Biofilms are medically important, accounting for over 80% of microbial infections in the body.
**BIOFILM**: Biofilm is a complex bacterial community protected by a self-produced polymeric matrix that adheres to a living or inert surface.

**BIOFILM FORMATION**

- Microbial adhesion to the surface (organic or inorganic one)
- Formation of microcolonies and synthesis of the matrix
- Stable intercellular adhesion
- Maturation of the biofilm and antibiotics resistance development
- Release of planktonic or aggregates of bacteria in the environment
Antibiotic resistance of bacteria in biofilm

• Antibiotic resistance of bacteria in the biofilm contributes to the chronicity of infections
• The mechanisms of resistance in biofilms are different from the now familiar plasmids, transposons, and mutations that confer innate resistance to individual bacterial cells
• In biofilms, resistance seems to depend on multicellular strategies

“Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century”.

OMS - Antimicrobial resistance: global report on surveillance 2014
THE BACTERIAL BIOFILM
# Implant- and Biofilm-Related Infections

<table>
<thead>
<tr>
<th>Implant-related</th>
<th>Not implant-related</th>
</tr>
</thead>
<tbody>
<tr>
<td>PJIs</td>
<td>Osteomyelitis</td>
</tr>
<tr>
<td>Post-osteosynthesis</td>
<td>Otitis media</td>
</tr>
<tr>
<td>Mechanical heart valves</td>
<td>Chronic rhinosinusitis,</td>
</tr>
<tr>
<td>Breast implants</td>
<td>Periodontitis &amp; dental plaque</td>
</tr>
<tr>
<td>Catheters</td>
<td>Chronic ulcers</td>
</tr>
<tr>
<td>Ventriculo-peritoneal shunts etc..</td>
<td>Chronic urinary or respiratory infections...</td>
</tr>
</tbody>
</table>
Implant- and Biofilm-Related Infections

The Journal of Arthroplasty, 2009; 24/2: e19

Lawrence D. Dorr Award Paper

The Epidemiology of Revision Total Hip Arthroplasty in the United States

Kevin J. Bozic, MD, MBA. Steven M. Kurtz, PhD. Edmund Lau, MS. Kevin Ong, PhD. Thomas P. Vail, MI. Daniel J. Berry, MD

51,345 revision THA procedures.

<table>
<thead>
<tr>
<th>Reason for revision</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Instability / dislocation</td>
<td>22.5</td>
</tr>
<tr>
<td>Implant loosening</td>
<td>19.7</td>
</tr>
<tr>
<td>Infection</td>
<td>14.8</td>
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</tbody>
</table>
# Implant- and Biofilm-Related Infections

**60,355 revision TKA procedures**

<table>
<thead>
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<th>Reason for revision</th>
<th>%</th>
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<tr>
<td>Infection</td>
<td>25.2</td>
</tr>
<tr>
<td>Implant loosening</td>
<td>16.1</td>
</tr>
<tr>
<td>Implant failure/breakage</td>
<td>9.7</td>
</tr>
</tbody>
</table>
Implant- and Biofilm-Related Infections

By 2030 over 60% of all revision total joint procedures will be due to PJIs.
Implant- and Biofilm-Related Infections
Implant- and Biofilm-Related Infections

- Chronic behavior or frequent recurrences
- Antibiotic resistance
- Complex and prolonged treatment
- Difficult diagnosis
- Poor prognosis
- High social and economical costs
Implant- and Biofilm-Related Infections – PJI NEW DEFINITIONS

PJI - DEFINITION

New Definition for Periprosthetic Joint Infection
From the Workgroup of the Musculoskeletal Infection Society

Javad Parvizi MD, Benjamin Zmistowski BS, Elie F. Berbari MD, Thomas W. Bauer MD, PhD, Bryan D. Springer MD, Craig J. Della Valle MD, Kevin L. Garvin MD, Michael A. Mont MD, Montri D. Wongworawat MD, Charalampos G. Zalavras MD

Clinical Infectious Diseases Advance Access published December 6, 2012

Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of America

Epidemiology and new developments in the diagnosis of prosthetic joint infection.
Corvec S¹, Portillo ME, Pasticci BM, Borens O, Trampuz A.

Proceedings of the International Consensus on Periprosthetic Joint Infection
J. Parvizi, T. Gehrke, and A. F. Chen
DEFINITE PJI EXISTS WHEN:

1) Pathogens fenothipically identic are isolated by two culture from prosthetic material; or
2) There is a sinus tract communicating with the prosthesis; or
3) At least three of the following five minor criteria exist:
   a) Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration
   b) Elevated synovial leukocyte count
   c) Elevated synovial neutrophil percentage (PMN%)
   d) Acute inflammation of periprosthetic tissues at histologic analysis
   e) Isolation of a microorganism in one culture of a single sample
**CLINICAL HISTORY**

**OBJECTIVE EXAM**

**RX**

**SCINTIGRAPHY**

**SAMPLES HARVEST**

**MICROBIOLOGICAL ANALYSIS**

**CRITICAL POINT:**
- false negative culture
- false positive culture
Samples Harvest

- From the infection site
- Sufficient quantity to allow necessary examinations
- Antibiotic therapy must be suspend at least 2 weeks before harvest

→ SAMPLES HARVEST, TRANSPORT AND PROCESS NEED CAREFUL WAYS

It is essential to determine the strain of bacteria actually responsible for an infection to diagnose and treat the infection and any resistance present. Diagnosis of implant-related infections requires dedicated microbiology to disclose adhering bacteria.
Implant scraping, sonication and tissue culture have been proposed to improve bacteria isolation from retrieved implants.
Implant- and Biofilm-Related Infections

IMPLANT SCRAPING

- Risk of contamination
- Operator-dependent procedures
- Superior risk to have false negative cultures comparing to sonication (swabs cannot destroy bacteria biofilm)
Implant- and Biofilm-Related Infections

**IMPLANT SCRAPING**

MORE THAN 20% cultural examination are **FALSE NEGATIVE** or **FALSE POSITIVE**

AND

THEIR USE IS NOT RECOMMENDED IN THE GUIDELINES
Implant- and Biofilm-Related Infections

SONICATION: THE GOLD STANDARD...

- Risk of contamination: multi-step procedures
- Operator-dependent procedures

1. Harvesting the samples in the operating theatre
2. Transporting the harvested samples to the lab
3. Inserting physiological solution in the container
4. Sonicating the samples
5. Collecting the fluid after sonication
6. Centrifuging the fluid
7. Culturing bacteria pellet in solid media or broth
**SONICATION**

1. Harvesting the samples in the operating theatre
2. Inserting physiological solution in the container
3. Placing the container on the shaker (5 min.)
4. Sonicating the samples
5. Collecting the fluid after sonication
6. Placing the container on the shaker (5 min.)
7. Collecting the fluid after sonication
Placing the container on the shaker (5 min.)

Inserting physiological solution in the container

Sonicating the samples

Collecting the fluid after sonication

…BUT

MORE THAN 20% cultural examination are **FALSE NEGATIVE** or **FALSE POSITIVE**
Implant- and Biofilm-Related Infections

SONICATION: THE GOLD STANDARD
- Risk of contamination: multi-step procedure
- Bacterial changes

AND

“By dislodging sessile bacteria from implants and using routine diagnostic systems, it may make species identification misleading (...) in particular when Gram - are involved.”
Implant- and Biofilm-Related Infections

What’s new?
DTT (Dithiothreitol): biofilm dissolvent → to dissolve the polysaccharide matrix of the biofilm and detach the bacteria.

Does Dithiothreitol Improve Bacterial Detection from Infected Prostheses? A Pilot Study

Lorenzo Drago PhD, Carlo Luca Romanò MD, Roberto Matina PhD, Valentina Signori BSc, Elena De Vecchi MSc

Use of Dithiothreitol to Improve the Diagnosis of Prosthetic Joint Infections

Lorenzo Drago,1,2 Valentina Signori,1 Elena De Vecchi,1 Christian Vassena,1 Elisa Palazzi,1 Laura Cappelletti,1 Delia Romanò,1 Carlo Luca Romanò1

1Laboratory of Clinical Chemistry and Microbiology, I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy. 2Laboratory of Medical Technical Sciences, Department of Biochemical Sciences for Health, University of Milan, Milan, Italy. 3Department of Reconstructive Surgery of Osteo-Articular Infections C.R.I.O. Unit, I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy.
Implant- and Biofilm-Related Infections

Confocal microscopy (PE surface)

Scraping

Biofilm remnants

Living bacteria
Implant- and Biofilm-Related Infections

Confocal microscopy (PE surface)

- DTT
- Sonication
Residual biofilm (red) and bacteria (green) on implant before (left) and after (right) treatment with dithiothreitol.
## Results

<table>
<thead>
<tr>
<th>Results</th>
<th>Tissue Cultures</th>
<th>Sonication</th>
<th>DTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>71.4%</td>
<td>71.4%</td>
<td>85.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>76.5%</td>
<td>94.1%</td>
<td>94.1%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>78.9%</td>
<td>93.7%</td>
<td>94.7%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>68.4%</td>
<td>72.7%</td>
<td>84.2%</td>
</tr>
</tbody>
</table>

**Methods:** Periprosthetic tissue samples (n=5-8). Removed implants aseptically divided into two parts and transported to the laboratory and randomly processed by sonication or DTT. Diagnosis of infection according Spangehl criteria.

SONICATION

DTT (DITHIOThREITOL)

to avoid risks of contamination by other bacteria not directly responsible for the infection (CROSS – CONTAMINATION)
AT THE HEART OF INFECTION CONTROL
the solution for microbiological examinations of explanted biomaterials and biopsies

Closed System for Intra-Operative Microbiological Samples Retrieval for Biofilm-Related Infection Detection

Transport the samples to be analyze under safe and sterile conditions

Process the samples quickly under safe conditions

Identify the bacteria responsible for infection
PVC chamber for collecting the 0.1% (w/v) DTT (Dithiothreitol) solution for dissolving the polysaccharide matrix of the biofilm and detaching the bacteria.

Valve with snap-off stem for connecting the chamber containing the explanted sample and the solution drawing system.

Chamber containing the sample to be analysed made of PVC (polyvinylchloride), sturdy and unpierceable, heat-sealable and equipped with a minigrip and adhesive seal for a watertight closure.

Valve connecting the two chambers, equipped with a snap-off opening stem, to allow the DTT solution to flow from the containment chamber into the collection chamber.

Access (closed selectively) for inserting special VacuTainer-Falcon test tubes to withdraw the solution.
1. EXPLANT THE BIOMATERIALS/BIOPSIES TO BE ANALYSED AT THE LABORATORY - multiple applications: possibility to analyzed different material (biological/biomaterial)

2. PLACE THE EXPLANTED SAMPLE INSIDE THE STERILE DEVICE
3. CLOSE THE DEVICE ERMETICALLY

4. SNAP OFF THE STEM OF THE VALVE CONNECTING THE TWO CHAMBERS

5. PLACE THE DEVICE ON THE MECHANICAL SHAKER
Bacterial biofilm dissolved in the DTT (Dithiothreitol) solution

Bacterial biofilm on a prosthetic surface

6. SNAP OFF THE STEM OF THE BLUE VALVE TO CONNECT THE SOLUTION DRAWING SYSTEM

7. WITHDRAW THE ELUTE IN THE TEST TUBES

8. CENTRIFUGE THE TEST TUBES

9. PERFORM BACTERIAL CULTURE IN ACCORDANCE WITH LABORATORY PROTOCOL
PATENTED EXCLUSIVE SYSTEM
FIRST (AND UNIQUE) COMPLETELY CLOSED SYSTEM FOR RETRIEVAL AND PROCESSING OF FAILED IMPLANTS AND BIOPTIC SAMPLES
EASY-TO-USE
TRANSPORT
SAFETY
EFFICACY
ACCURACY
LISTED BY THE AMCLI GUIDELINES
REFERENCES

2. Use of Dithiothreitol to Improve the Diagnosis of Prosthetic Joint Infections, JOURNAL OF ORTHOPAEDIC RESEARCH_2013
3. AMCLI Guidelines_2013
4. Sulfhydryl compounds reduce Staphylococcus aureus biofilm formation by inhibiting PIA biosynthesis, FEMS_2011
5. Algoritmo multidisciplinare di trattamento delle pseudoatrosi settiche, "LE FRATTURE DEL PILONE TIBIALE"_2015
7. Treatment with dithiothreitol improves bacterial recovery from tissue samples in osteoarticular and joint infections, JOURNAL OF ARTHROPLASTY_2016
8. Modeling Staphylococcus epidermidis-Induced Non-Unions: Subclinical and Clinical Evidence in Rats, PLOS ONE_2016