MENTORS IN ORTHOPEDICS

BACTERIA LIVING IN BIOFILMS IN FLUIDS: CAN WE IMPROVE OUR CULTURAL EXAMINATION OFSYNOVIAL AND OTHER ORGANIC LIQUIDS ?

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SUMMARY

Background: Biofilms represent the primary physiological state of microorganisms, characterized by surface-associated communities sequestered within an extracellular polymeric matrix. In clinical orthopedics and broader medical practice, these structures are implicated in 65% to 80% of human infections, including periprosthetic joint infections. While traditionally classified as planktonic cells, bacteria in synovial fluid, blood, and cerebrospinal fluid frequently exist as suspended aggregates. These aggregates exhibit heightened tolerance to antimicrobial therapy and host immune responses compared to isolated cells, contributing to the persistence of chronic infections and the failure of conventional treatments.

Objective: This review examines the presence of microbial biofilms within human biological fluids, focusing on their pathological roles and the resulting limitations of standard diagnostic culture techniques in identifying biofilm-associated infections.

Key Points: Biofilm aggregates in synovial fluid significantly reduce the sensitivity of microbiological investigations, with reported rates ranging from 41.6% to 90%. These structures alter bacterial phenotypes and virulence factors, hindering antibiotic penetration. Similar phenomena are observed in catheter-associated urinary tract infections, bloodstream infections, and cerebrospinal fluid shunt malfunctions. Conventional colony-forming unit counts often underestimate bacterial presence due to cellular aggregation. Chemical pre-treatment with dithiothreitol (DTT) facilitates the disruption of the biofilm matrix by reducing disulfide bonds. This process releases microorganisms into a planktonic state without compromising viability, thereby enhancing the sensitivity of subsequent cultures and molecular assays for both solid tissue and liquid biological samples.

Conclusion: The presence of biofilm aggregates in biological fluids complicates the diagnosis and management of chronic infections. Implementing DTT-based chemical disaggregation as a routine pre-treatment for liquid samples may improve pathogen detection and guide more effective therapeutic interventions in biofilm-associated infections.

KEYWORDS

Biofilms; Prosthesis-Related Infections; Dithiothreitol; Synovial Fluid; Microbiological Techniques

INTRODUCTION

Microorganisms are often considered as freely suspended cells, defined as planktonic, and classified on their growth characteristics in a culture media. However, Antonie Philips van Leeuwenhoek, already in the seventeenth century described the existence of surface-associated microorganisms, growing and living in communities. Biofilms-embedded microorganisms show typical mechanisms for initial attachment to a surface, development of a community structure, and detachment [1].

A biofilm is an assemblage of microbial cells, associated together on a surface and enclosed in a matrix of primarily polysaccharide material, living like in a proper ecosystem. Depending on the environment in which the biofilm is formed, non-cellular materials such as mineral crystals, clay and silt particles, corrosion particles, and blood components may also be present in the biofilm matrix. Organisms associated with biofilms also differ from planktonic with respect to transcribed genes. Within the ecosystem of the biofilm, bacteria have developed a communication system called "Quorum sensing", which allows them to coordinate their behavior using chemical molecules as signals. Quorum sensing communication system allows the microorganisms to orchestrate the group behavior, resulting in maximum benefit for the whole bacteria population living in the biofilm. [2]

As the knowledge of biofilms improved, it become more and more evident in the last decades that this is the predominant lifestyle of bacteria and fungi and an example of an extremely successful physiological adaptation, as they thrive in most natural environments as well as in harsh conditions.

Unfortunately, biofilms are also often associated with the majority of human infectious diseases and can negatively impact health. Indeed, biofilms offer to microorganisms an enormous capacity to resist host immune system defenses and antimicrobial therapy. In healthcare environments, the persistence of the microorganisms is extended by the formation of biofilms, being responsible for the onset and spread of hospital-care-associated infections (HCAIs) (also referred to as "nosocomial" or "hospital acquired" infections). HCAIs can result in prolonged hospital stays, long-term disabilities, enhanced resistance of the microorganisms to antimicrobials, enormous additional costs for the health care systems, the patients and their families, and increased mortality rates. The prevalence of HCAI is estimated to be between 5.7% and 19.1%.

According to some estimates, 65–80% of total human infections are associated with biofilm formation and include: periodontitis/dental caries, cystic fibrosis lung infection, chronic otitis media, infective endocarditis, chronic osteomyelitis, chronic rhinosinusitis, chronic tonsillitis, chronic peritonitis, chronic prostatitis, chronic wounds, recurrent urinary tract infections (UTIs), bloodstream infections (BSIs), ventilated-associated pneumonia and infections associated with indwelling medical devices (e.g., contact lenses, heart valves, joint prostheses, and other orthopedic implants, intrauterine devices, intravascular catheters, urinary tract catheters, peritoneal catheters, etc.) [3]

In humans, bacterial and fungal biofilms may form on a wide variety of surfaces, including living tissues, foreign bodies and biomaterials; the solid-liquid interface between a surface and an aqueous medium (e.g., water, blood) provides an ideal environment for the attachment and growth of microorganisms. The spreading of the biofilm on a surface is made possible by dispersion, a process through which bacterial cells leave the biofilms, return to an independent planktonic lifestyle and eventually colonize new surfaces to establish new biofilm-based communities. [4]

Recently, the ability of bacteria to form biofilms in fluids, like synovial fluid, blood, urine, cerebrospinal fluids, has been reported. Biofilm formation in liquids proceeds through an initial adhesion process of bacterial cells one to the other, by forming an extracellular polymeric substance, that provides a three-dimensional structure for the bacterial cell's aggregates, protecting them from the external environment. [5]

Aim of this article is to provide an overview of the bacteria living in human fluids, with a focus on the associated pathologic conditions and the impact that this phenomenon may have on the cultural examination of fluid samples.

SYNOVIAL FLUID

Periprosthetic joint infections (PJIs) represent a great challenge for the patients and the healthcare systems, due to the increased length of hospital stay, need for complex and expensive surgeries and prolonged antibiotic treatments. [6],[7] Recently, the presence of bacteria and biofilm aggregates floating in the synovial fluids of PJIs has been reported and associated to the resistance of bacterial joint infections to common treatments [8],[9]. The ability of bacteria to live in biofilm aggregates may explain the limited efficacy of current microbiological investigations of synovial fluids, with reported sensitivities as low as 41.6 to 90% [10],[11]. Moreover, the formation of fluctuating biofilm aggregates may compromise the ability of antibiotics to reach and kill the microbial cells, as it happens in bacteria living in biofilms adhering on a surface. Furthermore, the bacterial-protein interactions in these aggregates changes the production of virulence factors and the phenotype, inducing a marked tolerance to antibiotics [12]. Bacteria living in synovial fluids of PJI may substantially contribute to the development of a chronic condition difficult to diagnose and to reat and thus requiring suitable antibiofilm strategies.

URINARY TRACT

Urinary tract infections (UTIs), one of the most common infections sustained by bacteria, represents a severe public health issue. The operating costs of these infections are estimated around US$3.5 billion per year in the US. UTIs may manifest in different forms such as cystitis, pyelonephritis, prostatitis, urethritis. Ideal environment for attachment and colonization by uropathogens are urinary catheters. The most common agents responsible for complicated UTIs are Escherichia coli, Enterococcus spp., Klebsiella pneumoniae, Candida spp., Staphylococcus aureus, Proteus mirabilis and Pseudomonas aeruginosa. All these microorganisms have already been linked to biofilm formation [13]. Large fragments of the biofilms and high concentrations of microbial cells can detach from the catheter and flow into the bladder spreading the infection and leading to bacteriuria. In addition, uropathogens can form biofilm in the bladder and kidney, reducing antibiotic susceptibility and causing recurrent infections. Biofilms play a central role in catheter associated UTIs especially in patients with prolonged catheterization, leading to increased morbidity and mortality [14]. Bacterial populations living in biofilms show a more efficient and adapted behavior, compared to planktonic bacteria, with improved chance of survival while the biofilm community sheds planktonic cells able to further colonize adjacent tissues [15].

BLOOD SYSTEM

Not less relevant and worrying than UTIs are the blood system infections (BSIs), ranked as the 12th cause of death in the USA, with the estimated mortality rate of 15–30%. Through the bloodstream microorganism can spread from a local infection (endocarditis, meningitis, osteomyelitis...) to distant sites. In addition, intravenous catheters are an important risk factor for BSIs. In fact, bacterial biofilm can easily develop on the surface of this devices, and biofilm fragments or planktonic microorganism may spread into the bloodstream. The most often isolated pathogens in bacteremia are S. aureus, E. coli, K. pneuomoniae, P. aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Enterobacter cloacae, Streptococcus pneumoniae, Enterococus faecium and Acinetobacter baumannii [15],[16],[17]: all well-known biofilm-producers. As it happens with cultural examination in other fluids, blood cultures may often result in false negative findings.

CEREBROSPINL FLUID (CSF)

Thousands of CSF shunts are implanted every year as a treatment of hydrocephalus. To relieve cranial pressure in fact, CSF is generally shunted, from the cerebral ventricle into the peritoneal cavity. Common complications of this procedure are intraventricular hemorrhage, obstruction, over drainage of CSF and infection. Clinical signs range from local manifestations as ventriculitis, peritonitis, to nephritis or septicemia, leading to high risk of seizures, decreased intellectual performance, and mortality [18]. Correct diagnosis and treatment of device-related infections are notoriously difficult, because of bacteria forming biofilms. Diagnostic cultures of fluid aspirates and swabs are often falsely negative, presumably because of the very low chance to find cells in the planktonic state. [19],[20].

SALIVA

Van Leeuwenhoek, simply using his microscope, observed for the first-time microorganisms on tooth surfaces, the dental plaque [1]. Saliva under normal circumstances is sterile until it leaves the salivary duct and enters the oral cavity, where it is quickly contaminated by biofilm-producer microorganisms. Bacterial growth in the form of biofilm has been associated with most ear, nose, and throat infections [21]. Implanted biomaterials and other inert surfaces with poor host defense, such as salivary calculi, are subject to bacterial attachment and biofilm formation and also in this environment, pharmaceutical treatment and immune system have limited effect on bacteria living in biofilm. Biofilm growth has been associated with chronic otitis media and mastoiditis as well as chronic infections of the adenoid tissue. Planktonic bacteria may shed from mature biofilm and being the cause of an acute phase of infection such as a chronic and recurrent otitis media. Furthermore, the microbial diagnostics in oral cavity are always complicated as the saliva in mouth is contaminated with the oral microbiome [21],[22], which currently form large amount of biofilms.

TRACHEAL ASPIRATE

Nosocomial pneumonia represents about one quarter of all nosocomial infections and represents the first cause of nosocomial infection in Intensive care unit, contributing to extend the length of hospitalization, mortality and costs of treatment. Tracheal intubation in patient under mechanic ventilation in fact, increases the risk for infection ranging from six to twenty times higher. Bacteriological diagnosis through specific specimen brush, bronchoalveolar lavage, and endotracheal aspirates have been nowadays standardized but lack of specificity, as it is based on the identification of bacteria growing in tracheal secretion. It is necessary to consider that in ventilator-associated pneumonia biofilm plays an important role in the diagnosis as in the treatment. The endotracheal tube allows direct entry of microbial colonization of dental plaque and oropharynx, that has natural ability to form biofilm, into the lower respiratory tract. Then, bacteria within the biofilm can infect the lungs by several ways: through detachment of biofilm portions that then reaches the lungs and by aspiration into deeper airways of aerosolized planktonic pathogens detached from the biofilm [23],[24].

DRAINS

In a wide variety of surgical specialties, closed suction drains are used for prevention of haematoma and fluid accumulation. Nevertheless, more and more evidence, in different surgical field are showing that not always drains are effective, instead they are unnecessary or counterproductive, encouraging local wound complications and infections [25]. A recent in vivo drain study demonstrated a significant biofilm formation, as soon as two hours after drain insertion, cocci within clumps of fibrin adherent to the surface of the drain were detected. This finding suggests that drains are contaminated very early, and considering the ideal culture medium that clotted blood represents, combined with a foreign body in the form of the drain, biofilm formation is obviously able to evolve very rapidly [26]. In matter of prevention, even for this reason the drains should be used for the shortest time and if possible no later than 24 hours under strict surveillance.

IS IT POSSIBLE TO DETACH BACTERIA IN FLUIDS FROM THEIR BIOFILMS? DITHIOTHREITOL (DTT) AS A DIAGNOSTIC TOOL

The presence of biofilm-bacterial aggregates may have a strong impact on pathogen identification, on the bacterial count and antibiogram evaluation performed with traditional cultural techniques, that were designed for planktonic, isolated microorganisms (Figure 1). While physical (sonication) and chemical (Dithiothreitol, DTT) systems have been proposed to dislodge bacteria adhering on a surface from their biofilms, less is known about the possibility to improve microbiological sensitivity by antibiofilm pre-treatment of fluid samples. In fact, to the best of our knowledge, only DTT has been tested to dislodge bacteria from their biofilms in synovial fluid samples with promising results.

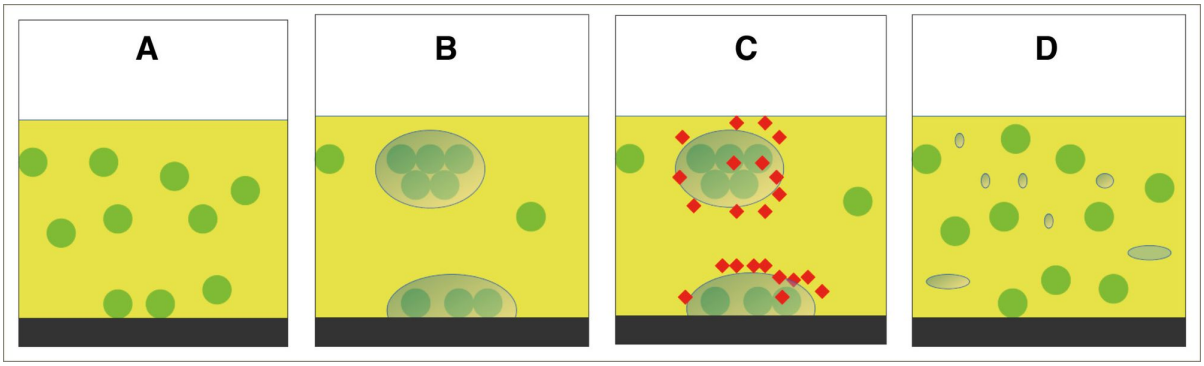

Figure 1: Schematic representation of (A) planktonic bacteria (green circles) floating free in a fluid and adhering on a surface; (B) after few hours, the microorganisms aggregate in biofilms both on the surface and in the fluid; (C) the application of a chemical antibiofilm agent (e.g. dithiothreitol, DTT) (red diamonds) breaks the biofilm, without killing the bacteria, that (D) may hence return free to float in the surrounding fluid. Biofilm remnants can be found in the fluid. Free living bacteria may then easily be cultured and/or analyzed with molecular methods with increased sensitivity.

As DTT has been demonstrated to be able to release microorganisms from the biofilm produced on prosthetic implants and on human tissues, it has also recently been demonstrated to be effective in disrupting biofilm-bacteria aggregates in fluids, and more specifically it has been shown to be effective in the synovial fluid [5] (Figure 2). DTT is a chemical agent that reduces disulfide bonds in peptides and can indeed alter the matrix of biofilm releasing bacteria without affecting their viability. Through this procedure, bacterial culture is possible and so identification and antibiotic susceptibility tests are easier to perform [27],[28],[29],[30].

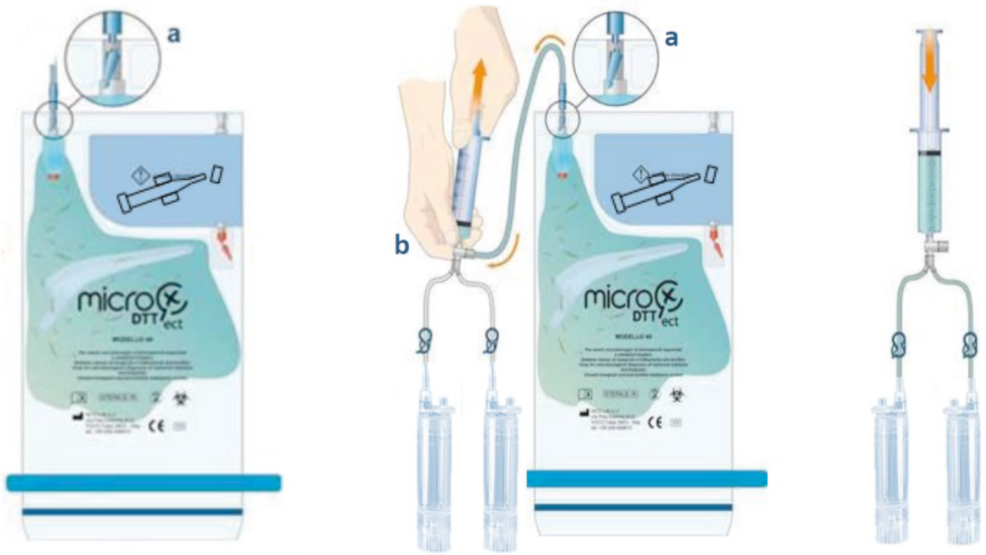

Figure 2 shows a the last generation of a completely closed system and procedure for chemical antibiofilm pretreatment of solid and liquid biological samples with dithiothreitol (DTT).

CONCLUSION

Diagnosis for biofilm-associated infection (BAI) can be challenging, and even with the correct diagnosis, therapy can be particularly difficult, long and expensive. The fact that bacteria may live in biofilms even in fluid should be taken into account both as to concern our diagnostic and treatment approach. In particular, as to concern microbiological diagnosis, the spontaneous tendency of many bacterial species to aggregate must be considered. In fact, cell disaggregation is often omitted when analyzing bacterial samples and the number of Colony Forming Units (CFUs) is usually taken as the golden standard. However, the CFU count is not an absolute measure of bacteria cells; instead, it represents the number of colonies that can form on an agar plate from a given sample. If bacteria aggregates are cultured as a single cell, the CFU will be falsely low and even false negative results may be reported.

Molecular approaches may at least partially overcome this difficulty; nevertheless, their use is limited due to high costs of the procedure, the level of expertise required and the inability to differentiate living from dead microroganisms. Antibiofilm chemical pre-treatment of fluid samples using DTT can be a low-cost and simple-to-use alternative in BAIs, and probably it should be included as a routine not only for any solid or tissue biological sample but also for the liquid ones. In fact, as antibiofilm pre-treatment of synovial fluid with DTT has been shown to increase the sensitivity of cultural examination by freeing the microorganisms from the biofilm aggregates, it may be expected that a similar result could be obtained by applying the same system to other fluids such as saliva, urine, blood, and cerebrospinal liquid, paving the way to a complete change in the results of the microbiological examinations. Given the high social and economic costs of chronic biofilm-related infections in nearly all the fields of Medicine, we believe that more research on this subject would be greatly beneficial.

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