

Utility of microcalorimetry in describing the growth curve of *C. albicans* at different temperatures – indentifying the optimal growth temperature

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Abstract

In comparison with other medical specialties, Orthopedics and Traumatology are continuously impressively developing, intrinsically connected to technological evolution. More than in other fields, in Orthopedics and Traumatology, the technological progress becomes obvious also by the frequent use of implants or osteosynthesis materials. By performing a simple search in the specialty literature, an exponential growth of arthroplasty surgeries in correlation with the life span of the population and implying a need that presupposes the increase of the quality of life, also by maintaining or improving mobility at a satisfying level, can be observed. At the same time, due to the increase in the number of traffic and sports accidents, the use of artificial joints and implants for osteosynthesis will constantly rise. The prostheses and the osteosynthesis materials used for the treatment of degenerative, traumatic or even esthetic pathologies are made of materials with a good biocompatibility.

Candida albicans is an optional anaerobic microorganism so the experiments performed with different volumes of environment were most likely influenced by the amount of oxygen, nutrient.

In the described research, we demonstrated that, using microcalorimetry, it is possible to identify this fungus in half the time required for classical microbiological identification. In addition, as our team has shown, there is the possibility of real-time antifungigram by using microcalorimetry.

Keywords: microcalorimetry, growth curve of *C. albicans*, optimal growth temperature

Introduction

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technological progress becomes obvious also by the frequent use of implants or osteosynthesis materials [1]. By performing a simple search in the specialty literature, an exponential growth of arthroplasty surgeries in correlation with the life span of the population and implying a need that presupposes the increase of the quality of life, also by maintaining or improving mobility at a satisfying level, can be observed [2]. At the same time, due to the increase in the number of traffic and sports accidents, the use of artificial joints and implants for osteosynthesis will constantly rise [3,4]. The prostheses and the osteosynthesis materials used for the treatment of degenerative, traumatic or even esthetic pathologies, are made of materials with a good biocompatibility [5,6].

Infectious pathology is an important problem in any health system and in any country in the world. It is all about material costs, the involvement of specialized and hyperspecialized human resources, but also the efforts of social reintegration of the affected patients, who frequently associate different degrees of depression. In the case of Orthopedics, but also of the other surgical specialties, infection of a wound leads to the slowing down of the healing process, accompanied by an increase of the psychological stress suffered by the patient [7,9,10-12]. As such, patients describe pains of greater intensity as compared to those with the same pathology, who cured without infection. Correct and rapid diagnosis followed by proper treatment is essential. The infection of an operated wound, especially in the case of arthroplasty or orthopedic implants, can have disastrous effects, the removal of the foreign material being necessary. Post-operative infections interrupt the normal healing process, increasing the risk of some associated pathologies or decompensation of the pre-existent ones, but also prolong the hospitalization time with significant additional costs for health systems [12-14].

The source of the infection may be a contaminated implant, may depend on errors in disinfection and sterilization, or it may be found at distance. For these reasons, in the

case of suspected surgery, it is mandatory to investigate, identify and treat any infectious outbreak [15,16]. Studies have shown that orthopedic infections are more common in patients with dental abscess or an infectious pathology in the tegument or digestive tract. There are numerous implicated microbial agents, of which, the following should be mentioned: *Staphylococcus aureus* (*S. aureus*), coagulase-negative staphylococci, *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*. Among these, *S. aureus* is the most frequently involved [7,8,10-12,15,16].

Taking into account the increased mortality and morbidity related to the situations in which the infections are associated with the medical act, it is necessary to develop rapid and efficient diagnostic methods. The speed of obtaining a positive, accurate diagnosis is important because, in the case of the Orthopedics field, but not only, targeted treatment can save a limb and restore the mobility of an affected patient with few resources.

One method that has managed to gain ground, in the age of technological evolution, is microcalorimetry. It represents a method that is frequently used in inorganic chemistry and has begun to develop also in medicine. Microcalorimetry can now be used successfully, with a high sensitivity, detecting temperature changes of the size of micro-nanojoules. It is also worth noting that at present, there are both data acquisition systems and software programs for data analysis and corroboration. We can undergo precise studies in a relatively short time. The ability of bacteria to release heat through metabolic activity is the foundation of our research, the microcalorimeter being able to record the energy released due to exothermic biochemical reactions and based on them through the integration program to generate growth curves that will be interpreted later in the clinical context [17-20].

Material and Methods

In order to carry out the experiments, our

team used two microcalorimeter devices that have a differential scanning mechanism, and to protect the 3D sensor we used pure Argon in gaseous state (99.99% SIAD - TP). During the experiments, samples and references were introduced into 1ml microcalorimeter cells.

Bacterial population

To undergo the experiments we used a freeze-dried *Candida albicans* strain provided by "Cantacuzino" National Research Institute.

The culture environment

For fungal development we used Sabouraud medium both in liquid and in solid form, both being autoclaved before use and tested for microbiologic purity. With the help of the plagues with solid environment, the fungus was repopulated, obtaining isolated colonies and testing in order to maintain the purity of the liquid medium. The liquid medium was used for replication and for the preparation of the fresh samples needed for microcalorimetry experiments.

Working protocol

1. To separate the liquid from solid (fungal mass), centrifugation was performed at 3000 rpm for 5 minutes. This process was repeated 3 times, with washing with fresh liquid medium, after each step.
2. After obtaining a fungal concentrate diluted in 200 µl, the McFarland index increased to 0.1.
3. Due to the slower growth kinetics of *C. albicans*, microcalorimetric samples were performed without further dilution.

1. Preparation of the sample

a. 3.000 µl of fresh medium at room temperature are added in a nephelometric tube.

b. The nephelometric tube is wiped with acetone to eliminate the risk of refractive defects.

c. McFarland index is measured.

d. Depending on the microbial agent studied, about 5 µl of the Eppendorf tube are

pipetted in the nephelometric tube until the McFarland index increases (reaches) to the proposed value.

e. Depending on the microbial agent used, dilutions are performed, stage that will be described according to the experiment.

f. The proposed volume of the solution is extracted and inserted into the microcalorimetric cell which is sealed using aseptic technique with a silicone o-ring and is afterwards inserted into the microcalorimeter.

2. Preparation of the reference

a. A solution of distilled water and antibiotic powder is prepared.

b. The fresh medium volume proposed is introduced into the microcalorimetric cell over which, an amount of solution containing antibiotic is added in a volume of 10%.

c. The cell is sealed with a silicone o-ring and it is inserted into the microcalorimeter.

Results

The same quantity of fungal agent was used for the proposed study, with changes being made to the volume used and the growth temperature. In order to obtain correct data and to observe any changes in the fungal growth pattern, changes of one parameter per experiment were made. Following the completion of these experiments, specific changes of the microcalorimetric growth curve were identified according to the temperature or volume used.

As the ATCC strain is a clinical isolate, the growth temperature used in the initial experiments was 37°C (although the recommended temperature for fungal development is 28°C). These experiments showed that *C. albicans* developed best at temperatures between 30°C and 37°C, too high or too low temperatures leading to a decrease in quantitative parameters such as maximum intensity of heat low (the peak of microcalorimetric growth), the total growth time (until the signal returns to the baseline).

Candida albicans is an optional anaerobic microorganism so the experiments performed

with different volumes of environment were most likely influenced by the amount of oxygen, nutrient (dependent on the smaller or larger volume of medium used). Considering this discussion, following the experiments with different volumes of medium, it could be observed that with the increase of the volume both the quantity of heat released by the fungal growth and the duration of the growth increased.

As observed in the microcalorimetric experiments with various types of bacteria, changes in the sample volume and the temperature at which the experiment is carried out produce changes during the fungal growth, in the intensity of the heat released (implicitly of the heat flux between the sample and the reference), but, in all experiments, the morphological pattern is preserved, underlining

the ability of microcalorimetric to identify a microorganism after its growth curve. Following the attached growth curves, an almost linear progression of the heat released depending on the incubation temperature could be observed.

For all increases, the microcalorimetric curve differs in terms of the quantitative parameters of the curves generated by the studied bacteria, the first peak being less well expressed for many of the investigated volume-temperature pairs, while the second peak was practically non-existent. We could see that *C. albicans* develops best at temperatures of 34 and 37°C. In all images, the most important increases are observed at 800 µL, but it should be taken into account that at 600 µL they are almost identical.

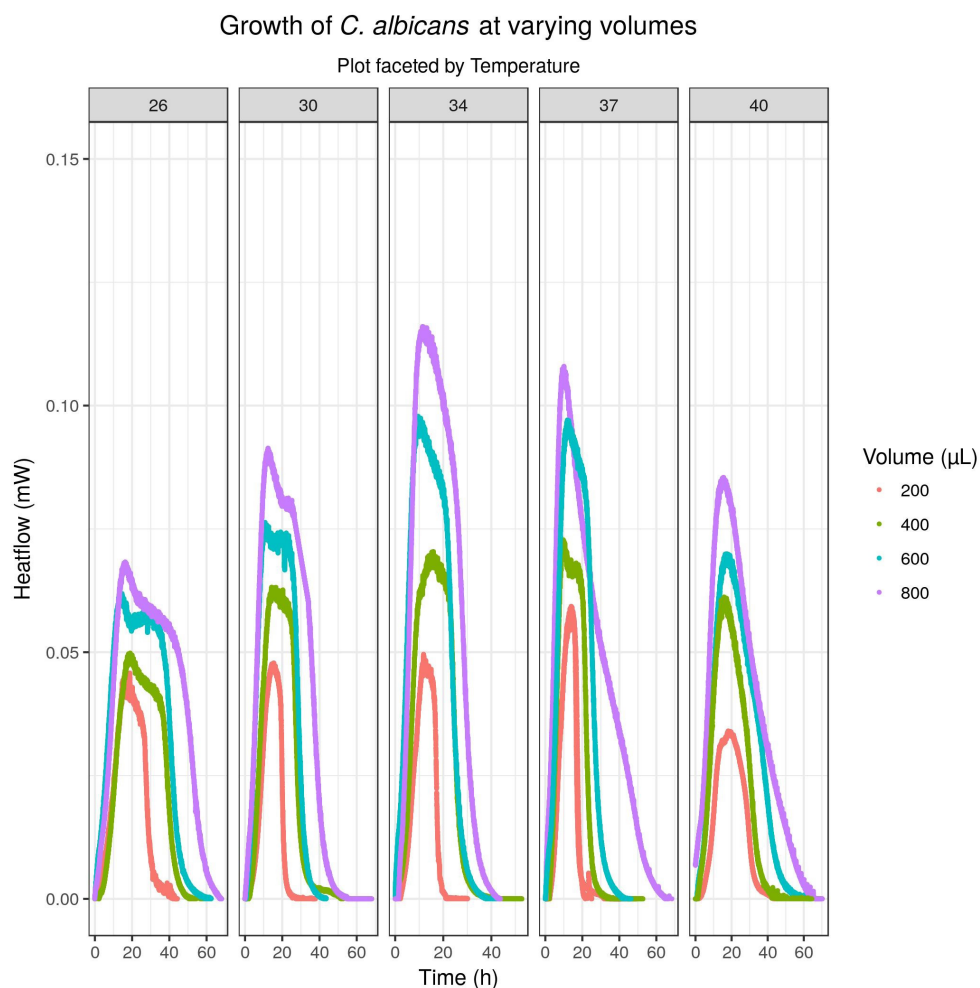


Fig. 1 Growth of *C. albicans* at different volumes

Discussions

Orthopedic infectious pathology, difficult to treat, is increasing due to the numerous microorganisms that develop resistance to the usual antibiotics. In most cases, this acquired resistance appears by administering certain classes of antibiotics without making the correct microbiological diagnosis (pathological product harvesting, correct and complete testing and without adapting the treatment to the identification and antibiogram results) [21]. Maybe the best-known case of antibiotic-resistant *Staphylococcus aureus* with particular problems in hospital units [22]. Another example is that of multi-resistant Gram-negative bacteria, where therapeutic options are becoming increasingly limited [23]. At present, many strains of multi-resistant *C. albicans* have not been described, but a major factor in the emergence of antifungal antibiotic resistance is the long time to obtain an antifungigram. In the described research, we demonstrated that, using microcalorimetry, it is possible to identify this fungus in half the time required for classical microbiological identification. In addition, as our team [24-26] has shown, there is the possibility of real-time antifungigram by using microcalorimetry.

Conclusions

Experiments undergone using *C. albicans* in different volumes of Sabouraud medium and at different temperatures showed the versatility of the method. These studies can help optimize the parameters of growth of fungal strains if the method can be widely used, both for identification and for determining susceptibility to antifungals.

Conflict of Interest statements

Authors state no conflict of interest.

Informed Consent and Human and Animal Rights statements

Informed consent has been obtained from all individuals included in this study.

Authorization for the use of human subjects

Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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