J Therm Anal Calorim (2014) 116:747–752
DOI 10.1007/s10973-013-3351-5

Differential scanning calorimetric measurement of cartilage destruction caused by Gram-negative septic arthritis

T. Sillinger · Dénes Lőrinczy · B. Kocsis · L. Kereskay · L. G. Nöt · N. Wiegand

Received: 15 May 2013 / Accepted: 23 July 2013 / Published online: 15 August 2013

Abstract The treatment of the bacterial arthritis of the joints is still a great challenge for orthopedic surgeons and rheumatologists. Aerobic Gram-negative bacteria are involved only in 20–25 % of cases. The inadequate therapy can cause cartilage destruction and can result in severe osteoarthritis of the affected joint. The aim of this study was to demonstrate and follow the destruction of the joints' hyaline cartilage by calorimetric method. We induced experimental septic arthritis in knee joints of seven New Zealand rabbits by a single inoculation of *Escherichia coli* ATCC 25922 culture (0.5 mL cc. $10^8 \pm 5$ % c.f.u.). The duration of this experiment was 7 days from the first to the last injection. After euthanizing the first subject, all other animals were given an overdose of anesthetics and samples were isolated from the cartilage of the femurs by surgical intervention for calorimetric measurements. The DSC scans clearly demonstrated the development of infective structural destruction in the cartilage from the first to the tenth day of incubation. In case of healthy control the melting temperatures (T_m) were: 57 and 63.1 °C and the total calorimetric enthalpy change (ΔH) was 0.37 J g^{-1}. After the third day, the enthalpy increased extremely (3.67 J g^{-1}), the two transition temperatures shifted toward lower temperature: 47.7 and 62.3 °C. At the fifth day, the effect of infection is culminated with $T_m = 62.2$ °C and a further elevation in ΔH (3.75 J g^{-1}). These results can indicate a dramatic change of the structure of rabbit cartilage between the third and fifth days. Therefore, the time elapsed seems to be critical and possesses clinical relevance, since by the sixth day, ΔH decreased to 2.6 J g^{-1} with a practically unchanged melting temperature. Between the sixth and tenth days, significantly increased melting temperatures (64.9 °C) were observed with decreased (3.38 J g^{-1}) calorimetric enthalpy. In conclusion, calorimetric measurements have been proven to be a reliable method in the measurement of cartilage destruction, caused by Gram-negative septic arthritis.

Keywords DSC · Rabbit cartilage · Septic arthritis · *Escherichia coli*

T. Sillinger
Orthopedie Landesklinikum Neunkirchen, Neunkirchen, Austria

D. Lőrinczy (✉)
Department of Biophysics, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti str. 12, Pécs, Hungary
e-mail: denes.lorinczy@aok.pte.hu

B. Kocsis
Department of Medical Microbiology and Immunology, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti str. 12, Pécs, Hungary

L. Kereskay
Department of Pathology, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti str. 12, Pécs, Hungary

L. G. Nöt · N. Wiegand
Department of Traumatology, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti str. 12, Pécs, Hungary

Introduction

Septic arthritis, also known as infectious or suppurative arthritis, may represent a direct invasion of joint space by various microorganisms, most commonly caused by a variety of bacteria. However, viruses, mycobacteria, and fungi have also been implicated in the pathomechanism. Failure to recognize and to appropriately treat septic arthritis results in significantly higher rates of morbidity and may even lead to death. The mortality rate depends primarily on the causative organism. *Neisseria gonorrhoeae*-caused septic arthritis

carries an extremely low mortality rate, whereas that of *Staphylococcus aureus* can approach 50 % [1]. Approximately 20,000 cases of septic arthritis occur in Europe each year (7.8 cases per 100,000 person years^{-1}) [2]. *N. gonorrhoeae* remains the most common pathogen (75 % of cases) among younger sexually active individuals [3], *S. aureus* infection is the cause of the vast majority of cases of acute bacterial arthritis in adults and in children older than 2 years [4]. The increased incidence of this pathogen parallels with the increased number of prosthetic joints and also, increased of the use of immunosuppressive agents. *S. aureus* represents the cause in 80 % of infected joints that are affected by rheumatoid arthritis. Streptococcal species, such as *Streptococcus viridans*, *S. pneumoniae* [5], and group B streptococci [6], account for 20 % of the cases.

Aerobic Gram-negative rods, like *Escherichia coli* or *Pseudomonas aeruginosa* are involved in 20–25 % of cases [7]. These bacteria can contaminate the open wounds of joints from the environment after an accidental trauma or during a surgical intervention. As Gram-negative bacteria, they produce endotoxic lipopolysaccharides. These toxic molecules can induce inflammation of the injured joints, fever, and immunologic reactions. Furthermore, they may lead to the development of endotoxic shock in septic patients. Most of these infections occur in young or in elderly patients [8]. Additionally, infections are related to diabetes, immune suppression, and intravenous abuse of drugs [9]. Polymicrobial joint infections (5–10 % of cases) and infections caused by anaerobic organisms (5 % of cases) are usually known as a result of traumas or abdominal infections.

Organisms may invade the joint by direct inoculation, by contiguous spread from the infected periarticular tissue, or most commonly via the route of the bloodstream [10]. The healthy joint has several protective components: synovial cells possess significant phagocyte activity and synovial fluid normally has significant bactericidal effect. The major consequence of bacterial invasion is the damage to articular cartilage. This may be due to the particular organism's pathologic properties, such as the chondrocyte proteases of *S. aureus*, as well as to the host's polymorphonuclear leukocytes response. The cells stimulate synthesis of cytokines and other inflammatory products, resulting in the hydrolysis of essential collagens and proteoglycans. As the destructive process continues, pannus formation begins, and cartilage erosion occurs at the lateral margins of the joint. Large effusions, which can occur in the infected joints, impair the blood supply and result in aseptic necrosis of bone. These destructive processes are well advanced as early as 3 days into the course of untreated infection [11].

The goal of the treatment involves elimination of the infection, decompression and restoration of the joint's function. In native joint infections, antibiotics are usually needed to be administered parenterally for at least 2 weeks [12]. The choice of the empirical antibiotic therapy is based on results of the Gram stain, the clinical symptoms, and background of the patient. The antibiotic therapy alone could not stop the cartilage destruction, but can decrease it [13, 14]. The other important factor in the treatment of the septic arthritis is the drainage of the joint. The choice of the type of drainage, whether percutaneous or surgical, has not yet been completely resolved [9, 15–17]. Surgical drainage is indicated when the appropriate choice of antibiotic and vigorous percutaneous drainage fails to clear the infection after 5–7 days or the adjacent soft tissue is infected. Routine arthroscopic lavage is rarely indicated. However, arthroscopic drainage can replace open surgical drainage. Using arthroscopic drainage, the surgeon can visualize the interior of the joint and clean it of pus, debris; adhesions can also be managed by arthroscopic technique. Publications regarding the beneficial effects of arthroscopic debridement usually do not focus on the cartilage destruction and the time elapsed since the infection started [18, 19].

The degeneration of human knee joint cartilage caused by osteoarthritis has already been described in the literature. In our previous studies, we investigated these degenerative changes of the cartilage with the help of calorimetry and we could determine the calorimetric standards of human hyaline cartilage [20, 21]. In our earlier experiments, besides describing the calorimetric standards of healthy cartilage of the rabbit knee [22], we investigated the thermodynamic features of the cartilage in experimentally induced osteoarthritis.

In the recent study, we induced experimental Gram-negative septic arthritis in the knee joints of seven New Zealand rabbits by single inoculation of *E. coli* ATCC 25922 culture (0.5 mL cc. $10^8 \pm 5$ % c.f.u.). We proved by calorimetry that the arthritis would arise sooner and hyaline cartilage destruction eventuates earlier than in the cases of *S. aureus*-induced experimental arthritis [23].

Materials and methods

Experimental protocol and sample preparation

All of the animal experiments were conducted in accordance with the current ethical regulations and approved by the ethical committee of the University of Pécs.

The *E. coli* ATCC 25922 strain was cultivated on Mueller–Hinton agar plate (CM 0337, Oxoid Ltd., UK) and controlled by biochemical tests [24]. For the inoculation experiments, the test strain was cultivated on Mueller–Hinton broth (CM 0405 Oxoid Ltd, UK) [25] overnight at 37 °C and washed in normal saline solution three times.

Concentration of the test strain was checked by a Perkin-Elmer spectrophotometer (A: 0.100 at 600 nm in a 1 mL cuvette) and the colony-forming units (c.f.u.) were determined by tube dilution method [26]: cc. $10^8 \pm 5$ % c.f.u. mL^{-1}. The volume of injected bacterial solution was 0.5 mL of the prepared solution.

Eight *New Zealand rabbits* (mean body mass: 2.5 kg) were kept under indifferent circumstances for 2 weeks prior to the experiments. Seven of the animals were subjected to 0.5 mL intra-articular injection of diluted *E. coli* ATCC 25922 strain into the left knee joint on the same day. One additional rabbit that was not subjected to any interventions served as a healthy Control. After the injection, the subjects were observed closely, the swelling of the joint as a clear sign of synovitis was registered. The injected animals were euthanized and femur cartilage samples were collected on the first, second, third, fifth, sixth, eighth, and tenth consecutive days after intra-articular injections.

At least four different, 2 × 2 mm-sized cartilage samples were collected from each of the examined joints. The synovial fluid collected from the infected joints was cultivated on blood agar plates to confirm the presence of the original inoculating *E. coli* ATCC 25922 strain. Samples collected for DSC measurements were stored on 4 °C in phosphate buffer and processed in 6 h. Samples taken for histological examinations were fixed in 4 % formaldehyde and analyzed later.

Histological examinations

Following proper fixation, decalcification with EDTA-containing solution was performed in 37 °C with an incubation period of minimum 2 days. Longitudinal and cross section slides were prepared and stained with hematoxylin–eosin. Samples were analyzed with light microscope and digital images of 100× and 200× original magnification of the slides were obtained by a Canon digital photo machine.

DSC measurements

The thermal denaturation of cartilage samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K min^{-1}. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments were between 15 and 40 mg. Phosphate buffer solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. The data analyses were performed with OriginPro 7.5 after ASCII conversion.

Results

Histological examination

On the slide taken from the control intact sample, smooth cartilage surface is shown with no inflammatory reaction (Fig. 1). At the end of the first day, the earliest histological changes start in the articular synovial membrane with cuffing of the vessels, granulocyte emigration, edema, and hemorrhage. In the next step, this process turns into a dense polymorphonuclear granulocytic infiltration and spread of the inflammation toward cartilage surface occurs, mainly in the second day. On the third day the previously smooth surface of the articular cartilage becomes irregular and the first slight erosion occurs (Fig. 2). While the granulocytic inflammation spreads from the marginal into the central area of the cartilage, granulocytes cover and then invade the cartilage, forming first microscopically, later, on the fourth and fifth day larger abscesses. Parallel with the inflammation, on the second and third day, bone necrosis starts with the presence of aseptic and later septic type with granulocytes. By the fifth day, an abscess with bone destruction developed inside the bone (Fig. 3). After the fifth day, some reparation starts with capillary emigration and granulation tissue formation. However, due to the lack of proper therapy, it was not able to completely stop the progression of the process.

In the period of observation, after inoculation, not only the behavior of rabbits have changed, but also the swelling

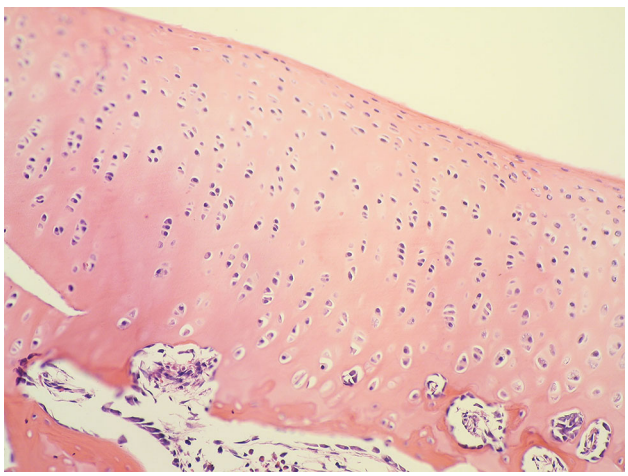

Fig. 1 Normal control with intact, smooth cartilage surface. No inflammatory reaction is visible

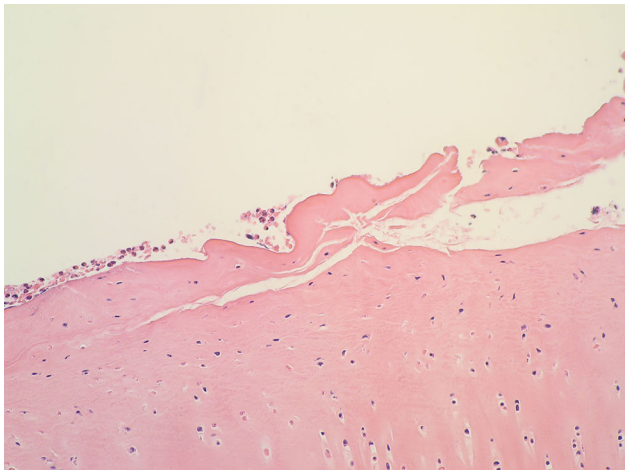

Fig. 2 Early (third day) phase of inflammation with erosion and irregularity of cartilage surface. An overlaying granulocytic inflammatory reaction is visible as well

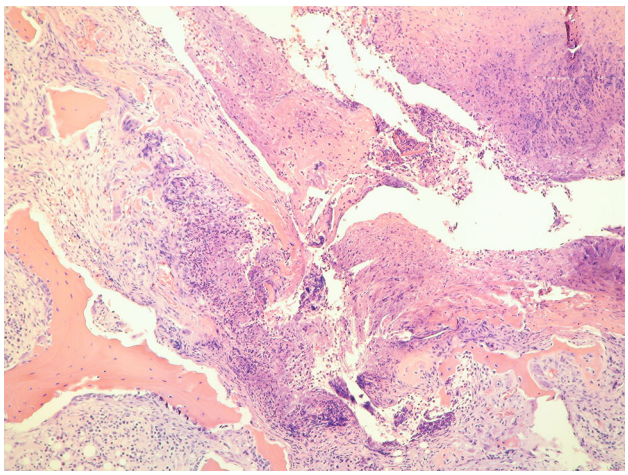

Fig. 3 Advanced (fifth day) phase of inflammation: inside the bone an abscess is visible with bone destruction, the tissue is rich in granulocytes

of the knee joint developed soon, within 23 (16.5–28) h in average.

DSC measurements

The thermal characteristics and thermal denaturation curves of rabbit cartilages after experimentally induced arthritis are shown in Fig. 4 and Table 1.

On the DSC scan of healthy controls, similar to our previous data [23], we could separate at least two big melted domains with 57 and 63.15 °C melting temperatures (T_m) and a 0. 375 J g^{-1} total calorimetric enthalpy change (ΔH). The main transition had a wide $T_{1/2}$ (transition temperature interval at the half of melting temperature), which are the signs of a low degree of cooperation of those structural elements that melt in this range.

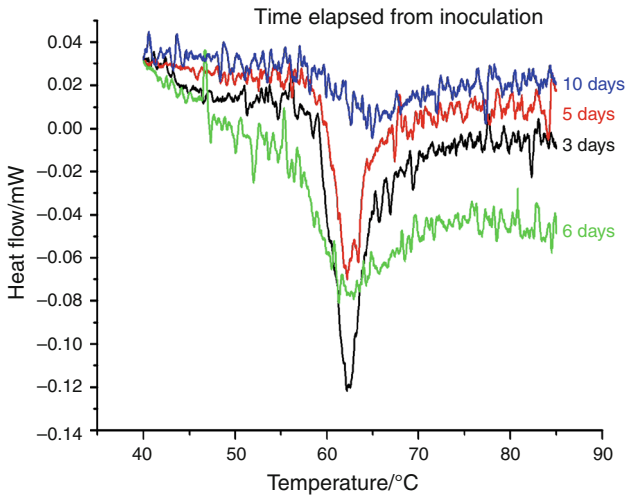

Fig. 4 Thermal denaturation curves of rabbit cartilages with experimentally induced arthritis

Table 1 The changes of melting temperatures/T_m and calorimetric enthalpy/ΔH of healthy and infected rabbit cartilages in correlation of the elapsed time of inoculation

Days after inoculation	T_m/°C		ΔH/J g^{-1}
Control	57.1	63.1	0.37
1	**54.2**	**62.8**	**0.85**
2	**52.1**	**62.4**	**1.24**
3	**47.7**	**62.3**	**3.67**
5	–	62.2	**3.75**
6	**–**	**62.5**	**2.62**
8	–	**63.8**	**3.25**
10	–	**64.9**	**3.38**

Bold values refer the remarkable deviation from the healthy one

In the infected cases, after the injection from the first day, the macroscopically observed swelling of the knee joint appeared as highly noisy DSC scans, compared to our previously published results [23]; indicating that the pronounced structural changes of the affected subunits were caused by the infection (Fig. 4).

On the first and second days, the melting endotherms were decreased and the calorimetric enthalpies were increased moderately. On the third day, the two melting endotherms were shifted down to 47.7 and 62.3 °C, while ΔH increased roughly tenfold (3.67 J g^{-1}). On the fifth day, the effect of infection is culminated with $T_m = 62.2$ °C, while the calorimetric enthalpy was $\Delta H = 3.75$ J g^{-1}; indicating a dramatic change of the structure of rabbit cartilage between the third and fifth days.

Importantly, by the sixth day, the ΔH further decreased to 2.6 J g^{-1}, meanwhile the melting temperature remained unchanged (Table 1). From the sixth to tenth day, the T_m increased up to 64.9 °C and the calorimetric enthalpy to 3.38 J g^{-1}.

Discussion

Here we have demonstrated for the first time that in response to Gram-negative bacterial infection, the destruction of hyaline cartilage develops early, during the first few days and this destruction can be measured by calorimetric examinations.

Calorimetric examinations have been recently utilized in a variety of different studies, evaluating changes of thermal characteristics of human connective tissue and cartilage samples [23, 27]. Furthermore, our workgroup has successfully utilized DSC scan to study the local and global changes in globular and fibrous actin following the nucleotide exchange [28, 29].

In our recent studies, thermal characteristics of human hyaline cartilage [20, 21] and calorimetric standards of healthy rabbit knee cartilage have already been described. Additionally, calorimetric analyses has been proven to be a useful tool in the analyses of cartilage destruction in an experimental model of septic arthritis [23].

With special importance, here we have found a marked change in the thermal characteristic of infected cartilage, occurring between the third and fifth day. It could indicate that the time elapsed between the third and the sixth day is the critical period in the development of Gram-negative bacteria-caused experimental septic arthritis.

It is likely that this period represents the "turning point," when the structural changes become irreversible. Meanwhile, the remaining time indicates the last stage of the development of experimental arthritis.

However, a possible limitation of our results is that no medical treatment was applied; therefore, we were unable to evaluate whether the hyaline destruction is reversible or not. To further explore the tissue degradation caused by infection, more experiments are needed, including histological examinations. Furthermore, additional examinations are necessary to determine the time span after which the intra-articular septic process becomes irreversible and consequentially, irresponsive to drugs or surgical interventions.

Conclusions

Our results indicate that DSC scan could be a useful, additional diagnostic method in the determination of clinical stages of cartilage destruction in Gram-negative septic arthritis. These findings underline the importance of the early treatment of infected joints, which might prevent the irreversible damage of the hyaline cartilage that can later cause osteoarthritis.

References

1. Kaandorp CJ, Krijnen P, Moens HJ, Habbema JD, Shaandenburg D. The outcome of bacterial arthritis: a prospective community-based study. Arthritis Rheum. 1997;40:884–92.
2. Goldenberg DL, Cohen AS. Acute infectious arthritis. A review of patients with nongonococcal joint infections (with emphasis on therapy and prognosis). Am J Med. 1976;60:369–77.
3. McGuire NM, Kauffman CA. Septic arthritis in the elderly. J Am Geriatr Soc. 1985;33:170–4.
4. Smith JW, Piercy EA. Infectious arthritis. Clin Infect Dis. 1995;20:225–30.
5. Raad J, Peacock JE Jr. Septic arthritis in the adult caused by *Streptococcus pneumoniae*: a report of 4 cases and review of the literature. Semin Arthritis Rheum. 2004;34:559–69.
6. Corvec S, Illiaquer M, Touchais S, Boutoilee D, Van dre Mee-Marquet N, Quentin R, Reynaud A, Lepelletier D, Berner P. Clinical features of group B Streptococcus prosthetic joint infections and molecular characterization of isolates. J Clin Microbiol. 2011;49:380–2.
7. Ohl CA. Infectious arthritis of native joints (Chapter 102). In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 7th ed. Edinburgh: Elsevier; 2010. p. 1443–56.
8. Margaretten ME, Kohlwes J, Moore D, Bent S. Does this adult patient have septic arthritis? JAMA. 2007;297:1478–88.
9. Manadan AM, Block JA. Daily needle aspiration versus surgical lavage for the treatment of bacterial septic arthritis in adults. Am J Ther. 2004;11:412–5.
10. Rice PA. Gonococcal arthritis (disseminated gonococcal infection). Infect Dis Clin North Am. 2005;19:853–61.
11. Jerosch J, Hofstetter I, Schröder M, Castro WH. Septic arthritis: arthroscopic management with local antibiotic treatment. Acta Orthop Belgica. 1995;61:126–34.
12. Wise CM, Morris CR, Wasilauskas BL, Salzer WL. Gonococcal arthritis in an era of increasing penicillin resistance. Presentations and outcomes in 41 recent cases (1985–1991). Arch Intern Med. 1994;154:2690–5.
13. Riegels-Nielsen P, Frimodt-Moller N, Sorensen M, Jensen JS. Antibiotic treatment insufficient for established septic arthritis. *Staphylococcus aureus* experiments in rabbits. Acta Orthop Scand. 1989;60:113–5.
14. Smith RL, Kajiyama G, Schurman DJ. Staphylococcal septic arthritis: antibiotic and nonsteroidal anti-inflammatory drug treatment in a rabbit model. J Orthop Res. 1997;15:919–26.
15. Garcia-De La Torre I. Advances in the management of septic arthritis. Rheum Dis Clin North Am. 2003;29:61–75.
16. Koeppe J, Johnson S, Morroni J, Siracusa-Rick C, Armon C. Suppressive antibiotic therapy for retained infected prosthetic joints: case series and review of the literature. Infect Dis Clin Pract. 2008;16:224–9.
17. Broy SB, Schmid FR. A comparison of medical drainage (needle aspiration) and surgical drainage (arthrotomy or arthroscopy) in the initial treatment of infected joints. Clin Rheum Dis. 1986;12:501–22.
18. Ivey M, Clark R. Arthroscopic debridement of the knee for septic arthritis. Clin Orthop Relat Res. 1985;199:201–6.
19. Jackson RW, Parsons CJ. Distention–irrigation treatment of major joint sepsis. Clin Orthop Relat Res. 1973;96:160–4.
20. Than P, Vermes C, Schäffer B, Lőrinczy D. Differential scanning calorimetric examination of human hyaline cartilage. A preliminary study. Thermochim Acta. 2000;346:147–51.
21. Than P, Domán I, Lőrinczy D. Differential scanning calorimetry in the research of degenerative musculoskeletal disorders. Thermochim Acta. 2004;415:83–7.

22. Than P, Lőrinczy D. Differential scanning calorimetric examination of the osteoarthritic hyaline cartilage in rabbits. Thermochim Acta. 2003;404:149–53.
23. Sillinger T, Than P, Kocsis B, Lőrinczy D. Differential scanning calorimetric measurement of cartilage destruction caused by septic arthritis. J Thermal Anal Calorim. 2005;82:221–3.
24. Enterobacteriaceae (Chapter 6). In: Washington CW Jr, editor. Koneman's Color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 211–302.
25. Bridson EY. The oxoid manual, vol. 2. Basingstoke: Oxoid Ltd.; 2006. p. 252–5.
26. Czirók É. Handbook of clinical and epidemiological bacteriology. Budapest: American Society for Microbiology; 1999. p. 144.
27. Nőt LG, Naumov I, Vámhidy L, Lőrinczy D, Wiegand N. Comparison of thermal characteristics of degenerated and inflamed human collagen structures with differential scanning calorimetry. J Thermal Anal Calorim. 2013;113:273–9.
28. Türmer K, Könczöl F, Lőrinczy D, Belágyi J. AMP.PNP affects the dynamical properties of monomer and polymerized actin. A DSC and an EPR study. J Thermal Anal Calorim. 2012;108:95–100.
29. Könczöl F, Belagyi J, Lőrinczy D. Thermal and dynamic behaviour of the actin monomer in case of different cations. J Thermal Anal Calorim. 2013;111:1717–23.