Single-cell MALDI-TOF based diagnostics of prosthetic joint infections

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Introduction

Diagnosis of prosthetic joint infection (PJI) still remains a serious clinical challenge, whereas proper diagnosis is crucial in selecting the right treatment strategy. Loosening of an implant can be caused by both infectious causes as non-infectious causes, e.g. mechanical problems. In non-infectious cases, replacement of the implant can be performed during the same operation (one-stage surgery). In case of infection the patient will be extensively treated with antibiotics for up to several months before a new implant is placed in a second operation (two-stage surgery). Rapid assessment of the presence or absence of infection thus has major consequences for the patient.

Single-cell MALDI-TOF (SC-MALDI-TOF) is a newly developed platform, able to identify strains without previous culturing. This technique is capable of presenting bacterial cells individually to the ionization unit of the mass-spectrometer. Each cell produces a classifiable mass spectrum, enabling a quantitative analysis of a contaminated sample containing a mixture of bacterial species. This strategy provides the opportunity to analyse clinical samples without culturing.

This study investigates the feasibility of SC-MALDI-TOF as a fast PJI diagnostic, using direct aliquots of sonication fluids collected during surgery.

Method

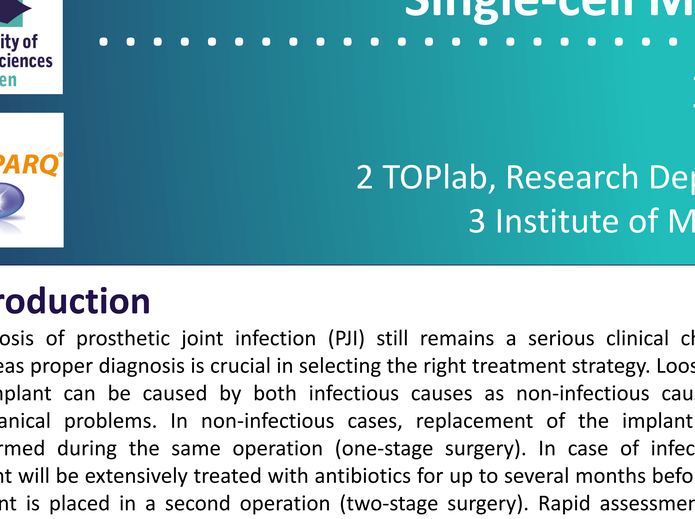

Fig. 1. *Sample processing method for SC-MALDI-TOF and culture-based identification*

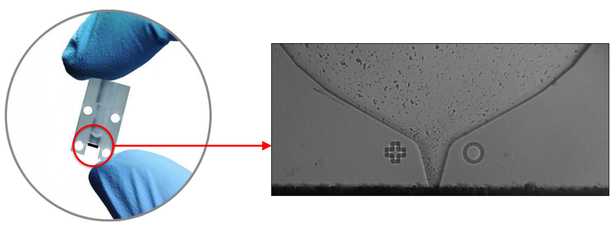

Fig. 2. *SC-MALDI-TOF*; Each cell is presented in a separate particle to the MALDI-TOF, resulting in a mass spectrum for every cell. Image courtesy of cytena.

Results

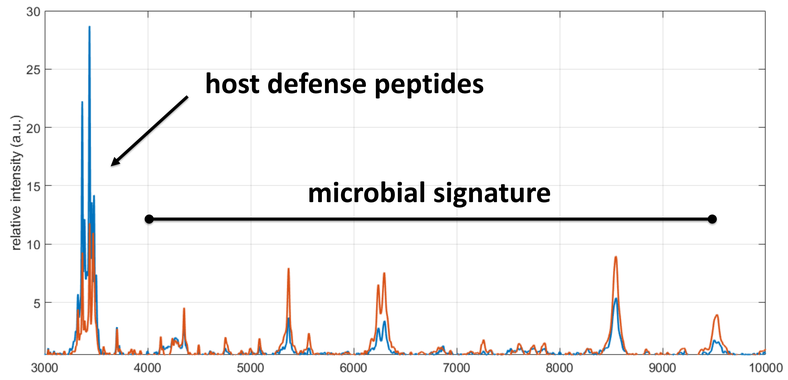

Fig. 3. *Mass spectra of S. epidermidis obtained directly from sonication fluid. In addition to the microbial signature (4 – 10 kDa), several peaks with masses corresponding to α-defensins 1-4 are observed (resp. 3373, 3445, 3488, and 3716 Da). The intensity of the α-defensins is inversely correlated with intensity of the microbial signature.*

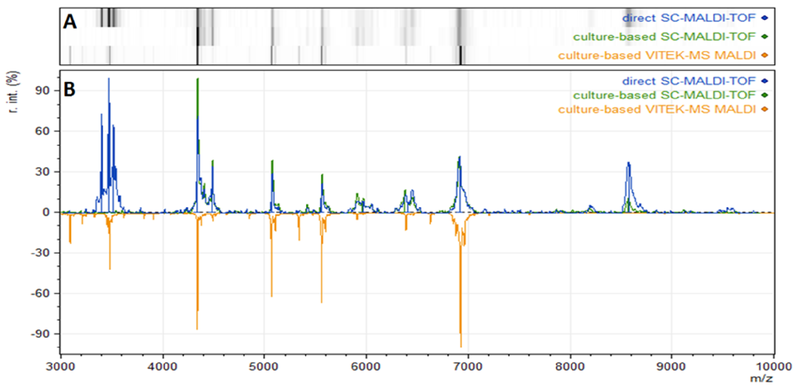

Fig. 4. *Pseudo-gel (A) and mass spectrum (B) of S. aureus directly from sonication fluid (blue) and culture-based single-cell MALDI-TOF (green) and conventional MALDI-TOF (yellow). The α-defensins are solely observable using direct MALDI-TOF .*

Table. 1. *Summarized results of direct SC-MALDI-TOF MS of 27 clinical sonication fluid aliquots*

Reference identification	presence of α-defensins (%)	microbial signature (%)	signal suppression⁺ (%)
E. coli (n=1)	1 (100)	1 (100)	1 (100)
Enterococci (n=3)	3 (100)	2 (67)	3 (100)
P. acnes (n=3)	3 (100)	3 (100)	2 (67)
S. aureus (n=2)	2 (100)	2 (100)	1 (50)
S. epidermidis (n=6)	6 (100)	4 (67)	5 (83)
Culture negative (n=12)	11 (92)	4 (33)	7 (58)

⁺*mass spectra with high intensity α-defensin peaks associated with a reduced intensity of the microbial signature*

Conclusions

This study shows the technical feasibility of a new MALDI method, that can analyse sonicates within minutes after collection during surgery by avoiding the time-consuming and potentially false negative culture step:

- Bacteria can be observed directly from sonication fluid: in 80% of the positive culture samples and in 33% negative culture samples a microbial signature was observed.
- Mass spectra obtained using direct SC-MALDI-TOF do contain extra information about host-pathogen interaction: peaks corresponding to α-defensins were found in 100% of the positive culture samples and 92% of the negative culture samples.
- 80% of the mass spectra obtained from positive sonication aliquots showed intense α-defensin peaks in combination with a (severely) reduced microbial spectrum or no microbial spectrum at all. For 58 % of the negative sonication aliquots this was the case

Discussion

A relatively high number of microbial signatures were observed from culture negative sonicates. Non-culturable bacteria cannot be analysed by culture-depended MALDI-TOF, while bacteria originating from biofilms, as is the case in most PJIs, have been found to exist in a viable but non-culturable state[1]. In addition, bacteria could be severely injured by host defence peptides (e.g. α-defensins) or preoperative antibiotic administration, and therefore non-viable or non-culturable.

The high amount of α-defensins in some samples implies the presence of an infecting pathogen, although in some cases no bacterial signature was found. α-defensin has received a lot of attention in the recent years as biomarker for PJI and a recent developed assay has shown to be an useful tool to confirm the absence of PJI[2].

The intensity of the α-defensins is in most mass spectra inversely correlated with intensity of the microbial signature. The cationic α-defensins have a relatively low ionization potential and are therefore likely suppress other proteins in MALDI-TOF. This phenomenon has been described in literature before in the case of direct MALDI-TOF of urine samples[3].

Acknowledgments

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