Which of the following treatments has been shown to assist in penetration of various topical agents in biofilm?

A) Ultrasound therapy
B) Electrical stimulation
C) Enzymatic debriders
D) B and C

19) Which of the following topical agents have been shown to work against biofilm?

A) Bacitracin
B) Metronidazole
C) Silver
D) B and C

20) Enzymatic debriders help eradicate biofilm by

A) Disrupting quorum-sensing
B) Digesting extracellular proteins
C) Killing bacteria
D) All of the above

See answer sheet on page 189.

PM's CPME Program

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(3) All answers should be recorded on the answer form below. For each question, decide which choice is the best answer, and circle the letter representing your choice.

(4) Complete all other information on the front and back of this page.

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2. The answers to the test
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Charge to: _____Visa _____ MasterCard _____ American Express

Card #_____Exp. Date_____

Note: Credit card is the only method of payment. Checks are no longer accepted.

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State License(s)_____ Is this a new address? Yes_____ No_____

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ENROLLMENT FORM & ANSWER SHEET *(continued)*

EXAM #6/11
The Chronic Wound and the Role of Biofilm (Steinberg and Siddiqui)

Circle:

1.	A	B	C	D	11.	A	B	C	D
2.	A	B	C	D	12.	A	B	C	D
3.	A	B	C	D	13.	A	B	C	D
4.	A	B	C	D	14.	A	B	C	D
5.	A	B	C	D	15.	A	B	C	D
6.	A	B	C	D	16.	A	B	C	D
7.	A	B	C	D	17.	A	B	C	D
8.	A	B	C	D	18.	A	B	C	D
9.	A	B	C	D	19.	A	B	C	D
10.	A	B	C	D	20.	A	B	C	D

LESSON EVALUATION

Please indicate the date you completed this exam

_____

How much time did it take you to complete the lesson?

_____ hours _____minutes

How well did this lesson achieve its educational objectives?

_____Very well _____Well

_____Somewhat _____Not at all

What overall grade would you assign this lesson?

A B C D

Degree_____

Additional comments and suggestions for future exams:

_____

_____

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_____

_____

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