MENTORS IN ORTHOPEDICS

NOVEL DIAGNOSTIC APPROACH TO BIOFILM-RELATED INFECTIONS USING DITHIOTREITOL (DTT)

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SUMMARY

Background: Implant-associated infections (IAI) and prosthetic joint infections (PJI) present significant clinical challenges due to bacterial biofilm formation on inert surfaces. These biofilms protect pathogens from host immune responses and conventional microbiological detection, contributing to increased mortality and diagnostic inaccuracy. Standard culture methods often fail to identify sessile microorganisms, necessitating specialized techniques for biofilm disruption.

Objective: This review evaluates current microbiological tools for dislodging biofilms to improve the diagnostic accuracy of PJI, specifically comparing the efficacy, methodology, and clinical implications of physical sonication and chemical dithiothreitol (DTT) treatments.

Key Points: Effective diagnosis requires the disruption of biofilms from periprosthetic tissues and explanted components. Sonication utilizes ultrasound (30-40 KHz) for physical dislodgment but carries risks of cross-contamination and bacterial inhibition. Alternatively, 0.1% DTT chemically reduces disulfide bonds to release embedded microorganisms. Meta-analytical data indicate comparable diagnostic accuracy between DTT (86.7%) and sonication (83.9%), with no statistically significant difference in sensitivity or specificity. A closed-circuit DTT system has been developed to minimize exogenous contamination during sample transport and processing. Cost-benefit analyses suggest that while antibiofilm techniques increase direct laboratory costs compared to traditional tissue cultures, they offer superior cost-effectiveness by reducing processing time and mitigating the clinical consequences of false-positive or false-negative results.

Conclusion: Accurate identification of pathogens in PJI necessitates specialized pre-analytical biofilm disruption. The implementation of standardized protocols and closed-system chemical dislodgment may enhance diagnostic reliability, reduce laboratory-acquired contamination, and optimize clinical management in orthopedic revision surgery.

KEYWORDS

Prosthesis-Related Infections; Biofilms; Dithiothreitol; Sonication; Arthroplasty, Replacement

INTRODUCTION

The implants generally used to surgically treat or manage several patients may act as a biotic surfaces in the human body, thereby facilitating the colonization and the settle of many microbial species. Microbes are often cleared by the host's innate immune mechanisms, but sometimes they can cause a devastating life threatening infection, generally called Implant-associated Infection (IAI). The main reason of these infections is because bacteria adhered to the implant surfaces are less susceptible to killing/elimination by the immune system (1). In addition, these bacteria may survive on the implant surfaces and develop biofilms that reduce the effect of antimicrobial agents and result in a persistent colonization which confers them an “embedded biofilm status”, then consequently difficult to be dislodged and identified. In order to detect the true pathogens, disruption and demolition of the biofilms should indeed precede the standard microbiological methods (2).

Recently, Wildelman et al. reported that all-cause 10-year mortality is higher for patients with PJI (45%) compared with patients undergoing THA without PJI (29%). This can be due to the natural evolution of the implants, but also to the difficulty to manage these infections even microbiologically (3). As matter of fact, it is important to point out that inaccurate diagnosis should be worth of attention in order to avoid any misunderstanding in the laboratory setting as well as in the clinicians. False positive and negative results are very deleterious for the patients and frustrating for the surgeons.

Biofilm- and implant-related infections are constantly looking for definitive guidelines and resolutive diagnostic approaches, it is therefore necessary to pay the utmost attention when dealing with these topics, certainly controversial and to be further improved. This paper summarizes the main microbiological tools to improve diagnosis and avoid unreliable results.

MICROBIOLOGICAL DIAGNOSIS

According to the WAIOT Guidelines (4), the culture and isolation of the microorganisms is the main useful approach for the diagnosis of prosthetic and joints infections. Samples suitable for the microbiology Laboratories can be periprosthetic tissues, joint fluid and/or prosthetic components removed during the revision procedure. Swabs must be avoided and a minimum of 3-6 periprosthetic tissue samples and prosthetic components should be collected on the basis of clinical stage, the type of suspected infection (low-grade or high-grade) or microorganism (low or high virulence). Microbiological procedures, from the sample collection until the microbiological report, requires specific and mandatory conditions:

a) to avoid samples contamination;

b) to use closed and sterile transportation systems;

c) certified laboratory processing methods to avoid false positives or false negatives results.

The pre-analytical phase and samples preparation is very relevant to perform a reliable diagnosis. So, the use of antibiofilm techniques for explanted biomaterials and for biopsies is definitively mandatory. The formation of biofilms is intrinsic to the pathogenesis of PJIs, so many diagnostic tools have been used but many biases remain

still open so far. The pre-analytical phase is very important to increase bacterial retrieving after biofilm dislodgement.

PRE-ANALYTICAL TOOLS USED TO IMPROVE DIAGNOSIS

Authors have conducted several analyses of the various microbiological methods to diagnose implant-related infections, outlining the advantages and disadvantages of the various techniques today available. Today, the physical (Sonication) and the chemical treatment (Dithiothreitol at 0.1%) can be used to pre-treat samples and dislodge bacteria from their biofilm.

Sonication

The containers with prosthetic components need to be handled under a laminar air flow cabinet and covered for at least 90% of its volume with Ringer's solution or sterile physiological solution. The samples are vortexed for 30 seconds, sonicated at 30-40 KHz 0.22 ± 0.04 W / cm2 for 5 minutes and vortexed again for additional 30 seconds. All these procedures must be performed with attention in order to minimize possible contamination (5).

Dithiothreitol (DTT)

An alternative to the sonication is the use of a solution of dithiothreitol (DTT), which reduce the disulphide bounds in biofilm, providing a chemical debonding [68]. In this case a sterile solution of 0.1% (w: v) of dithiothreitol (DTT, formula $C_4H_{10}O_2S_2$, molecular weight: 154.2) in phosphate buffer saline (PBS) will add to cover the prosthetic components. The container with prosthetic components and DTT solution will be shaken up at about 80 rpm for 15 min (6,7).

Comparison between the two methods

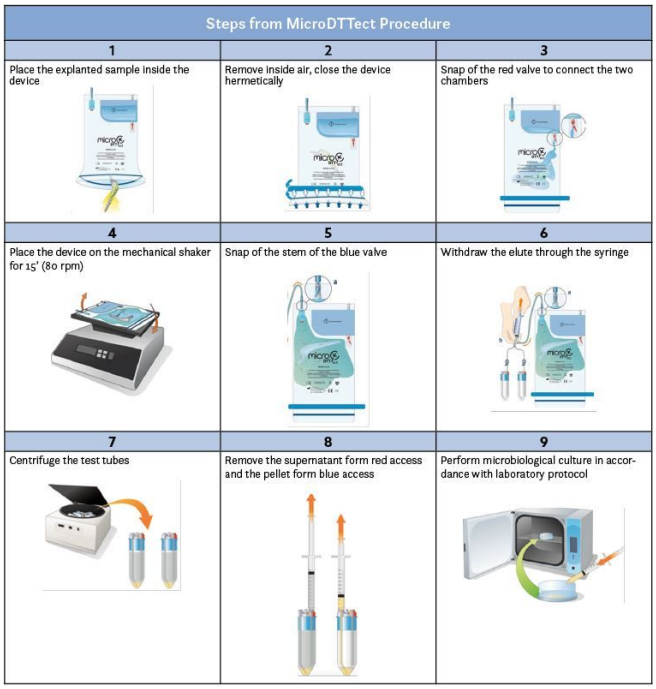
The literature on sonication has demonstrated satisfactory results especially in the setting of a non-suspected PJI and proven of superior diagnostic capacity over traditional cultures. However, the aforementioned method has some drawbacks, especially the high cost of equipment to many parts of the world and cross-contamination risks. The introduction of chemically based biofilm breaking techniques (i.e., dithiothreitol) has been developed to avoid risk of contamination and improve diagnosis. A recent meta-analysis (8), collecting data from comparative studies with 726 implants, demonstrated that the diagnostic accuracy of DTT and sonication were 86.7% (95% CI 82.7 to 90.1) and 83.9% (95% CI 79.7 to 87.5), respectively. Pooled sensitivity and specificity showed no statistically significant differences between DTT and sonication, and that sonication is not superior that DTT.

NEW DTT TECHNOLOGY IN A STERILE AND CLOSED CIRCUIT

A completely closed circuit from the surgical field to the microbiological lab have been recently developed, which prevents sample contamination, thus increasing the specificity. MicroDTTect (Figure 1, kindly provided by NovaHealth srl, Italy) represents indeed the first and the only patented system for sample microbiological collection, transport and processing, that combines the increased sensitivity of the DTT with a completely closed system.

Figure 1: MicroDTTect system

This system could have the advantage to allow any lab and any clinic to maximize microbiological testing without the need for special training or expensive machines. It also has the advantage of processing simultaneously or separately multiple samples, including tissue samples, fluids, implants and biomaterials. Figure 2 describes the necessary steps to be followed from the Operative Room to the Laboratory.



COST BENEFIT ANALYSIS OF ANTIBIOFILM METHODS

Cost-benefit analysis of antibiofilm microbiological techniques for peri-prosthetic joint infection diagnosis has been published (9). This analysis highlights the potential economic advantage to hospitals associated with the

routine introduction of antibiofilm techniques for microbiological diagnosis of PJI. Authors considered an average of five samples per patient, processed separately with traditional tissue culture with or without sonication of prosthetic components, or pooled together using the MicroDTTect device. They calculated that the overall mean direct cost per patient was € 397 and € 393 for sonication or MicroDTTect, respectively, compared to € 308 for traditional tissue cultures. In terms of opportunity costs, MicroDTTect was the most effective technique, allowing for a 35% or 55% reduction in time required for sample treatment, compared to tissue cultures combined or not with sonication, respectively. Pooling together direct and indirect costs associated with false positive and negative results of the different diagnostic techniques or unnecessary medical treatments and possible medical claims, MicroDTTect or sonication become increasingly cost-effective when the extra-costs, generated by diagnostic inaccuracy of traditional tissue culture, took place, respectively, in 2% or 20% or more of the patients.

DISCUSSION

One of the main challenges of Implant Associated Infections (IAIs) comes from the striking ability of bacteria to adhere to the inert surfaces, forming biofilms which make the microorganisms much less susceptible to killing/elimination by the immune system. Bacteria embedded in biofilms are also difficult to be dislodged and identified by traditional microbiological techniques. In order to detect the true pathogens, disruption and demolition of the biofilms has then been proposed by different means in literature.

Procedures for samples pre-treatment in order to dislodge bacteria, such as sonication of retrieved implants, is better than conventional cultures for the diagnosis of device-related infections. However, the significance of some isolates in patients without clinical infection remains uncertain, and the risk of false positive as well as false negative is still high and need to be further defined. The over antibiotics treatment can be counterproductive when a contamination occurs and the true pathogens are not isolated. This is why research on the field is still looking for alternative methods that are less laborious and with streamlined and fast algorithm from the surgery room (where the sample are collected) to the Laboratory (where the samples are processed).

A recent paper by Oliva A et al. (10) has performed an acute analysis of the various microbiological methods to diagnose implant-related infections, outlining the advantages and disadvantages of the various techniques today available. However, inside this paper some points that may have a great relevance for the daily clinical activity, need to be better clarified and discussed.

Drago et al. (11) have recently underlined that “Dithiothreitol Assay” should be considered a “Culture based” methods, as the sonication one.

Both methods are indeed “culture-based”, since they both aim at dislodging bacteria from a given sample (DTT by chemical means, sonication by physical action), with the resulting processed fluid from both procedures requiring further culture to identify the pathogen(s).

No substantial difference there are indeed between sonication and Dithiothreitol as to regard the need for microbiological examination and concerning the possible choice of the microbiological technique used to identify the pathogen (traditional culture in many cases, or molecular or other methods in fews).

In fact, both antibiofilm processing methods often require a subsequent bacterial cultural examination, that can be chosen among all of those currently and routinely available in laboratories, as both sonication and Dithiothreitol only provide bacteria dislodgment from the biofilms prior to culture or the direct examination by molecular methods.

Dithiothreitol is effectively used since decades in the analysis of sputa for the diagnosis of broncho-pneumonia, and Streptococcus pneumoniae is well known among microbiologists as one of the most labile bacteria. So, the hypothesis that Dithiothreitol may have a toxic effect is clearly contradicted by the same literature that the Oliva et al. cite, including the large clinical trials performed by Sambri et al (7).

Even one of the most recent studies, performed on collection strains and not on clinical isolates, did show how planktonic bacteria viability after exposure to Dithiothreitol is exactly the same as that is found after exposure to sonication and even to NaCl 0.9% alone (12).

Randau et al (13), demonstrated that DTT was inferior in sensitivity when diagnosing PJIs compared to sonication fluid cultures and tissue biopsies, but only when pH in the DTT was low. When improperly used (unstable solution, very low pH, DTT-samples contact for a long time or days, higher concentrations), DTT fluids correlated indeed with false-negative results.

This is very similar to what can be found for sonication, that is known to have the ability to kill bacteria (14) and requires an accurate choice of the ultrasound parameters to avoid bacterial growth inhibition.

In addition, Randau et al (13), evidenced that sonication had better sensitivity but lower specificity. The Authors concluded that the closed system of the DTT kit avoids contamination and false-positive results, and that DTT can be an alternative where sonication is not available.

False positive results and samples contamination during collection, transportation and laboratory procedures remain challenging and not fully solved so far. Contamination is often overlooked in the surgery rooms during samples collection and in most laboratories during the processing or biofilm pretreatment.

All procedures which require standards of hygiene and proper and useful disposables or equipment when handling samples and special care is then required to be taken as per recommendations in microbiological testing laboratories.

In conclusion, we need to build specific procedures/guidelins and/or to apply dedicated devices for avoiding contaminations and any false positive results, as well as the negative ones. In this scenario the DTT device represents the first completely closed system to collect, transport and process retrieved biomaterials and tissues to diagnose implant- and biofilm-related infections.

CONCLUSION

Orthopaedic infections are common and devastating issues in the clinical setting. Prosthetic and orthopaedic infections are often neglected by the scientific community, but their incidence is increasing as well as the difficulty to treat these types of infections. Diagnostics tools are often not correctly applied, the antibiotic treatments fail to have success because the increase of bacteria resistance and the presence of biofilms is able to protect the pathogens. Infections, especially those iatrogenic and nosocomial, are receiving increasing attention and visibility because the high rate of legal litigations and the negative outcomes for the patients which impair their life's quality. A prompt and true diagnosis is essential at any level of suspicion of infection to avoid delay of medical or surgical treatments. For these reasons, infection prevention, the right and quick recognition of the infection, and the prompt attention by the healthcare personnel are of paramount importance. Accurate diagnosis remains difficult, as most signs of infection are subjective, so a multidisciplinary approaches and new tools are necessary to finally improve this challenging disease.

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