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Efficacy of thermal analysis in the detection of bacterial strains in periprosthetic joint infections

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Abstract

In musculoskeletal surgery, joint and periprosthetic infections are rare but significant complications with a high risk of morbidity. Rapid diagnosis, surgical intervention and definitive antibiotic therapy are key factors to the success of the treatment and the recovery of former limb function. In addition to the current diagnostic procedures, there is a need to introduce new, therapy-centered diagnostic procedures in view of the increase in antibiotic resistance. In our current study, we set out to demonstrate the practical utility of differential scanning calorimetry and the isoperibolic calorimetry in diagnostics of joint and periprosthetic infections. We have shown significant differences among the different grade of arthritis. Under experimental conditions, synovial fluid samples were artificially superinfected with the most frequently occurring bacteria at a concentration of 103–105 CFU mL^{-1} and monitored in isothermal mode at 37 °C. The isoperibolic tests clearly showed a specific, concentration-dependent representative curve (duration of proliferation and its maximum rates) for each bacterial strain of the study. In our opinion, the different thermal analytical methods can be useful in the diagnosis of septic inflammatory processes and, in the rapid selection of definitive antibiotic therapy.

Keywords Isoperibolic test · Calorimetry · Bacteria · Synovial fluid · Antibiotic resistance

Introduction

In addition to the changing environmental factors, more and more harmful effects on the human body caused by bacteria and viruses appear. Fortunately, pharmaceutical research and modern medicine had undergone significant changes by the end of the twentieth century. In addition to advances in medicine, after introduction of antibiotic therapies, child mortality decreased and contributed to an increase in life expectancy [1–13][14]. After the discovery of penicillin, despite the initial successes, it had become known that due to the genetic mutation ability of bacteria, a chance of survival against the available antibiotics is offered to some mutant strains due to moderate antibiotic sensitivity and resistance [7]. Long before the discovery and use of antibiotics, gene mutations responsible for natural resistance to certain classes of antibiotics already existed in nature [1, 6–8, 15–17].

Due to the immunization of the body to microorganisms and the fight against pathogenic bacteria, development of new antibiotics is essential due to moderate sensitivity and the emergence of new multidrug resistant strains. While the twentieth century was called the era of the discovery of antibiotics, in the following decades, only existing antibiotics were modified [2, 18–20].

There are efforts to set stricter application conditions for the further usability of existing antibiotics. If antibiotics are applied properly, we can contribute to reducing the

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development of multidrug resistant strains. Unfortunately, in the case of an unknown pathogen, current clinical protocols use combined broad-spectrum antibiotic treatments "blindly," often unnecessarily, at the start of treatment in order to control sepsis caused by bacteria.

In addition to the stricter application of human medicine, many forums have tried to regulate the uncontrolled use of antibiotics in the crop and livestock sectors [8], which indirectly contributes to the mutation of bacteria.

The treatment of septic symptoms is mainly determined by the clinical appearance and the microbiological culture of the pathogens based on the laboratory results. Prompt, aggressive antibiotic therapy and appropriate surgical treatment are essential to ensure a good prognosis. Despite rapid diagnosis and treatment, there is still a high mortality rate [21–23], which in many cases can be decisively influenced by appropriately chosen antibiotic therapy.

Based on some studies, it would be an important criteria that, if possible, the patient should not receive systemic antibiotics before sampling [24–27] and that the patient only receives the therapeutic or prophylactic dose of antibiotics afterward [28].

One of the main goals could be a fast, reliable method to identify bacterial strains and drug resistance during surgery. In the early management of periprosthetic joint infections, under certain criteria, there is a chance for preserving the implant by applying DAIR protocol (debridement, antibiotics and implant retention), followed by prolonged antibiotic treatment. However, this therapeutic approach has a relatively high failure rate, reported between 10% and 40%. It is known that *Staphylococcus aureus* or coagulase-negative staphylococci (CNS) are accounted for two-third of all PJI cases and susceptibility to methicillin (methicillin-resistant *Staphylococcus aureus* [MRSA] and methicillin-sensitive *Staphylococcus aureus* [MSSA]) could definitely improve the efficacy of antibiotic management [29].

In order to increase effectiveness, Trampuz et al. recommend the sonication procedure during which, after the removal of the implant, the bacteria are extracted from the biofilm in an ultrasonic sonicator, which is later examined by microbiological methods [30, 31].

In order to reduce periprosthetic joint softening, under the leadership of *Deirmengian* and *Parvizi*, the alpha defensin protein was recommended to be subjected to further tests in the diagnosis of periprosthetic joint infection [32]. As a development of this work, a rapid test is available that can also be used in clinical practice (Synovasure® Alpha Defensin Test), which clearly shows αD positivity after 10 min [26, 27].

Despite efforts to reduce drug resistance, the identification and isolation of the causative agent and the early use of targeted antibiotic therapy are still crucial for the success of treating bacterial infections.

Objective: *We aimed to demonstrate the practical utility of differential scanning calorimetry (DSC) used in our research project* [9–11] *to isolate the pathogen bacteria in joint and periprosthetic infections.*

Materials and methods

In accordance with the norms of the responsible Regional and Institutional Research Ethics Committee (*number of permission: 6737*) and the Helsinki Declaration of 1975, revised in 2008, using our previous research results [9–11], in order to verify and isolate the identity of the most common pathogens in locomotor septic pathologies. The research was performed in the DSC laboratory of the Institute of Biophysics, University of Pécs, in the frame of our interdisciplinary working group.

When creating the septic joint model, the sterilely collected synovial fluid samples were permanently stored at − 70 °C under experimental conditions, taking into account the physiological environment and basic reproduction conditions of the bacteria. On the day the experimental model was assembled, taking into account the rules of sterility and the risk of cross infection, during septic musculoskeletal infections, we monitored the proliferation of bacteria in isothermal mode at 37 °C. We used the most common bacteria (*Escherichia coli*, MRSA (methicillin-resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* [10, 12]) under in vitro conditions, by superinfecting the synovial fluid with a concentration of 10^3 or 10^5 CFU mL^{-1}. Depending on the tested concentration (10^3/10^5 CFU mL^{-1}), in the case of the used bacterial strains, their heat production was recorded during their reproductive cycle, and the maximum of the heat rates was monitored [10, 11].

Similarly to our previous research project, synovial samples were collected in cases without arthrosis and, in cases of different degrees of arthrosis, based on the modified Kellgren–Lawrence classification [13] (Table 1). Then, we examined and analyzed the factors influencing the reproduction of bacterial strains with isoperibol calorimetry, all for unequivocal test results. The reproductive cycle of bacterial strains that occur more often in septic locomotor pathologies was investigated with the SETARAM Micro DSC-II calorimeter, based on the chosen experimental model, using SETARAM produced conventional and mixing batch vessels (see Fig. 1).

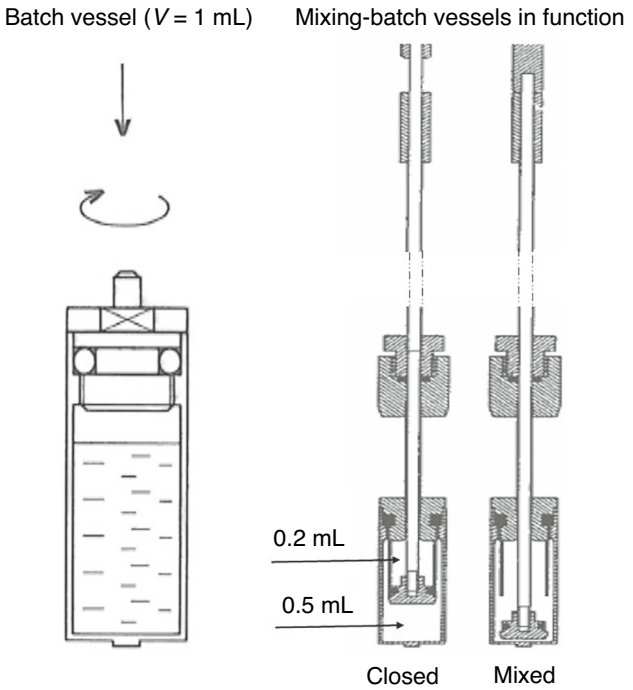

Fig. 1 For the denaturation (left) as well as for the proliferation test used, specially designed mixing cells, in closed and open (after mixing) positions (SF in the lower, bacteria in the upper compartment)

Results

Denaturation results of the examined synovial samples

Similar to our previous results [9–11], the denaturation curves of the synovial samples are influenced by the progression of the degree of arthritis, their chemical composition and primarily their protein content (see Fig. 2). As arthrosis progresses, an important parameter change can be detected, which can be seen in the change in the half-value width ($T_{1/2}$) of the denaturation curve (see Table 2), which is the temperature range of the structural transformation at half of the maximum heat flow. (It gives information about the cooperativity among the different thermal domains being the constituent elements of the tested sample.)

We performed a deconvolution of the scans too, because on the basis of the shape and "shoulders" of the curves it is obvious the presence of a multidomain structure. (We have taken into consideration the components with highest contribution to the total enthalpy, see Fig. 3 and Table 3.)

We have checked the effect of more aggressive bacteria on the denaturation behavior of the infected SF too. After the mixing, we have waited the end of the proliferation, and in the same arrangement, we denatured the infected SF. The

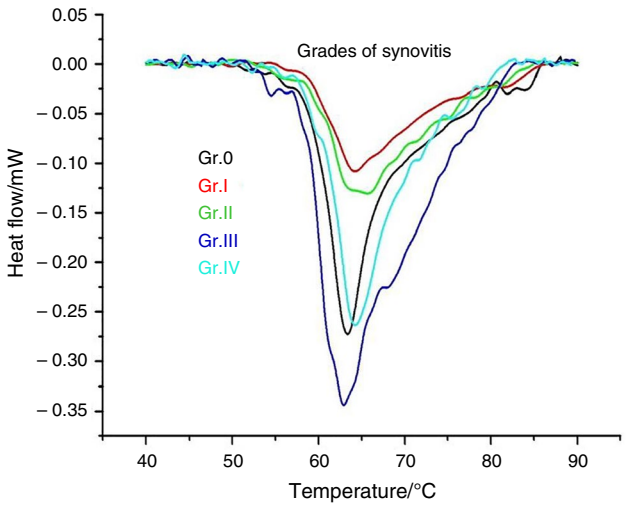

Fig. 2 The denaturation scans of different grades of synovitis (the curves are average of the independent experiments)

results are shown in Fig. 4 and Table 4, after deconvolution in Fig. 5 and Table 5.

Results of the proliferation cycle of the tested bacteria

After reaching the thermal equilibrium between the sample mixing vessel (filled with the tested bacterial strain and the separated medium synovial fluid) and the reference vessel (in both compartment the same amount of SF), after the mixing, the heat released during the proliferation process of the bacterial strains was recorded as a function of the time elapsed, which at the tested concentrations (10^3–10^5 CFU mL^{-1}) was characteristic of the given bacterial strain, had different time courses as well as different total heat production (see Table 6) [9–12].

The maximum heat flow time and the calorimetric enthalpy are bacteria-dependent parameters. The run of the scans prohibited a multistep proliferation, so we performed a deconvolution (see Figs. 6 and 7, and Table 7 and 8). In the tables we took into consideration only three main steps in the heat production. (The smaller contributions which were in the vicinity of the detectable limit of the instrument were neglected in the repeated deconvolution.)

During the calorimetric identification of most frequently infecting bacteria, the time elapsed to reach the maximum of the proliferation heat flow could be a monitor, as shown in Table 9. After mixing the proliferation starts maximum in 18–20 min. The quickest proliferation's peak is around 3 h 30 min, the smallest at 12 h 40 min.

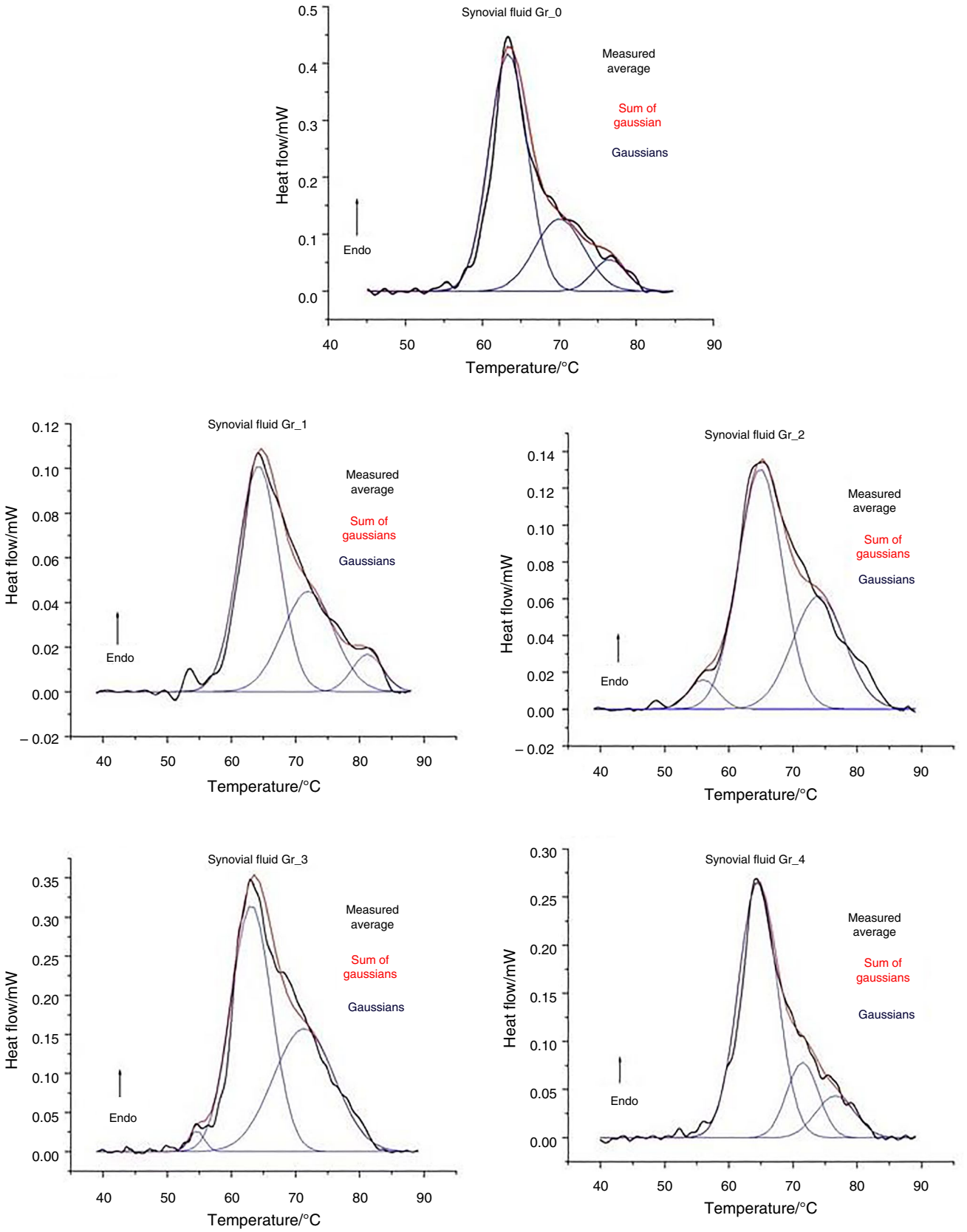

Fig. 3 Deconvoluted DSC scans of SF with different severity (Gr.0-Gr.IV.). Colors in figures are; black: experimental average curve, red: sum of the individual transitions, blue stands for the main contributors of denaturation (1st-albumin, 2nd and 3rd to α_1-α_2, β and γ globulins [33–35]). Endotherm deflection upwards

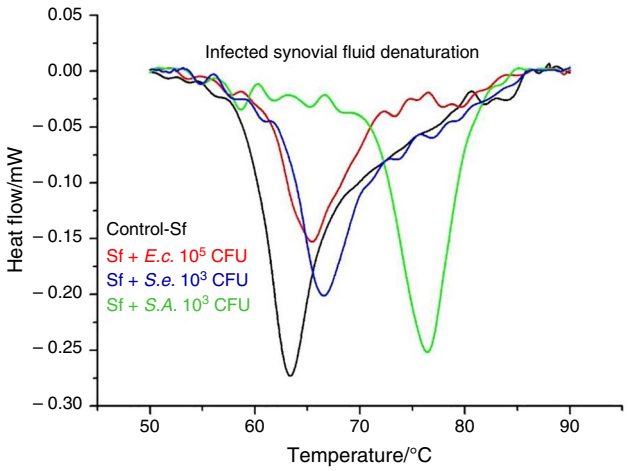

Fig. 4 Denaturation scans of infected SF samples. Endotherm deflection downward

Discussion

The aim of our current study was to demonstrate the applicability of thermal analysis in evaluation of the grade of osteoarthritis or the infection of knee joint using synovial fluid samples. Our primary goal was to gain reliable information about the bacterial growth in a relatively short time.

Based on the literature, two positive cultures or the presence of a sinus tract are considered as major criteria for diagnosis of periprosthetic joint infection [36]. While tasking samples with swabs are a widely used practice, they are less reliable and nowadays more sophisticated methods are recommended for detections of pathogens [37]. Generally, intraoperatively collected tissue samples, sonication and synovial fluid should be utilized instead of swabs.

Besides standard culturing techniques, matrix-assisted LASER desorption/ionization time-of-flight (MALDI-TOF)

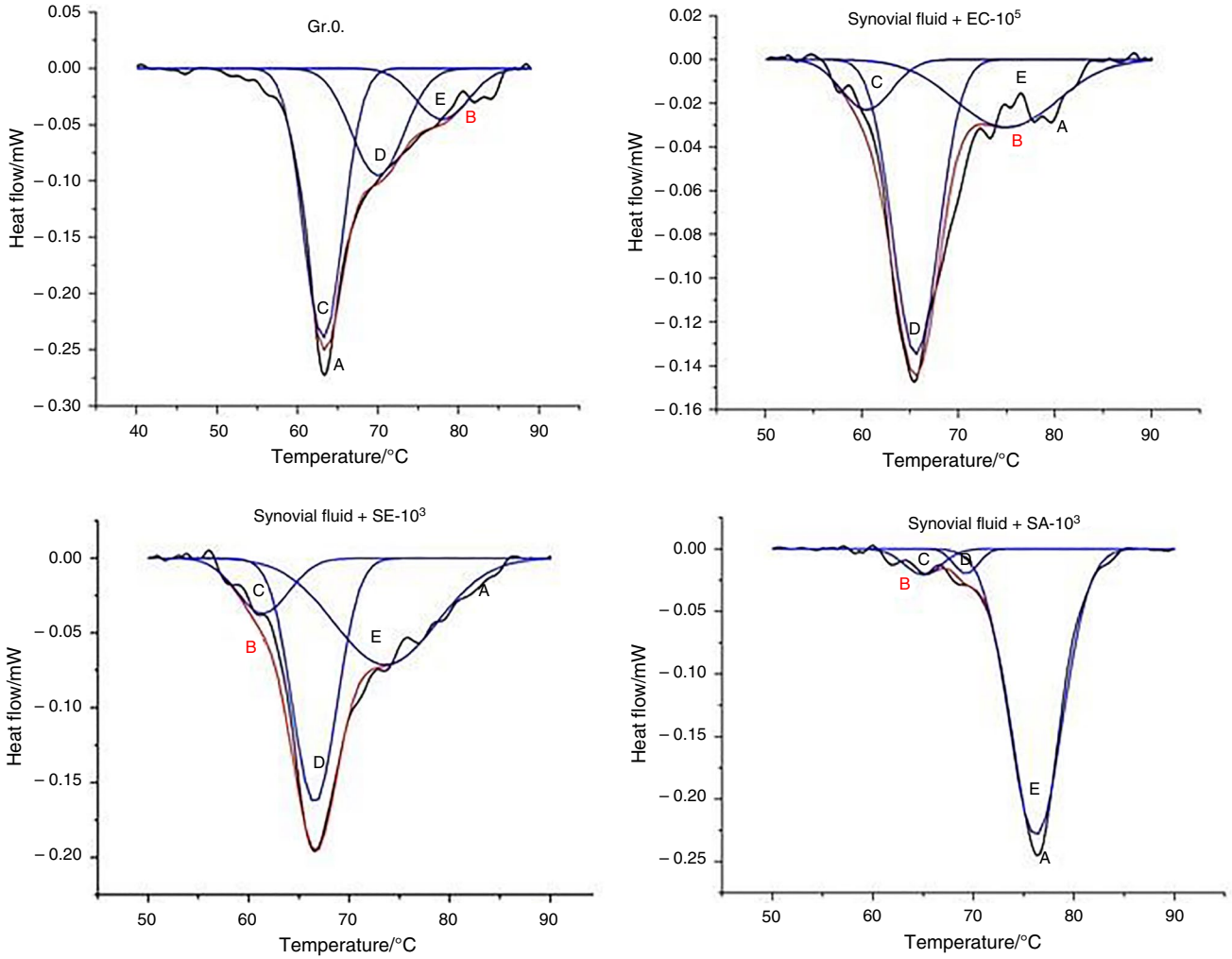

Fig. 5 Deconvoluted DSC scans of SF infected with different bacteria. Symbols in figures are: **A** experimental average curve, **B** sum of the individual transitions, the **C-D-E** curves stand for the main contributors of denaturation (C-albumin, D and E to α_1-α_2, β and γ globulins [33–35]). Endotherm deflection downward

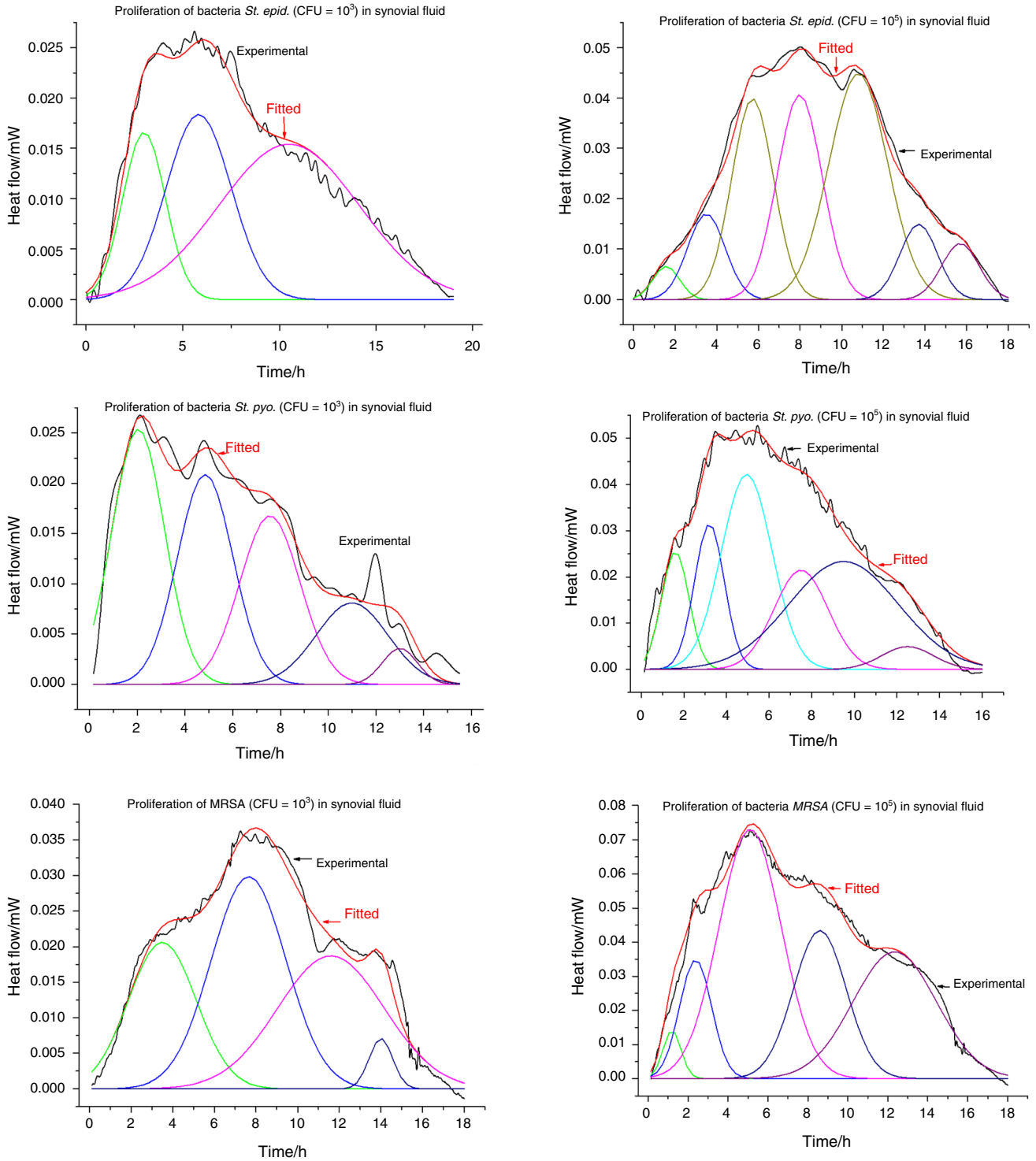

Fig. 6 Isoperibolic heat flow scans of less effectively proliferating bacteria

spectroscopy and an automated antimicrobial susceptibility testing systems can be used to identify pathogens and test antibiotic sensitivity [38].

While the established microbiological tests are still the "gold-standards" in the detections of pathogens, these methods are also time-consuming. However, in case of treatment of periprosthetic infections, rapid diagnosis and availability of antibiotic sensitivity are essential. Therefore, one

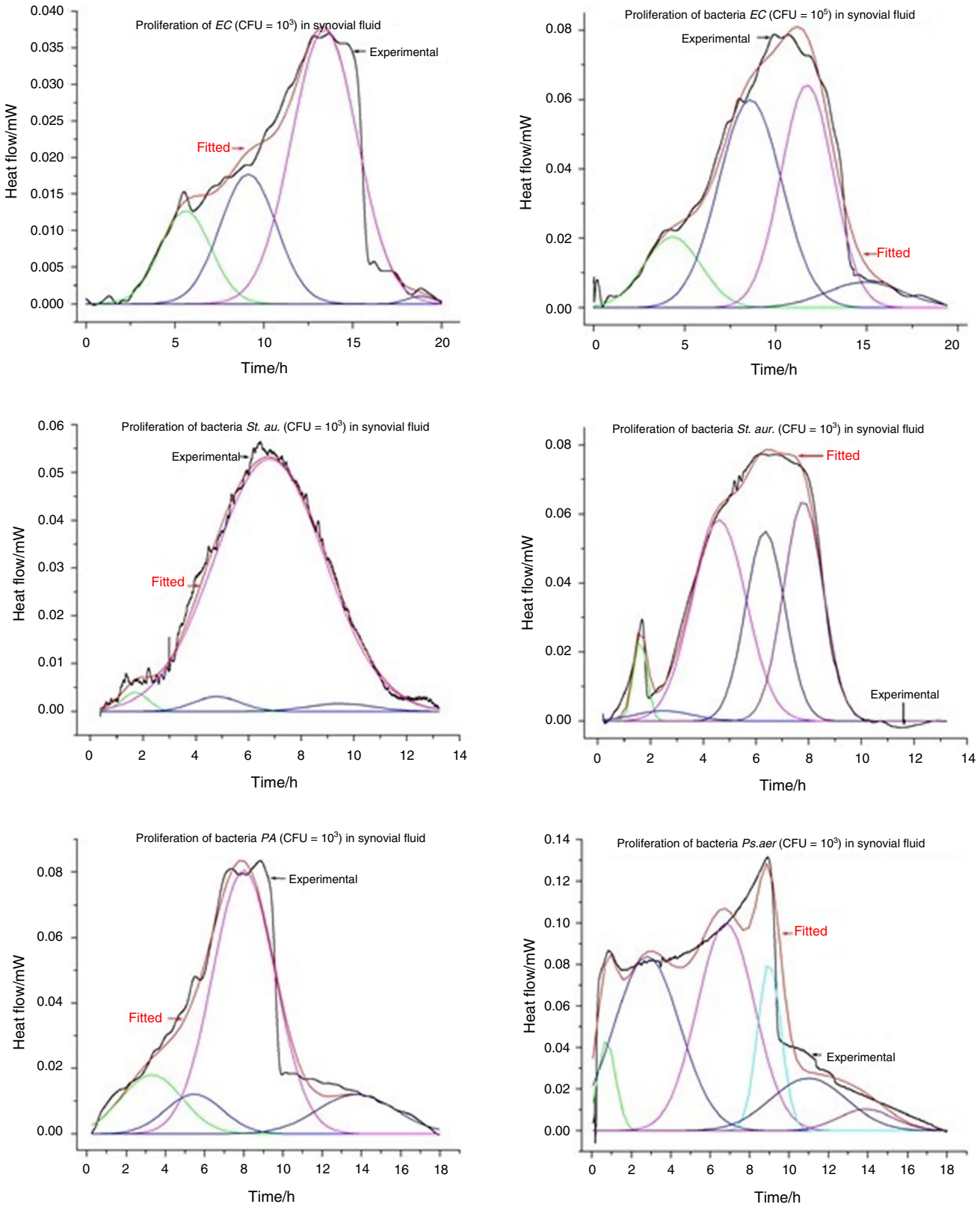

Fig. 7 Isoperibolic heat flow scans of more effectively proliferating bacteria

Table 1 Description of Kellgren–Lawrence and the modified Kellgren–Lawrence classification systems [13]

Classification	Kellgren–Lawrence	Modified Kellgren–Lawrence
Gr-0	No osteoarthritis lesions	No osteoarthritis lesions
Gr-I	Narrowing of the joint gap is suspected, osteophytic "lip formation" is possible	Osteophyte is questionable
Gr-II	Clear osteophytes and possible narrowing of joint gap	Osteophytes are clearly detected
Gr-III	Moderate, multiple osteophytes, clear narrowing of joint gap, some sclerosis and deformity at the bone ends possible	Narrowing of the joint gap
Gr-IV	Large osteophytes, the joint gap is explicit narrowing, severe sclerosis, the bone ends have determined deformity	Apparent disappearance of the joint gap ("bone on bone")

Table 2 Denaturation parameters of synovial fluids in different severity of arthritis

Degree of severity	T_m/ °C	$T_{1/2}$/ °C	ΔH_{cal}/Jg^{-1}
0	63.4	4	0.46
I	64.3	11.5	0.32
II	63.7 ± 0.6	12.4 ± 0.4	0.54 ± 0.06
III	63.5	10.9	0.58
IV	64.2 ± 0.7	8.2 ± 0.3	0.4 ± 0.05

T_m, stands for maximum temperature of denaturation (50% of the sample is in unfolded state); $T_{1/2}$ for the half width of denaturation; ΔH, for calorimetric enthalpy change; normalized for the total sample mass (s.d. was calculated only in case when the number of samples was $n \geq 5$)

of the greatest advantages of thermal analysis that it can be performed in a few hours, instead of days.

The early diagnosis of osteoarthritis would be a key factor to prevent further progression of these degenerative diseases. Beside the conventional imaging methods, such as X-rays, CT-scan and MRI, only a few alternative tests are found in the literature for the detection of a developing osteoarthritis. To find a reliable biomarker to diagnose the osteoarthritis in the early phase, Ab Aziz et al. have recently utilized attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to test serum samples of patients with symptomatic primary knee osteoarthritis [39]. Another possible target could be the biochemical analysis of synovial fluid, due to the inflammatory processes and oxidative stress occurring in arthritis [40] (Table 1).

In regard of osteoarthritis samples, Fig. 2 and Table 2 clearly demonstrate that the alteration of DSC scans is a good fingerprint of the severity of disease. The $T_{1/2}$ increases in all stages (the biggest is in case of Grade II, similar the change in calorimetric enthalpy) compared to the healthy control, demonstrating, that the cooperativity among the fluid components decreases in all cases [9–11].

During the denaturation, the actual stage of SF proteins is the key factor. In a healthy SF, it is in the range of 9–22 g L^{-1}, but in inflammatory stage, it can reach 44 g L^{-1}. Roughly 60% of the protein content is albumin, the others are globulins, proteoglycans and hyaluronic acid [41–43]. The denaturation scans of SF exhibit a good similarity to the blood plasma curves [33, 44–49], where 4–5 thermal domains can be separated by deconvolution of scans. The albumin gives the biggest contribution. Generally, the enthalpy resolution of a commercial DSC equipment is in the range of 5%; therefore, we followed the Briere method [34], where in case of SF only three main compound was used (see Fig. 3, where the fitting was better than 95%). Table 3 shows that the albumin contribution (T_m) was the biggest one, and exhibited a severity dependence, compared to the control.

Those SF samples which undergone to bacterial infection produced strong bacterial dependence (see Fig. 4 and Table 4). The melting temperature and calorimetric enthalpy exhibit a fingerprint character. Having a big DSC scan pool, with the aid of a good informatic database, it could be used as a quick test of the type of infecting bacterium (within a

Table 3 Maximum denaturation temperature (T_m) values of the three main overlapping transitions (made by deconvolution) and relative contribution of each one to the total enthalpy (using average DSC scans of different synovial fluids with altering degree of severity)

Sample	T_{m1}/ °C	Relative abundance %	T_{m2}/ °C	Relative abundance %	T_{m3}/ °C	Relative abundance %
Gr. 0	63.2	56.3	70	29.4	78	14.3
Gr. I	64.1	47.9	69.6	34.8	78	17.3
Gr. II	64.9	65.9	71.55	18.2	77.3	15.9
Gr. III	62.9	54.9	68.1	16.2	73.5	28.9
Gr. IV	64	63.1	68.6	16.4	74.5	20.5

Table 4 Denaturation parameters of SF after inoculation with different bacteria

Samples	T_m/ °C	ΔH_{cal}/Jg^{-1}
Healthy synovial fluid (SF)	63.4	0.46
SF + *E. coli* CFU 10^5	65.3	0.54
SF + *S. epidermidis* CFU 10^3	65.7	0.56
SF + *S. aureus* CFU 10^3	76.4	0.6

T_m, stands for maximum temperature of denaturation; and ΔH_{cal}, for calorimetric enthalpy change; normalized for the sample mass [because of a few samples (less than 5), there was no statistical evaluation performed (s.d. is omitted)]

few hours scanning the T_m can be reached for the identification). The deconvolution of the DSC curves (see Fig. 5 and Table 5) also demonstrate the shifting of denaturation temperature of the biggest compound albumin to the higher range, representing a strong structural rearrangement.

The proliferation experiments performed with the six more frequently infecting bacteria exhibited also the ability to use the isoperibol technique to identify the infecting agent. On the basis of Table 6, we can see that the smallest calorimetric enthalpy is produced by *Streptococcus pyogenes*, but it quickest to reach the maximum heat flow, while the biggest *ΔH* by *Pseudomonas aeruginosa*. Two groups can be separated in the bacterium line: moderately proliferating (see Fig. 6 and Table 7) as well as more effectively proliferating (on the basis of total calorimetric enthalpy) bacteria (see Fig. 7 and Table 8). This can be also a relatively quick method to identify the bacterium and start the antibiotic treatment.

Table 9 presents data for a further identification of bacteria. To reach the maximum heat flow during the proliferation *Streptococcus pyogenes* leads, and the slowest is *Escherichia coli*. It is interesting that in case of *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* at 10^5 CFU mL^{-1} infecting concentration the proliferation heat flow reaches the maximum value later than in case of 10^3 CFU mL^{-1}. Comparing the synovial media with similar but still varying protein content, we did not notice any significant differences in the bacterial reproduction cycle, the chemical composition of the synovial samples mainly did not affect the initial

Table 6 Thermal proliferation characteristics of bacteria causing frequent joint infections

Tested bacterial strains	Thermal parameters			
	to	*te*	*tm*	DHcal/*Jg*^{1}
Streptococcus pyogenes				
CFU 10^3	10 min	15 h 30 min	2 h	− 0.64
CFU 10^5	12 min	15 h 50 min	5 h 30 min	− 2.45
Staphylococcus epidermidis				
CFU 10^3	8 min	19 h	5 h 37 min	− 0.66
CFU 10^5	10 min	17 h 50 min	8 h 20 min	− 1.58
MRSA				
CFU 10^3	15 min	18 h	7 h 16 min	− 0.91
CFU 10^5	8 min	18 h	5 h 5 min	− 3.67
Staphylococcus aureus				
CFU 10^3	26 min	13 h 22 min	6 h 27 min	− 1.48
CFU 10^5	13 min	13 h 30 min	7 h	− 1.98
Escherichia coli				
CFU 10^3	8 min	20 h	13 h	− 1.56
CFU 10^5	9 min	19 h 35 min	9 h 55 min	− 3.22
Pseudomonas aeruginosa				
CFU 10^3	16 min	18 h	8 h 50 min	− 2.48
CFU 10^5	16 min	17 h 50 min	9 h	− 3.32

t_o, onset; t_e, end point; t_m, time maximum of proliferation rate; ΔH_{cal}, calorimetric enthalpy of proliferation [negative sign stands for exoterm process]; data are averages, $n < 5$)

reproduction kinetics of the bacteria. The sharp decrease in the growth rate of the tested bacteria can be influenced by the depleting culture medium level.

During the analysis of the reproduction cycle of bacterial strains, when differentiating the different proliferation kinetics, in some cases, sample superinfection also arises, which results in the heat flow an irregular run, the sum of the heat flow can be produced by two or more bacteria during a unit of time. With the present investigation, despite the sterility and the strict treatment of the bacterial strains, we could not confirm or exclude possible superinfection of the samples.

Table 5 Maximum temperature (T_m) values of the three main overlapping transitions (made by deconvolution), and their relative contribution to the total enthalpy

Sample + CFU	T_{mC}/ °C	Relative abundance %	T_{mD}/ °C	Relative abundance %	T_{mE}/°C	Relative abundance %
Gr.0	63.2	56.3	70	29.4	78	14.3
SF + SA 10^3	65	5.7	69.25	3.5	76.2	90.8
SF + SE 10^3	61.4	11.7	66.55	43.8	73.6	44.5
SF + EC 10^5	60.5	11	65.6	59.4	75	29.6

SF, synovial fluid; *SA*, *Staphylococcus aureus; SE*, *Staphylococcus epidermidis; EC*, *Escherichia coli*)

Table 7 The proliferation time maxima (t_ms) and relative abundance (in %, higher than 5%, rounded to one decimal) to the total enthalpy of the deconvoluted curves in case of the bacteria that produced the smallest calorimetric enthalpy during the proliferation

Sample CFU mL^{-1}	t_{m1}/h	Relative abundance %	t_{m2}/h	Relative abundance %	t_{m3}/h	Relative abundance %	t_{m4}/h	Relative abundance %
St. epid.								
10^3	2.2	17.4	5.8	29.4	10.5	53.2	–	–
10^5	3.5	8.1	5.7	21.1	8	23.1	10.8	33.3
St. pyo.								
10^3	2	31.7	4.9	27.6	7.6	24	11	13.8
10^5	3.5	12.1	5	27.6	9.5	32.9	12.5	10.6
MRSA								
10^3	3.5	24.1	7.7	38.6	11.6	34.4	–	–
10^5	2.4	9.8	5.1	38.6	–	–	–	–

Table 8 The proliferation time maxima (t_ms) and relative abundance (in %, rounded to one decimal) to the total enthalpy (higher than 5%) of the deconvoluted curves in case of the bacteria that produced the highest calorimetric enthalpy during the proliferation

Sample CFU mL^{-1}	t_{m1}/h	Relative abundance %	t_{m2}/h	Relative abundance %	t_{m3}/h	Relative abundance %	t_{m4}/h	Relative abundance %
E. coli								
10^3	5.6	17.7	9.1	26.8	13.4	66.7	–	–
10^5	4.3	13	8.6	42.8	11.7	37.9	14.6	6.3
St. aer.								
10^3	–	–	6.8	94.3	–	–	–	–
10^5	4.6	37.9	6.4	25.9	7.8	30.3		
Ps. aer.								
10^3	3.3	14.1	5.5	8.8	8	49.7	13.7	12.1
10^5	3.3	14.1	5.5	40.3	8.6	20.2	12.3	27.4

Table 9 Ranking of the bacteria according to the time of reaching the maximum heat rate

Tested bacterial strains	t_0	Proliferation time t_e	t_m
Streptococcus pyogenes 10^3 CFU mL^{-1}	10 min ± 2 min	16 h 56 min ± 18 min	3 h 21 min ± 42 min
Streptococcus pyogenes 10^5 CFU mL^{-1}	11 min ± 2 min	15 h 58 min ± 68 min	4 h 20 min ± 63 min
Staph. epidermidis 10^3 CFU mL^{-1}	8 min ± 1 min	20 h 12 min ± 12 min	3 h 35 min ± 75 min
Staph. epidermidis 10^5 CFU mL^{-1}	9 min ± 2 min	20 h 5 min ± 23 min	7 h 38 min ± 63 min
Staph. aureus 10^3 CFU mL^{-1}	18 min ± 5 min	15 h 12 min ± 21 min	7 h 28 min ± 67 min
Staph. aureus 10^5 CFU mL^{-1}	13 min ± 2 min	13 h 40 min ± 45 min	6 h 58 min ± 68 min
Pseudomonas aeruginosa 10^3 CFU mL^{-1}	15 min ± 2 min	15 h 32 min ± 84 min	7 h 42 min ± 116 min
Pseudomonas aeruginosa 10^5 CFU mL^{-1}	14 min ± 3 min	18 h 10 min ± 52 min	11 h 5 min ± 65 min
MRSA 10^3 CFU mL^{-1}	14 min ± 3 min	17 h 31 min ± 64 min	9 h 5 min ± 118 min
MRSA 10^5 CFU mL^{-1}	7 min ± 2 min	17 h 20 min ± 37 min	7 h 28 min ± 114 min
Escherichia coli 10^3 CFU mL^{-1}	7 min ± 2 min	15 h 18 min ± 76 min	12 h 38 min ± 89 min
Escherichia coli 10^5 CFU mL^{-1}	8 min ± 2 min	18 h 8 min ± 55 min	11 h 15 min ± 74 min

Conclusions

Given that the number of antibiotics discovered and used in clinical practice in recent decades is significantly exceeded by the number of resistant strains that have developed [28], we need a significant strategic change in approach in order to be effective in treatment with the existing set of antibiotics. Despite the high sensitivity and specificity of the biomarkers, antibiotic sensitivity and resistance remain an open question, for the detection of which the isoperibolic calorimetric test is a reliable tool, based on the work of *Trampuz* and *Braissant* [50, 51] and our research.

Until now, in clinical practice, we have been able to achieve effective treatment based on the combined use of serological markers, synovial tests and microbiological vaccinations [28]. However, it could be revolutionized by utilizing antibiotic sensitivity specified with microcalorimetrical test.

Our experimental series using DSC and isoperibolic calorimetry proves that it is an excellent method for verifying the identity and reproductive capacity of the pathogen. In future studies, the effectiveness of broad-spectrum antibiotics could be tested in our experimental settings in a relatively short time duration.

Author contributions Dénes Lőrinczy was involved in investigation, writing—original draft and funding acquisition. Árpád Dandé participated in investigation. Laszlo G. Nöt performed writing—review and editing and supervision. "All authors have read and agreed to the published version of the manuscript."

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Declarations

Conflict of interest "The authors declare no conflict of interest." "The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results."

Ethical approval The study was designed in accordance with the norms of the responsible Regional and Institutional Research Ethics Committee (*number of permission: 6737*) and the Helsinki Declaration of 1975, revised in 2008.

Informed consent "Informed consent was obtained from all subjects involved in the study."

References:

1. Adedeji WA. The treasure called antibiotics. Ann Ibadan Postgrad Med. 2016;14(2):56–7.
2. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol. 2010;1:134.
3. Baldoni D, Hermann H, Frei R, Trampuz A, Steinhuber A. Performance of microcalorimetry for early detection of methicillin resistance in clinical isolates of *Staphylococcus aureus*. J Clin Microbiol. 2009;47:774–6.
4. Murphy SL, Kochanek KD, Xu J, Arias E. Mortality in the United States. NCHS Data Brief. 2020;2021:1–8.
5. Xu J, Murphy SL, Kockanek KD, Arias E. Mortality in the United States. NCHS Data Brief. 2018;2020:1–8.
6. Aminov RI, Mackie RI. Evolution and ecology of antibiotic resistance genes. FEMS Microbiol Lett. 2007;271:147–61.
7. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74:417–33.
8. Larsson DGJ, Flach C-F. Antibiotic resistance in the environment. Nat Rev Microbiol. 2022;20:257–69.
9. Dandé Á, Nöt LG, Wiegand N, Kocsis B, Lőrinczy D. DSC analysis of human synovial fluid samples in the diagnostics of non-septic and septic arthritis. J Therm Anal Calorim. 2017;130:1249–52.
10. Dandé Á, Nőt LG, Bűcs G, Wiegand N, Kocsis B, Lőrinczy D. Examination of typical bacterial strains in septic arthritis by isoperibol calorimeter. J Therm Anal Calorim. 2018;131:2041–8.
11. Dandé Á, Kocsis B, Lőrinczy D. Thermal analysis of synovial fluids in different stages of osteoarthritis and after bacterial infections. J Therm Anal Calorim. 2020;142:797–808.
12. Dandé Á, Nöt LG, Bűcs G, Kocsis B, Lőrinczy D, Wiegand N. Efficacy of microbiological culturing in the diagnostics of joint and periprosthetic infections. Injury. 2021;52(1):S48-52.
13. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16:494–502.
14. Murphy SL, Xu J, Kochanek KD, Arias E. Mortality in the United States. NCHS Data Brief. 2017;2018:1–8.
15. Lipp EK, Huq A, Colwell RR. Effects of global climate on infectious disease: the cholera model. Clin Microbiol Rev. 2002;15:757–70.
16. Aminov RI. The role of antibiotics and antibiotic resistance in nature. Environ Microbiol. 2009;11:2970–88.
17. Bush K. Past and present perspectives on β-Lactamases. Antimicrob Agents Chemother. 2018;62(10):10–1128.
18. Rogers GB, Carroll MP, Bruce KD. Enhancing the utility of existing antibiotics by targeting bacterial behaviour? Br J Pharmacol. 2012;165:845–57.
19. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009;325:1089–93.
20. Fernandes P. Antibacterial discovery and development—the failure of success? Nat Biotechnol United States. 2006;24:1497–503.
21. Talbot GH, Bradley J, Edwards JEJ, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline from the antimicrobial availability task force of the infectious diseases society of America. Clin Infect Dis Off Publ Infect Dis Soc Am. 2006;42:657–68.
22. von Ah U, Wirz D, Daniels AU. Rapid differentiation of methicillin-susceptible *Staphylococcus aureus* from methicillin-resistant *S. aureus* and MIC determinations by isothermal microcalorimetry. J Clin Microbiol. 2008;46:2083–7.
23. Weston VC, Jones AC, Bradbury N, Fawthrop F, Doherty M. Clinical features and outcome of septic arthritis in a single UK health district 1982–1991. Ann Rheum Dis. 1999;58:214–9.
24. Toms AD, Davidson D, Masri BA, Duncan CP. The management of peri-prosthetic infection in total joint arthroplasty. J Bone Joint Surg Br. 2006;88:149–55.
25. Gallo J, Smizanský M, Radová L, Potomková J. Comparison of therapeutic strategies for hip and knee prosthetic joint infection. Acta Chir Orthop Traumatol Cech. 2009;76(4):302–9.
26. Zahar A, Webb J, Gehrke T, Kendoff D. One-stage exchange for prosthetic joint infection of the hip. Hip Int J Clin Exp Res hip Pathol Ther. 2015;25:301–7.
27. Gehrke T, Zahar A, Kendoff D. One-stage exchange: it all began here. Bone Joint J. 2013;95(B):77–83.

28. Ali F, Wilkinson JM, Cooper JR, Kerry RM, Hamer AJ, Norman P, et al. Accuracy of joint aspiration for the preoperative diagnosis of infection in total hip arthroplasty. J Arthroplast. 2006;21:221–6.
29. Scheper H, Gerritsen LM, Pijls BG, Van Asten SA, Visser LG, De Boer MG. Outcome of debridement, antibiotics, and implant retention for staphylococcal hip and knee prosthetic joint infections, focused on rifampicin use: a systematic review and meta-analysis. Open Forum Infect Dis. 2021;8:ofa298.
30. Krenn V, Perino G, Krenn VT, Wienert S, Saberi D, Hügle T, et al. Histopathological diagnostic work-up of joint endoprosthesis-associated pathologies. Hautarzt. 2016;67:365–72.
31. Krenn V, Kölbel B, Huber M, Tiemann A, Kendoff D, Wienert S, et al. Revision arthroplasty : histopathological diagnostics in periprosthetic joint infections. Orthopade Ger. 2015;44:349–56.
32. Frommelt L. Principles of systemic antimicrobial therapy in foreign material associated infection in bone tissue, with special focus on periprosthetic infection. Injury. 2006;37(2):S87-94.
33. Garbett NC, Mekmaysy CS, Helm CW, Jenson AB, Chaires JB. Differential scanning calorimetry of blood plasma for clinical diagnosis and monitoring. Exp Mol Pathol. 2009;86:186–91.
34. Briere L-AK, Brandt J-M, Medley JB. Measurement of protein denaturation in human synovial fluid and its analogs using differential scanning calorimetry. J Therm Anal Calorim. 2010;102:99–106.
35. Ahrer K, Buchacher A, Iberer G, Jungbauer A. Thermodynamic stability and formation of aggregates of human immunoglobulin G characterised by differential scanning calorimetry and dynamic light scattering. J Biochem Biophys Methods. 2006;66:73–86.
36. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, et al. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. J Arthroplast. 2018;33:1309-1314.e2.
37. Fröschen FS, Randau TM, Franz A, Molitor E, Hischebeth GT. Microbiological profiles of patients with periprosthetic joint infection of the hip or knee. Diagnostics. 2022;12(7):1654.
38. Yusuf E, Roschka C, Esteban J, Raglio A, Tisler A, Willems P, et al. The state of microbiology diagnostic of prosthetic joint infection in Europe: an in-depth survey among clinical microbiologists. Front Microbiol. 2022;13: 906989.
39. Aziz AA, Selvaratnam V, Fikri YF, Sani MS, Kamarul T. Diagnosis of osteoarthritis at an early stage via infrared spectroscopy combined chemometrics in human serum: a pilot study. Processes. 2023;11(2):404.
40. Myers SL. Synovial fluid markers in osteoarthritis. Rheum Dis Clin N Am. 1999;25(2):433–49.
41. Oates KMN, Krause WE, Jones RL, Colby RH. Rheopexy of synovial fluid and protein aggregation. J R Soc Interface. 2006;3:167–74.
42. Blewis ME, Nugent-Derfus GE, Schmidt TA, Schumacher BL, Sah RL. A model of synovial fluid lubricant composition in normal and injured joints. Eur Cell Mater. 2007;13:26–39.
43. Mazzucco D, Scott R, Spector M. Composition of joint fluid in patients undergoing total knee replacement and revision arthroplasty: correlation with flow properties. Biomaterials. 2004;25:4433–45.
44. Michnik A, Michalik K, Kluczewska A, Drzazga Z. Comparative DSC study of human and bovine serum albumin. J Therm Anal Calorim. 2006;84(1):113–7. https://doi.org/10.1007/s10973-005-7170-1.
45. Garbett NC, Miller JJ, Jenson AB, Miller DM, Chaires JB. Interrogation of the plasma proteome with differential scanning calorimetry. Clin Chem. 2007;53:2012–4.
46. Todinova S, Krumova S, Gartcheva L, Robeerst C, Taneva SG. Microcalorimetry of blood serum proteome: a modified interaction network in the multiple myeloma case. Anal Chem. 2011;83:7992–8.
47. Fekecs T, Zapf I, Ferencz A, Lőrinczy D. Differential scanning calorimetry (DSC) analysis of human plasma in melanoma patients with or without regional lymph node metastases. J Therm Anal Calorim. 2012;108:149–52.
48. Zapf I, Fekecs T, Ferencz A, Tizedes G, Pavlovics G, Kálmán E, et al. DSC analysis of human plasma in breast cancer patients. Thermochim Acta. 2011;524:88–91.
49. Todinova S, Krumova S, Kurtev P, Dimitrov V, Djongov L, Dudunkov Z, et al. Calorimetry-based profiling of blood plasma from colorectal cancer patients. Biochim Biophys Acta. 2012;1820:1879–85.
50. Trampuz A, Salzmann S, Antheaume J, Daniels AU. Microcalorimetry: a novel method for detection of microbial contamination in platelet products. Transfus. 2007;47:1643–50.
51. Braissant O, Müller G, Egli A, Widmer A, Frei R, Halla A, et al. Seven hours to adequate antimicrobial therapy in urosepsis using isothermal microcalorimetry. J Clin Microbiol. 2014;52:624–6.