Microbial Biofilms in Osteomyelitis of the Jaw and Osteonecrosis of the Jaw Secondary to Bisphosphonate Therapy
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A biofilm is a complex community of sessile microbes attached to a substrate, often composed of bacterial and fungal organisms. Biofilm organisms differ considerably from their planktonic (free-floating) counterparts because they are characterized by a community of cells that are attached to a surface; are embedded in a matrix of extracellular polymeric substances that they have produced to connect to and communicate with each other; and exhibit an altered phenotype with respect to growth rate, gene transcription and antimicrobial resistance.1,2

Biofilm theory has emerged to explain the etiology of the infections that constitute between 65 and 80 percent of the microbial diseases treated by physicians in the developed world.3 Orthopedic surgeons have adopted the biofilm theory to explain the etiopathogenesis of long-bone infections and to guide their treatment.4,5 In long-bone osteomyelitis, the causative microbes—in most cases, Staphylococcus aureus and Staphylococcus epidermidis—grow as bacterial biofilms on the bone surface and are

**ABSTRACT**

**Background.** The authors report their observations with respect to microbial biofilms in osteomyelitis of the jaw (OMJ), compare these findings with those for osteonecrosis of the jaw (ONJ) secondary to bisphosphonate therapy and discuss recent findings that the pathogenesis of ONJ may represent a biofilm-mediated infectious disease in the context of bisphosphonate therapy.

**Methods.** In 2004, a program was established at the University of Southern California, Los Angeles, to evaluate, treat and monitor patients who have OMJ and ONJ. Twenty people from this cohort of study patients who were scheduled to undergo surgical debridement or sequestrectomy and who met the authors’ inclusion criteria gave informed consent for the study. The authors examined bone samples histopathologically and via scanning electron microscopy, a technique applicable to biofilm characterization.

**Results.** Specimens from all patients with OMJ and ONJ exhibited large surface areas of bone occluded with well-developed biofilms comprising microbial organisms embedded in an extracellular polymeric substance. Actinomyces predominated in OMJ cases, whereas ONJ cases represented more diverse bacterial organisms in addition to fungal organisms not seen in OMJ. The authors observed resorption pits, septic clots, putative nanowires and host inflammatory cells in all specimens.

**Conclusions.** The findings of this study support a role for microbial biofilms in both disease processes.

**Clinical Implications.** Microbial biofilms are a potential target for therapy that includes antibiofilm modalities in the treatment and prevention of OMJ and ONJ.

**Key Words.** Osteomyelitis; osteonecrosis; jawbones; bisphosphonates; biofilms.

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notably resistant to antibiotics and host defenses. Furthermore, laboratory diagnosis and clinical management of osteomyelitis are predicated on the identification and elimination of planktonic bacteria, which are inappropriate for the evaluation or treatment of biofilm-mediated disease.

Osteomyelitis can be associated with significant morbidity. Most cases of the disease occur after trauma to bone or bone surgery or as a complication of vascular insufficiency. Antimicrobial therapy and surgical debridement are the mainstay of therapy. Treatment of chronic osteomyelitis may require a tremendous commitment by the patient and clinician, placing a significant temporal and financial burden on patients and the health care system. Despite surgical and chemotherapeutic advances, osteomyelitis remains difficult to treat, cure rates are marginal, no universally accepted therapeutic protocols exist and expensive antibiotic regimens sometimes do little more than stabilize the disease.

Most osteomyelitis research pertains to long bones, which develop through endochondral ossification and rarely come in contact with bacteria except during bone exposure or septicemia. The jawbones are different from long bones in that they are flat bones derived from intramembranous ossification and regularly are exposed to many oral pathogenic and nonpathogenic organisms through the oropharyngeal cavity, saliva and odontogenic structures. Therefore, osteomyelitis of the jaw (OMJ) is a unique condition meriting distinction from long-bone osteomyelitis.

Osteonecrosis is a condition that shares many similarities with osteomyelitis, but until recently it has not been considered to have an infectious etiopathogenesis. Osteonecrosis generally develops when damage to bone interrupts the osseous nutrient supply or as a result of a medical condition that undermines bone health. Recently, osteonecrosis affecting only the jawbones—osteonecrosis of the jaw (ONJ)—has been described as a unique complication secondary to bisphosphonate therapy.

Bisphosphonates are a class of pharmaceuti- cals used in the treatment of numerous bone disorders, including osteoporosis, cancer metastases to bone and multiple myeloma. They have antiosteoclastic, antiangiogenic and antineoplastic properties. Millions of Americans are receiving treatment with bisphosphonate medications. Most cases of ONJ are associated with long-term use of bisphosphonates; thus, researchers believe that a dose- and time-dependent relationship exists with respect to the disease process.

The reasons why only the jawbones are affected in patients with bone necrosis secondary to bisphosphonate therapy remain unclear. Clinical and epidemiologic studies of ONJ are lacking, and diagnostic criteria are not well established. Ruggiero and colleagues concluded that preventive dentistry is the only measure to prevent ONJ, but this is not always successful. Significant research into ONJ and OMJ is necessary at both the basic science and clinical levels to elucidate the pathogenesis and provide a rationale for treatment.

The purpose of this article is to report our observations of microbial biofilms in OMJ, to compare these findings with those pertaining to ONJ, and to discuss recent findings that the pathogenesis of ONJ may represent a biofilm-mediated infectious disease in the context of bisphosphonate therapy. These findings have important preventive and therapeutic implications and support a role for microbial biofilms in both disease processes.

PARTICIPANTS, MATERIALS AND METHODS

We obtained appropriate institutional review board approval for this study. In 2004, a program was established at the University of Southern California, Los Angeles, to evaluate, treat and monitor patients with OMJ and ONJ. This program represents a multidisciplinary collaboration between the School of Dentistry, Center for Biofilms, Center for Craniofacial Molecular Biology and the Keck School of Medicine. Twenty people from this cohort of study patients—10 with ONJ and 10 with OMJ—gave written informed consent to participate in the study. The total cohort size was approximately 50 patients, but we selected a convenience sample consisting of only patients scheduled for surgical debridement or sequestrectomy because we could evaluate their tissue according to the methods described below.

Inclusion criteria for this study included clinical and radiographic findings consistent with a diagnosis of OMJ and clinoradiographic findings
and a history of bisphosphonate treatment consistent with a diagnosis of ONJ.\(^8,9\) We excluded patients if they had systemic signs of infection or, at the time of the study, had cancer or were receiving chemotherapy, radiation therapy, antiretroviral therapy or steroid therapy (other presumed risk factors for some cases of bone necrosis in general).

Two of us (P.P.S., S.K.S.K.) sectioned all bone samples from affected patients immediately after surgery; we immersed one section in 10 percent formalin for conventional hematoxylin-eosin staining and histopathologic evaluation, and we placed the other section in 4 percent formalin for two hours and then stored it in phosphate buffered saline (PBS) at 4°C. To reduce bias, we included cases for further study only if two pathologists independently confirmed a histopathologic diagnosis of OMJ or ONJ (with a history of bisphosphonate use) according to routine microscopy, and no evidence of other disease was manifest.

Once the pathologists confirmed the samples for inclusion, two of us (A.G., C.S.) prepared the second section stored in PBS for scanning electron microscopic (SEM) evaluation. The investigators fixed the specimens for SEM in Karnovsky solution for 48 hours at 4°C. After washing them with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES solution), the investigators dehydrated the samples with graded ethanol, critical-point dried them with carbon dioxide, mounted them with silver adhesive and sputter-coated them with 30 nanometers of platinum. The investigators examined the samples by means of SEM with 5 kilovolts and obtained detailed micrographic documentation of the findings.

**RESULTS**

Patients with ONJ ranged in age from 60 through 84 years (with a female-to-male ratio of 7:3), and patients with OMJ ranged in age from 28 through 62 years (with a female-to-male ratio of 4:6). Six patients with ONJ (three women, three men) had received intravenous bisphosphonate therapy as part of earlier cancer treatments, and four patients with ONJ (all female) were receiving oral bisphosphonate treatment for osteoporosis.

Common comorbidities among patients with ONJ were hypertension, hypercholesterolemia and type 2 diabetes; in contrast, most patients with OMJ had few or no comorbidities.

All bone specimens from affected sites for both OMJ and ONJ exhibited large surface areas occluded with well-developed biofilms comprising microbial organisms embedded in an extracellular polymeric substance. Both OMJ and ONJ specimens exhibited multispecies microbial biofilms on all internal and external surfaces throughout the depth of the excised bone. In both conditions, different regions of bone exhibited different stages of biofilm development, ranging from individual bacterial cells to cells embedded in a thick amorphous matrix suggestive of a well-established chronic condition. In cases of OMJ, usually one bacterial morphotype consistent with the genus *Actinomyces* predominated, and it was characterized by pleomorphic rods measuring 2 to 6 micrometers in length (Figure 1).

*Actinomyces* are gram-positive organisms and are anaerobes or facultative anaerobes depending on the species. Some cases of OMJ revealed occasional multispecies bacterial colonization of bone in addition to monospecies colonization by *actinomyces*. In all cases of ONJ, we observed multiple bacterial morphotypes, which included short and long rods; cocci in clusters; and filamentous, crescent-shaped and branching forms. Specifically, bacteria from the genera *Fusobacterium*, *Streptococcus*, *Actinomyces*, *Selenomonas* and *Bacillus* were represented. These consist of gram-positive and gram-negative organisms, as well as both aerobes and anaerobes that usually are found in the oral cavity and often are associated with odontogenic and periodontal infections. The size of the bacterial cells in the biofilm ranged from 0.5 to 10 μm. In addition, we observed fungal organisms consistent with *Candida albicans* colonizing the bone in all cases of ONJ, often coaggregating with bacterial biofilm organisms. We did not observe fungal organisms in any of the OMJ specimens.

We observed active sites of bone resorption in all cases of OMJ and ONJ as resorption lacunae, or pits, on the surface of the bone (Figure 2). The pits contained large numbers of multispecies bacteria, and the advancing edge of the biofilm seemed to be responsible for the uneven edges along the bone. The depth of the resorption pits varied with the numbers of bacteria in them. In
both OMJ and ONJ specimens, we noted a moderate population of host inflammatory cells consisting of neutrophils, monocytes and lymphocytes, as well as septic clots consisting of fibrin and erythrocytes adjacent to the biofilm bacteria. These findings are consistent with a role for acute and chronic host inflammation in addition to, or in response to, biofilms in the disease process. We did not observe any eukaryotic cells such as osteoclasts or inflammatory cells in or near bacteria-laden resorption pits. Finally, both OMJ and ONJ specimens exhibited evidence of putative nanowires. These putative nanowires are thought to be communication networks between different types of organisms in the biofilm community.18

DISCUSSION

This is the first study, to our knowledge, showing evidence of microbial biofilms in the bone of patients affected by OMJ. In addition, it is the first time, to our knowledge, that bone specimens from patients with this condition have been examined by means of SEM, a method applicable to biofilm evaluation. In contrast to most reported cases of long-bone osteomyelitis, which are caused by staphylococci, the organisms we identified in OMJ specimens were consistent with oral microbial flora common in pathogenic states. Actinomyces have been identified in cases of OMJ,19,20 but a biofilm mode of growth exhibited by these organisms has not been recognized until now. Badros and colleagues21 reported an association...
between bacteria and ONJ. However, until our recent laboratory study and the work of Hansen and colleagues, most of these investigations involved the use of traditional microbiological methods of screening organisms on culture media, which cannot be used to identify unculturable biofilm organisms.

**Traditional screening methods.** Traditional methods of screening usually miss biofilm bacteria because they are predicated on planktonic bacterial growth. Oral bacteria have evolved across millions of years in mixed biofilm communities, and an organism dependent on another in such a community will not be able to grow in vitro as it does in vivo. Furthermore, cultures or swabs from the oral cavity have high rates of contamination, and exposure to oxygen can kill many anaerobic bacteria, such as the ones identified in this study.

In addition, the results of antibiotic sensitivity testing techniques are not applicable to biofilms and can lead to misguided antibiotic therapy. Susceptibility tests used with in vitro biofilm models have shown the survival of osteomyelitis-causing biofilms after treatment with conventional antibiotics at concentrations as high as 1,000, the minimum inhibitory concentration of the bacteria measured in bacterial culture. Such antibiotic concentrations are impossible to reach clinically without causing serious harm to patients, thus requiring novel approaches to therapy.

Before 2003, clinicians diagnosed most cases of ONJ as OMJ. In 2003, Marx first described the role that bisphosphonates play in the disease process. The misdiagnosis of ONJ was due mainly to the strikingly similar clinicopathologic features of both conditions despite their different etiologic mechanisms (Table). Our findings help explain why OMJ and ONJ can be acute or chronic in nature, share overlapping clinicopathologic features and are highly resistant to conventional treatment modalities. To control these difficult-to-treat diseases, we need to identify and eradicate biofilms, an approach that has been proposed by orthopedic surgeons and biofilm microbiologists.

**Resorption.** Bone resorption was evident in all specimens in our study, which potentially was caused by the microbial biofilms because there was no evidence of eukaryotic cells, such as osteoclasts, within or adjacent to the resorption pits. Furthermore, given that these patients were receiving an antiresorptive medication (bisphosphonate), the degree of resorption seen is unlikely until we consider the possibility of direct resorption by bacteria. Certain bacteria are capable of causing pathological bone loss through various mechanisms. Given the large numbers of gram-negative bacteria observed in the specimens in our study, it is conceivable that the porins they carry may play a major role in causing bone destruction and sequestration. Porins are beta barrel proteins located on the outer membrane of gram-negative bacteria that render the membrane permeable to metabolites and smaller molecules (< 1,500 daltons); in addition, porins are involved in the synthesis of proinflammatory mediators.

Furthermore, tissue destruction by bacterial enzymes like collagenases, with subsequent tissue invasion, may allow bacteria to evade host

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**TABLE**

Comparison of osteomyelitis of the jaw (OMJ) with osteonecrosis of the jaw (ONJ).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>OMJ</th>
<th>ONJ</th>
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<tbody>
<tr>
<td><strong>Etiology</strong></td>
<td>Spread of odontogenic or periodontal infection to jawbone</td>
<td>Bisphosphonates, jawbone exposure, comorbid conditions</td>
</tr>
<tr>
<td><strong>Pathogenesis</strong></td>
<td>Inflammation leading to abnormal remodeling, sclerosis, ischemia and necrosis; biofilm present</td>
<td>Antiosteoclastic and antiangiogenic activity leading to abnormal remodeling, sclerosis, ischemia and necrosis; biofilm present</td>
</tr>
<tr>
<td><strong>Clinical Features</strong></td>
<td>Swelling, pain, erythema, ulcer, pus and sequestra may be present; sepsis and/or fever possible; jawbones affected predominantly</td>
<td>Swelling, pain, erythema, ulcer, pus and sequestra may be present; rarely sepsis or fever; only jawbones affected</td>
</tr>
<tr>
<td><strong>Radiographic Findings</strong></td>
<td>Poorly defined lytic (radiolucent) lesion with or without sequestrum or involucrum (radiopaque)</td>
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<tr>
<td><strong>Macroscopy</strong></td>
<td>Healthy to dead bone (sequestrum) visible grossly</td>
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<tr>
<td><strong>Light Microscopy</strong></td>
<td>Vital to nonvital bone (sequestrum) with or without inflammation or organisms</td>
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<tr>
<td><strong>Scanning Electron Microscopy</strong></td>
<td>Mixed-species bacterial biofilms; presence of resorption pits filled with bacterial biofilms</td>
<td>Mixed-species bacterial and yeast biofilms; presence of resorption pits filled with bacterial biofilms</td>
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immune responses or gain access to more anaerobic sites deep within the host. Alternatively, the production of tissue-destroying enzymes may result in local impairment of the blood supply and the buildup of metabolites from protein degradation, resulting in a lowering of the redox potential at the infected site, thus favoring the growth of anaerobic bacteria. Once growth of bacteria is established, further colonization, biofilm growth and antimicrobial resistance may be conferred to the microbial community through conjugation, transformation, coaggregation and communication networks consisting of putative nanowires.

**Bone necrosis.** In the context of this study’s findings, it is understandable that most cases of bone necrosis secondary to bisphosphonate therapy occur in the jaws, where oral bacteria have access to bone (for example, through saliva), especially after exposure of bone following a dental procedure such as an extraction (the most common dental procedure associated with ONJ). Long bones are well protected from bacteria through layers of skin and connective tissue and rarely are exposed to such microorganisms. In the oral cavity, bone can be colonized easily by the abundant bacteria and yeast that have the potential to cause a biofilm-mediated disease. Although routinely exposed to oral microbes, the jaws usually are effective in preventing microbial colonization. Typically, such colonization requires a notoriously pathogenic organism such as actinomyces—which predominated in all OMJ cases in this study—to infect the jaws in susceptible patients.

Accordingly, jaw osteomyelitis is rare compared with long-bone osteomyelitis, with the latter usually occurring after trauma to bone that exposes it to skin bacteria. In the oral cavity, however, if a pathological condition such as an odontogenic infection or periodontal disease or trauma occurs that exposes the jaws to oral organisms, local defenses and repair mechanisms can wall off and eradicate the necrotic bone via sequestration.

Sequestration is the most common clinical feature in established cases of ONJ. Therefore, microbial biofilms could play an important role in the pathogenesis of ONJ in the context of bisphosphonate therapy, as has been implicated recently. This may explain the finding of numerous morphotypes in ONJ cases compared with monospecies-dominated OMJ cases (actinomyces), because the bone in ONJ is susceptible to a more diverse colonization by organisms that usually do not supplant bone, such as superficial fungal organisms (C. albicans, for instance) and some of the more benign bacterial morphotypes seen in the ONJ cases.

This susceptibility likely is derived from the antiosteoclastic (sclerotic) and antiangiogenic (ischemic) effects of bisphosphonates, as well as other known and some undetermined factors. For example, the finding of Candida organisms in the ONJ group but not in the OMJ group may be attributed to a greater risk of developing oral fungal infections associated with older age, poorly controlled diabetes or dry mouth resulting from use of hypertension medications in this population.

We should point out that this is a descriptive study, but a necessary first step in the characterization of microbial biofilms and their potential role in the pathogenesis of jawbone infections such as OMJ and ONJ. Future studies will need to focus on hypothesis-based approaches to the characterization and treatment of both conditions. The findings in this study support a role for microbial biofilms in both disease processes and indicate a potential target for clinical therapy that considers antibiofilm modalities in the treatment and prevention of OMJ and ONJ.

**CONCLUSION**

The clinical and microscopic findings in OMJ and ONJ cases suggest the presence of a biofilm-mediated infectious process that must be prevented and treated. Thus, some researchers favor conservative management with antimicrobial mouthrinses and minor sequestrectomy of the exposed necrotic bone instead of complete resection of the diseased bone unless necessary, with frequent and close follow-up evaluations.

**Disclosure.** None of the authors reported any disclosures.


