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Biofilm-Related Infection: Diagnosis, Treatment, and Prevention

Biyofilm İlişkili Enfeksiyonlar: Tanı, Tedavi ve Korunma

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Abstract

Biofilms can cause infections associated with orthopedic devices, endotracheal tubes, intravenous catheters, urinary catheters, and tissue fillers as well as chronic wound infections, and chronic lung infections in cystic fibrosis patients. The diagnosis and treatment of biofilm-related infections are difficult. For diagnosis, there are methods for imaging the biofilm at the site of infection, as well as microbiological methods for identifying the microorganism causing the biofilm infection. In recent years, different therapeutic approaches have been investigated to address difficulties in the diagnosis and treatment of biofilm-related infections, particularly problems such as antibiotic resistance. Together with various antibiotics, it is predicted that antimicrobial photodynamic therapy, antimicrobial lock therapy, and various bioactive molecules/enzymes with antibiofilm activity will become more widely used as therapeutic models in the future. The aim of this paper was to review methods of diagnosis, treatment, and prevention of biofilm-related infections.
Keywords: Echinocandins, chronic wound, prosthetic joint infections, "Quorum-sensing" inhibitors, antibiotic-coated implants

Öz

Biyofilmler ortopedik protez implantları, endotrakeal tüpler, vasküler kateterler, üriner kateterler, doku dolgu implantları gibi araç ilişkili enfeksiyonlara, kronik yara enfeksiyonlarına ve kistik fibrozisli hastalarda kronik akciğer enfeksiyonları gibi enfeksiyonlara neden olabilirler. Biyofilm enfeksiyonlarının tanı ve tedavisi genellikle güçtür. Tanıda biyofilmin yerinde tanımlanmasına yönelik görüntüleme yöntemleri ve biyofilmi oluşturan mikroorganizmanın tanımlanmasına yönelik mikrobiyolojik yöntemler bulunmaktadır. Günümüzde tanı ve tedavisinde yaşanılan güçlükler ve özellikle antibiyotik direnç sorunları gibi problemlere yönelik farklı tedavi yaklaşımları araştırılmaktadır. Çeşitli antibiyotiklerle birlikte fotodinamik tedavi, antibiyotik kilit tedavisi ve çeşitli biyofilm etkili biyoaktif molekül/enzim tedavilerin yakın gelecekte daha yaygın kullanılacağı öngörülmektedir. Bu derlemenin amacı biyofilm ilişkili enfeksiyonların tanı, tedavi ve korunma yöntemlerinin gözden geçirilmesidir.
Anahtar Kelimeler: Ekinokandinler, kronik yara, protez eklem enfeksiyonları, "Quorum sensing" inhibitörleri, antibiyotik kaplı implantlar

Introduction

Biofilm infections have an important place in the clinical practice due to the risk of microbial resistance resulting from recurrent, chronic, or long-term antibiotic use. Management can be very difficult in this patient group. Biofilm infections may be observed in clinical practice as device-related infections or tissue infections. The medical devices mainly associated with biofilm-related infections include central/peripheral vascular catheters, peritoneal dialysis catheters, ventricular devices, contact lenses, prosthetic cardiac valves, cardiac pacemakers, vascular grafts, breast implants, tissue filler implants, orthopedic prosthetic implants such as prosthetic joints, voice prostheses, and intrauterine devices. In terms of spesific tissue infections, recurrent lung infections in patients with cystic fibrosis, urinary tract infections, gallbladder infections, chronic sinusitis,

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otitis media, dental plaques, periodontitis, kidney stones, osteomyelitis, vaginal infections, and chronic wound infections are common[1,2].

Gram-positive pathogens with a high tendency to form biofilms are *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus* species, *Enterococcus faecium, Streptococcus mutans, Streptococcus sanguinis, Streptococcus mitis, Streptococcus pyogenes,* and *Actinomyces viscosus*, while Gram-positive pathogens include *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter* species, *Flavobacterium* species, *Alcaligenes* species, *Serratia marcescens, Aeromonas hydrophila, Brucella melitensis, Burkholderia cepacia, Proteus mirabilis, Enterobacter cloacae, Prevotella intermedia, Porphyromonas gingivalis,* and *Yersinia pestis. Candida albicans, Candida parapsilosis, Aspergillus* species, and *Fusarium* species are fungi with higher potential for biofilm formation[1-4].

The aim of this paper is to review the diagnosis, treatment, and prevention of biofilm-related infections.

What is Biofilm?

Biofilm, or slime layer, is formed when microorganisms adhere to and colonize a surface and produce an extracellular polymer-coated mass. The colonized surface can be a living tissue or a synthetic surface such as a catheter. The main feature of biofilm is the polymer layer that surrounds the microbial colony like a shell and protects it from external influences. The biofilm layer enables the microorganisms to colonize the surface, accelerate infection, evade the immune system, and demonstrate resistance to antimicrobial agents. The stages of biofilm formation are (1) adherence of microorganisms to the surface, (2) microcolony formation, (3) biofilm maturation, and (4) dispersal from the biofilm and restarting the cycle from the adherence stage. Biofilm includes 95-97% water. The other components of the biofilm are microorganisms (2-5%), DNA/RNA (1-2%), polysaccharide (1-2%), and protein (1-2%). Detailed analysis of biofilm composition revealed that it contains surface proteins such as polysaccharides, exopolysaccharides such as alginate, pel, and psl, poly-gamma glutamate, type 4 pili, CupA fimbriae, lectin-binding proteins, extracellular DNA, N-acetyl glucosamine proteins, and Baps (biofilm-associated proteins). Biofilm composition may vary depending on the microorganisms forming it[5].

In a health personnel survey about biofilm and its importance, analysis of responses from 1,223 people showed that most staff members knew the definition of biofilm, but the role of biofilm in chronic wounds, the effect of wound debridement and dressings as interventions against biofilm, diagnosis of biofilm, and antimicrobial agents for the treatment of biofilm were lesser known topics[6].

Diagnosis of Biofilm-forming Microorganisms

While the diagnosis of biofilm may be a matter of microbiology in terms of demonstrating and identifying the microorganism, it also concerns materials science and engineering as it includes subjects such as the monitoring and measurement of the surface-coating mass. Certain nonmedical imaging and measurement systems are used in the diagnosis of biofilm. Classic biofilm diagnosis involves microbiological culture-based methods. Cultured microorganisms can be identified using classical methods or novel, protein-based identification methods such as MALDI-TOF mass spectrometry. There are also microbiological methods based on visualization of the biofilm. Moreover, the genes responsible for biofilm formation may be demonstrated using molecular methods. Biofilm identification is imperative in device-associated infections and recurrent chronic tissue infections. An appropriate identification method should be chosen for samples according to the patient and site of infection[6-8].

1. Classical Methods

The 'tube adhesion method' described by Christensen et al.[9,10] in 1982 for *Staphylococcus epidermidis* is the most classical method used to show the slime factor forming the basis of a biofilm. In this method, the biofilm layer formed on a synthetic surface can be evaluated visually using dyes such as crystal violet and safranin. Spectrophotometric measurement of biofilms formed in microplates instead of test tubes is a new method resulting from adaptation and development of Christensen et al.'s[9,10] method. Slime-producing strains are differentiated using this method. When adhesion is evaluated visually, it can be scored from 1+ to 4+. While not an exact numeric measurement, visual grading of the slime layer is an accepted method in the literature. A fully numeric comparison is possible when measuring the biofilm. Spectrophotometric evaluation is a requirement, especially in studies evaluating the efficacy of antibiofilm approaches. In cases where a measured value is necessary, spectrophotometric comparison is recommended. Resazurin (7-hydroxy-3H-phenoxazine-3-one-10-oxide), also known as alamar blue, is a biological stain that does not damage living cells. It is converted to pink resorufin by cellular metabolic activity. Resorufin can be measured spectrophotometrically. Similarly, 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide salt (XTT) is converted to the water-soluble formazan[7].

If the question is whether an organism produces biofilm, methods that yield only yes/no results may be preferred. In 1989, Freeman et al.[11] described an agar-based method in which slime-positive organisms form dark-colored colonies and slime-negative colonies form colorless colonies on agar

plates containing congo red. However, this method is not quantitative[11].

In the 1990s, the Calgary Biofilm Device (CBD) was developed to demonstrate the efficacy of antibiotics on biofilm. The CBD is a model method that utilizes a two-piece microplate. The steps of the method are shown in Figure 1. There is a lid-like top piece featuring 96 pegs. These pegs serve as a surface that completely protrudes into the standard 96-well microplate that constitutes the bottom piece, allowing biofilm formed in the well to be transferred undisturbed to another liquid or solid environment. The CBD enables the inhibitory effect of any antimicrobial substance on the biofilm to be evaluated by colony counting, and is an indispensable classical method. It is still used today with various modifications[12-14].

Biofilms are not static. They form in an environment where body fluids and the microorganism colony generate a continuous flow. Due to its dynamic structure, biofilm models have been developed using perfusion models instead of static models. The biofilm perfusion model enabled the staging of realistic scenarios[15]. A liquid containing microorganisms, flows through a tube representing the catheter, and adherence to the catheter surface occurs over time. The perfusion model involves two pumps. The flow rate through the system may be regulated with the first pump, while the second pump controls delivery of the antimicrobial agent into the medium. The cells dispersed from within the tube catheter can be quantified using any counting method. Thus, this method enables the quantitative assessment of anti-biofilm approaches such as catheter locks[15].

2. Imaging Methods Used in the Diagnosis of Biofilm

Evaluating the adherence of microorganisms to cells by counting is a commonly used method. Since the light microscope offers a two-dimensional image, it does not allow precise measurement of the biofilm. In addition to classical imaging methods, new systems that have been recently developed can be used to visualize the biofilm[16]. Electron microscopy offers a three-dimensional, quantifiable image and has been long used to demonstrate the biofilm mass. The surface of a microcolony can be visualized using scanning electron microscopy (SEM), while the inner structure of the biofilm mass can be visualized using transmission electron microscopy. The atomic force microscopy can be considered a more advanced electron microscope. It is used for high magnification, especially for the evaluation of surfaces. Atomic force microscope is not commonly used in medicine, but enables visualization and measurement of surfaces in the engineering sciences. Because it includes a variable called surface roughness, it may also be utilized in biofilm studies[17].

Laser scanning microscopy (LSM), magnetic resonance imaging, and scanning transmission X-ray microscopy are recently developed imaging methods. These new methods are superior in that they not only demonstrate biofilm thickness, but also provide a detailed image of its internal structure. The addition

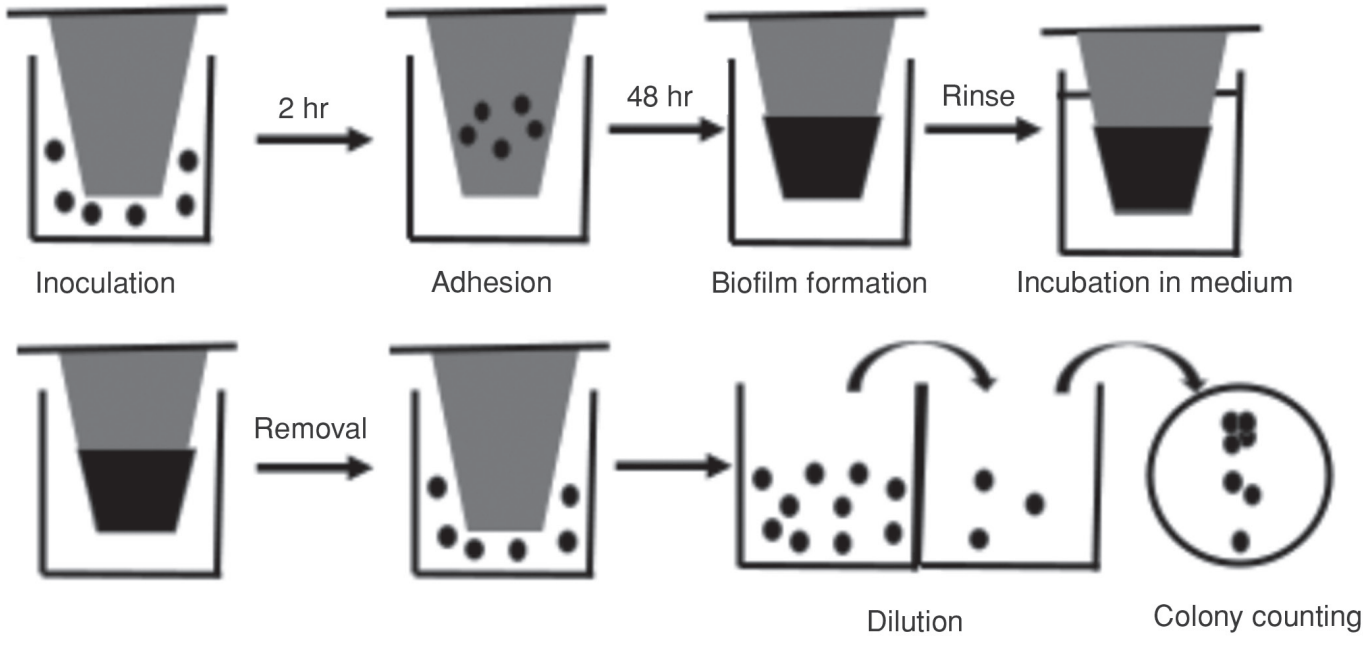

Figure 1. Calgary Biofilm Device

of hybridization and fluorescence techniques enables the characterization of all structures within the biomass[18].

3. The Role of Molecular Methods in Biofilm Diagnosis

Moleculer methods can be used for two purposes in the diagnosis of biofilm. The first is to identify the microorganisms in the biofilm via molecular target genes. Once the microorganism is known, the second purpose is to demonstrate the genes responsible for biofilm formation, adhesion, and slime production. Molecular method may be an amplification-based method with a single target, or a DNA array system that targets gene under- and overexpression in the biofilm. Demonstrating DNA requires RNA isolation from the biofilm and a transcription-based molecular method. The presence of DNA does not always mean that there is transcription. Biofilms utilize "Quorum-sensing" mechanisms. There are silent genes and RNA silencing occurs. The use of direct proteomic approaches would allow results to be obtained at the protein level. Genes responsible for adhesion vary depending on the species of microorganism. When planning a study of biofilm diagnosis, it is recommended to choose the biofilm genes and check the target gene sequence in a gene bank before designing primers. A molecular study can be performed after separating each microorganism in the biofilm mass, or the target genes can be studied after directly isolating biofilm DNA/RNA before dispersal.

4. Identification of Biofilm-related Infection at the Infection Site

Biofilm formation is the principle step in prosthesis infection development. Biofilm can be visualized and measured with the prosthesis while in place or after removing it[20]. Using the methods discussed above, the biofilm can be evaluated *in situ*. Without identifying the causative microorganism, the mass can be approached by visual and quantitative methods. A biofilm can be identified on a prosthesis by direct inoculation of the prosthesis to a liquid or solid media, obtaining samples from the prosthesis after sonication or rinsing, and performing inoculation[21], fluorescent microscopy and fluorescent *in situ* hybridization, confocal LSM, SEM, or DNA isolation and analysis from the prosthesis[22]. Advantages and disadvantages of methods for biofilm diagnosis and guideline recommendations are compared in Table 1.

Prevention and Treatment of Biofilm-related Infections

Prophylaxis is among the primary preventive methods against biofilm infections. Preemptive, empirical, and definitive treatment methods are more often performed during or after the development of infection[23,24].

Prevention of Biofilm Infections

Prophylactic Approach

Short-term prophylaxis: Surgical prophylaxis during the perioperative period is the short-term antibacteial prophylactic method most commonly used to prevent surgery-related biofilm infections. Besides surgical prophylaxis, short-term prophylaxis administered for one or two weeks can be used to delay biofilm infection in patients with urinary catheter/stent. However, since it involves systemic antibiotherapy, the prophylactic approach is not recommended in the guidelines due to the risk of superinfection with multi-drug resistant strains[1].

There is no evidence to support the use of systemic antibiotics as prophylaxis for catheter-related bloodstream infections (CRBSI) or to prevent the development of wound site biofilm. Moreover, there are no recommendations for preventing tissue filler material infections[1,25].

Prophylactic approaches to intermediate and long-term endotracheal tube (ETT) biofilm: The prophylactic use of topical, nonabsorbed antibiotics used oropharyngeally/gastrically for selective decontamination is not recommended. High-dose inhaled antibiotic (e.g., gentamicin) has been shown to protect against ETT biofilm. Silver-coated ETT shows maximum efficacy within the first 10 days after intubation and was shown to reduce mortality and cost in patients with ventilator-associated pneumonia (VAP). Use of silver-coated ETT is recommended as prophylaxis against biofilm[26]. Washing the tube with mucus cleansers is a method for mechanically removing biofilm and was shown to be effective in small patient groups. The efficacy of the inflatable balloon method in removing ETT biofilm in pediatric patients has been demonstrated in a limited number of studies [1].

Use of topical antibiotics

Materials impregnated with antibiotics (mostly gentamicin, tobramycin and vancomycin) are used to reduce the incidence of orthopedic device (prosthesis)-related biofilm infection[27]. The use of antimicrobial agents (nitrofurantoin) in short-term urinary catheters is not sufficient to prevent biofilm infections. Antibiotic-coated urinary catheters delay biofilm formation, but do not prevent it. As silver-coated and nitrofurantoin-impregnated catheters do not reduce the incidence of symptomatic urinary tract infection, they are not recommended for routine use[28]. The addition of antibiotics is recommended for intravenous catheters (e.g., silver/minocycline-coated), tracheal tubes (e.g., silver-coated), joint prostheses, and orthopedic surgery bone cements[1].

Prevention of CRBSI: A randomized controlled study showed that the use of chlorhexidine-impregnated sponges and

Table 1. Diagnostic methods for biofilm

Methods		Advantages	Disadvantages	ESCMID guideline
Classical methods	Tube adhesion method-visual evaluation	Easy	Subjective evaluation	None
	Microplate adhesion method-spectrophotometric assay	Provides quantitative data Comparable Antibiofilm efficacy can be measured	Requires spectrophotometer	None
	Congo red agar method	Easy Yes/no result	Not quantitative	None
	Calgary Biofilm Device	Provides quantitative data Comparable Antibiofilm efficacy can be measured	Requires special microplate	None
	Perfusion models	Lock therapies can be evaluated Anti-biofilm drugs can be evaluated	Requires expensive equipment	None
Imaging methods	Light microscope	-	Two-dimensional Cannot be evaluated	**Yes**
	SEM	Three-dimensional surface analysis	Internal mass not visible Requires equipment and experienced center	**Yes**
	TEM	Three-dimensional and internal mass can be visualized	Requires equipment and experienced center	None
	AFM	Can provide three-dimensional measurement Gives surface roughness	Requires equipment and experienced center	None
	LSM	Not only thickness but also internal structure can be evaluated	Requires equipment and experienced center	None
	MRI	Not only thickness but also internal structure can be evaluated	Requires equipment and experienced center	None
	STXM	Not only thickness but also internal structure can be evaluated	Requires equipment and experienced center	None
Molecular methods	Amplification-based tests	Easy Yes/no result	Only indicates gene presence	**Yes**
	Array-chip technology	Multiple targets can be selected	Requires expensive equipment	None
	Ohmic approaches	Results at protein level	Expensive requires equipment	None
In situ biofilm identification		Medical device is protected *in situ*	Lack of experience	**Yes**

SEM: Scanning electron microscopy, TEM: Transmission electron microscopy, AFM: Atomic force microscopy, LSM: Laser scanning microscopy, MRI: Magnetic resonance imaging, STXM: Scanning transmission x-ray microscopy, ESCMID: European Society of Clinical Microbiology and Infectious Diseases

chlorhexidine dressings in central venous catheter (CVC)-related bloodstream infections reduced the incidence of CRBSI by 60-67%[29]. The efficacy of catheters coated with silver sulfadiazine, minocycline, and rifampin has been demonstrated in trials and meta-analyses, but the considerable cost has limited its clinical use exclusively to very-high-risk patients[29-32].

Antibiotic lock therapy (ALT)

This method involves continuous administration of concentrated antibiotics in the catheter lumen for 12-24 hours. The ALT prophylactic approach is limited to patients with recurrent CRBSI. In hemodialysis patients with long-term catheter, application of minocycline-EDTA was found to be superior to heparin. Taurolidine-citrate is one of a growing number of local drugs introduced in recent years. It resembles biocides more than antibiotics, and its clinical use is recommended due to its antibacterial and antifungal properties. Reports comparing heparin and taurolidine-citrate in high-risk patients receiving total parenteral nutrition showed that taurolidine-citrate reduced CRBSI (number of bloodstream infections per 1000 catheter days was 1.3 in the heparin group and 0.3 in the taurolidine-citrate group)[30]. When used without heparin, it was shown to increase the risk of thrombosis[29-31].

In randomized controlled studies performed in high-risk pediatric hematology patients under chemotherapy and total parenteral nutrition, the application of 70% ethanol significantly reduced the rate of CRBSI compared to heparin. In one of those studies, the number of bloodstream infections per 1000 catheter days was 12.2±10.6 before ethanol lock therapy and decreased to 0.9±1.8 after ethanol lock therapy[33]. Meta-analyses have shown that ethanol lock therapy was more effective in reducing

CRBSI incidence than heparin lock therapy[32]. However, it may increase the risk of thrombosis. Amphotericin B, ethanol, or echinocandins are promising for the prevention of fungal infections. Since the application of antibiotic/antiseptic ointments may increase settlement by *Candida* species, it may lead to infection or colonization[33,34].

Preemptive treatment of Biofilm Infections

If the microorganism responsible for a biofilm infection has been isolated, eradication can be achieved by early preemptive antibiotic treatment before the onset of clinical signs or symptoms. In cystic fibrosis patients with chronic *P. aeruginosa* lung infection, intermittent colonization can be prevented by preemptive systemic or inhaled antibiotic eradication therapy. ALT (e.g., vancomycin) may be used for CVC colonization with recurrent growth of coagulase-negative staphylococci without clinical signs. There are no data or recommendations for VAP, chronic wound infection, orthopedic prosthesis-related infections, urinary catheter and urethral stent, or tissue filler applications (e.g., breast implants)[1].

Treatment of Biofilm Infections

Treating with antibiotics or combined therapies incorporating novel antibiofilm treatment methods is recommended to reduce the risk of antibiotic resistance developing in biofilm infections[1,3,4]. Current therapeutic doses are those known to prevent the spread of planktonic cells. The antibiotic concentrations required to suppress biofilm infections are unknown. Effectiveness of treatment can be evaluated using clinical signs, symptoms, culture-dependent or culture-independent methods, and imaging techniques. The risk of microorganism growth in the biofilm or relapse increases when treatment is discontinued. Antibiotic sensitivity of the biofilm-producing bacteria may decrease over the course of treatment[35].

Treatment of prosthesis infections: For prosthesis infections that have not developed a sinus tract and are caused by a susceptible agent, debridement followed by long-term biofilm-appropriate combined antibiotherapy is recommended, without removing the prosthetic. In particular, a combination of combined antibiotic and initial surgical debridement/immediate implant replacement and monotherapy were found to be effective. Rifampicin and fluoroquinolones may be preferable for combination therapy due to their efficacy against staphylococci and Gram-negative bacteria, respectively[35,36].

Treatment of catheter infections: In CRBSI, patients whose catheter cannot be removed due to thrombocytopenia and those with CRBSI due to *S. aureus* or *Candida,* should be treated with both ALT and systemic antibiotic/antifungal therapy[1,37,38]. In ALT, 100- to 1000-fold higher minimum inhibitory concentration values can be attained for 12-24 hours. Due to the hematogenous complications caused by *S. aureus*, catheter removal is recommended. The Infectious Disease Society of America (IDSA) recommends vancomycin or daptomycin for the treatment of methicillin-resistant *S. aureus* (MRSA)-associated catheter infection. The joint guidelines of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), International Society of Chemotherapy (ISC), and European Society of Cardiology (ESC) recommend vancomycin or teicoplanin (daptomycin if not responsive). In case of persistent MRSA bacteremia under vancomycin therapy, the joint recommendation of IDSA and ESCMID/ISC/ESC is to use treatment options effective against biofilm such as high-dose daptomycin (10 mg/kg/day) + gentamicin/rifampicin/linezolid/trimethoprim-sulfamethoxazol/beta-lactam antibiotic combinations[38,39]. ALT may be used in patients with candidemia whose catheter cannot be removed. In a study on ALT, liposomal amphotericin B was shown to be 70% effective in a model of silicone catheter biofilm caused by *C. albicans, C. glabrata,* and *C. parapsilosis*[37]. In recent years, the echinocandins class of antifungal drugs has been used in antifungal lock therapy and showed good *in vitro* efficacy against *Candida* biofilms. Amphotericin B and caspofungin are shown to have the best antifungal activity in *in vitro Candida* biofilm models, whereas azoles were shown to have weak activity[34,37].

Treatment of VAP: Systemic antibiotic therapy is not sufficient to remove ETT biofilm. Concentrations of systemically administered antibiotics are sufficient for planktonic bacteria in the respiratory compartments, but not for bacteria growing in biofilms in the bronchi and sputum. Combination antibiotics delivered systemically and by inhalation can reach high concentrations in the various layers of the lung[1].

Treatment of urinary catheter-related infections: Antibiotic treatment in urinary catheters is insufficient in the presence of biofilm. It only reduces the microbial load, which suppresses symptoms and urinary culture growth. If the catheter is not removed or is reinserted at the same location, relapse may occur after treatment. The use of renally excreted antibiotics is recommended together with catheter and stent replacement[1,24].

Treatment of chronic wound infections: The addition of nonantibiotic local treatments such as debridement and vacuum therapy are recommended for chronic wound infections. If systemic treatment is given, a combination of two systemic antibiotics with different mechanisms as well as local disinfectants is recommended. Debridement is recommended more to promote wound healing. Topical antimicrobial agents have been found effective in preventing the reformation of biofilm after debridement. Negative-pressure wound therapies and irrigation treatments prevent biofilm formation in chronic

wounds and reduce the bacterial load[1]. The majority of patients with chronic wound infections have diabetic foot infections. The use of local gels that contain borate (3% sodium pentaborate pentahydrate), which is effective against biofilm, was shown to increase the rate of wound healing in these patients[40].

New Antibiotics and Novel Treatments for Biofilm

Studies are ongoing to develop new antibiofilm drugs that will be effective antibiotic therapies against both the biofilm and planktonic cells. New drugs that combine "Quorum-sensing" inhibitors (e.g., antibiofilm substances such as lactonase, patulin, penicillic acid, baicalin hydrate) with antibiotics (e.g., tobramycin, gentamicin, ciprofloxacin) are promising. There are also ongoing studies about anti-inflammatory treatment approaches for tissue damage surrounding the biofilm infection due to the anti-inflammatory host response, drugs that disperse or degrade the biofilm matrix, and enzyme chelators or biofilm components[4,41,42].

Current research areas are very diverse, including new combinations of biofilm-degrading drugs and antibiotics, newly developed topical antibiotic regimens, combinations of antimicrobial agents with ultrasonography, electricity or ultraviolet light, "leukopatch" applications containing leukocytes and platelet-rich fibrins that can be used for local treatment of infected chronic ulcers, anti-*P. aeruginosa* vaccines against chronic biofilm infections in cystic fibrosis patients, and mechanical devices such as mucus slurpers placed in ETTs to prevent VAP[1].

In the future, there may be vaccines to prevent prosthetic infections, antibiofilm-coated prostheses, zinc nanocoating of prosthetic surfaces, and antibiotic-loaded fixed cements for arthroplasty in high-risk patients[43]. In a recent *in vivo* animal model, biopolymer chitosan sponges coated with ciprofloxacin and rifampicin were shown to inhibit *P. aeruginosa* (ATCC 27.317) and *S. aureus* (ATCC 12598)[44]. In a study published in Nature in 2015, it was reported that the combination of antibiotic therapy and shock waves can be used in the treatment of medical device-related biofilm infections. The study demonstrated that the combination of ciprofloxacin and shock waves yielded successful results in biofilm-related chronic *Pseudomonas* lung infection and staphylococcal wound infection[45].

Antimicrobial Photodynamic Therapy

This new antimicrobial application is based on the generation of an antimicrobial effect in the tissue by activating a nontoxic dye with a low-energy light source. The combination of photodynamic dye and light produces reactive oxygen species (ROS), which induces apoptosis of the infecting microorganism. This approach facilitates microbial eradication and biofilm clearance[46]. There are currently a few approved photodynamic antimicrobial agents that are mostly topical and locally effective, such as toluidine blue and methylene blue.

Photodynamic therapy is advantageous because it is broad-spectrum, eradicates pathogens in the biofilm, has no or minimal tissue toxicity, does not lead to resistance in microorganisms, does not induce release of proinflammatory cytokines, is harmless to mammalian tissue, its effect starts in a few minutes, and is practical and cost-effective[47].

Photodynamic therapy is among the new therapeutic approaches and is used more for dermatological diseases, dental treatments, and tumor cell inhibition in clinical practice. Due to its antimicrobial effects, it has potential as an alternative method that avoids drug resistance problem in the treatment of sinusitis, keratitis, otitis media, necrotizing fasciitis, intraabdominal abscess, burns/wounds/skin infections, cystitis, gastric *Helicobacter pylori* infections, localized tuberculosis, fungal infections, oral and dental infections caused by MRSA, vancomycin-resistant *Enterococci*, *C. albicans*, and herpes simplex virus. However, with the recently developed chemiluminescent photodynamic antimicrobial therapy, it is believed that photodynamic therapy may be used with endoscopy, fiberoptic devices, and transcutaneous needles in the treatment of infections in other sites and deep infections in the future[48,49]. In 2015, a bladder catheter that utilizes photodynamic therapy to prevent urinary infection in neurogenic bladders was produced as part of a medical innovation program. Furthermore, a study demonstrating that infrared light-activated thermosensitive liposomal therapy was synergistic to antibiotic treatment in an animal model of subcutaneous abscess was published recently[50].

It was reported that the addition of topical silver colloid to a two-week oral antibiotic therapy was effective against persistent chronic rhinosinusitis[51]. The application of nitric oxide was shown to be beneficial in wound infections caused by polymicrobial or resistant microorganisms. Nitric oxide caused a 2- to 10-log decrease in *S. aureus, P. aeruginosa, Acinetobacter baumannii, E. coli,* and *Candida* species, and 1.9- and 3.2-fold decreases in pyocyanin and elastase activity in *P. aeruginosa,* respectively[52].

Conclusion

Biofilm-related infections are important health problems. Diagnosis and treatment of these infections are difficult and require new technological advances. Novel treatment modalities that prevent biofilm formation on surfaces such as catheters are being sought[53]. Biofilm may be diagnosed *in situ* or based on the isolated microorganisms. Imaging and molecular methods

can be used in addition to microbiological culture methods[54]. New technologies aim to enable *in situ* diagnosis and eradication of biofilms on medical devices[55-57].

Researchers should plan studies investigating new technologies that can be added to antibiotherapy to prevent biofilm formation in particular. Natural antimicrobial compounds and photodynamic therapy are among the current research areas in this field. In addition, comparative clinical studies on *in situ* diagnosis and elimination of biofilms on medical devices are needed.

Ethics

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Authorship Contributions

Surgical and Medical Practices: A.K., Ö.G.T., Concept: A.K., Ö.G.T., Design: A.K., Ö.G.T., Data Collection or Processing: A.K., Ö.G.T., Analysis or Interpretation: A.K., Ö.G.T., Literature Search: A.K., Ö.G.T., Writing: A.K., Ö.G.T.

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