J Therm Anal Calorim (2012) 109:783–787
DOI 10.1007/s10973-012-2254-1

Differential scanning calorimetric examination of the interfacial membrane in failed hip joint replacements

I. Naumov · D. Lőrinczy · L. Vámhidy · P. Than · N. Wiegand

Bretsznajder Special Chapter

Abstract Aseptic and septic periprosthetic osteolysis following total hip arthroplasty has become increasingly recognized as a major clinical problem. An aggressive granulomatous tissue, the interfacial membrane, develops at the interface between the bone and the prostheses or the bone and the cement. Our hypothesis was that during the septic and aseptic loosening of the total hip arthroplasty, there is a clear pathological abnormality in the tissue elements building up the interfacial membrane, which is responsible for the different aetiologies of the disease and could be monitored besides the classical methods by differential scanning calorimetry. In our study, the interfacial membrane pieces removed during operations of revision hip arthroplasties in the cases of aseptic loosening and during prosthesis removals in the cases of septic implant loosening. We investigated stem parts of cemented hip arthroplasties only. Our measurements were carried out on eight septic and 12 aseptic samples. With our investigations, we could demonstrate that DSC is a useful and well-applicable method for the investigation of the interfacial membrane that develops in septic and aseptic loosening of hip arthroplasty. DSC scans clearly demonstrated significant differences between the different types and conditions of samples (aseptic membrane: $T_m = 62.2$ °C and $\Delta H_{cal} = 2.13$ J/g, septic membrane: $T_m = 60.2$ °C and $\Delta H_{cal} = 3.22$ J/g,). These investigations can help us make a correct diagnosis in the problematic cases of loosened total hip arthroplasty. To decide the possible septic feature of a given sample, calorimetry could serve as one of the quickest procedures available.

Keywords Periprosthetic osteolysis · Interfacial membrane · Hip arthroplasty · DSC

I. Naumov · L. Vámhidy · P. Than · N. Wiegand
Department of Musculoskeletal Surgery, University of Pécs, Pecs, Hungary

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

Background

Prosthesis loosening

Total hip arthroplasty is a final solution for millions of patients with end-stage hip joint arthritis. In contrast to the benefits of joint replacement, aseptic and septic periprosthetic osteolysis following total hip arthroplasty has become increasingly recognized as a major clinical problem in both cemented and cementless reconstructions. The destruction of bone around joint replacements is an adverse biological response associated with the generation of excessive wear particles. Wear can be a consequence of micro motions at the interface between implant and bone cement. Wear debris from the materials used for joint replacements stimulate a chronic inflammatory and foreign body reaction that leads to increased osteoclast differentiation and maturation and decreased bone formation [1]. An aggressive granulomatous tissue, the interfacial membrane, develops at the bone/prostheses or bone/cement interface is shown in Fig. 1. This membrane predominantly consists of fibroblasts, aggregates of macrophages and foreign body giant cells. The particulate wear debris from prosthetic materials and bone cement are phagocytized by fibroblasts of the interfacial membrane, and then these cells produce inflammatory mediators and proteolytic enzymes to

provoke a cascade of osteolytic events [2–5]. The released cytokines induce the human osteoblasts towards particulate debris; the osteoblasts increase the levels of interleukine (IL)-6 and IL-8. Furthermore, the de novo synthesis of type I collagen is reduced and the expression of the matrix metalloproteinase (MMP)-1 is considerably increased [6]. Instead of fibroblast and osteoblast cells, the synovial tissue-infiltrating natural killer immune cells play a central role in degenerative joint disease and particle-mediated periprosthetic osteolysis too [7].

Diagnosis

Differential diagnosis of a painful hip arthroplasty is a complex problem. Osteolytic changes may be caused by numerous pathologic processes, including periprosthetic osteolysis, infection, metabolic disease and neoplasia. Careful evaluation of standard roentgenograms should always be performed and possibly integrated with imaging modalities, such as CT, MRI and bone scintigraphy to increase diagnostic accuracy. The typical radiographic feature of osteolysis is a radiolucent area adjacent to an implant, sometimes associated with a soft tissue mass [8–10]. If uncertainty remains, then joint aspiration should always be considered to exclude infection. Synovial white blood cell count, C-reactive protein, Interleukin-6 and erythrocyte sedimentation rate are useful for diagnosing periprosthetic infections, but the sensitivity and specificity of these tests in the early postoperative period remain unknown as hemarthrosis, and postoperative inflammation may render standard cutoff values inaccurate [11, 12]. Successful treatment of an infected joint arthroplasty depends on identifying the responsible pathogens correctly. The combination of tissue biopsy and joint aspiration provides improved sensitivity and accuracy in the diagnosis of total joint infection [13].

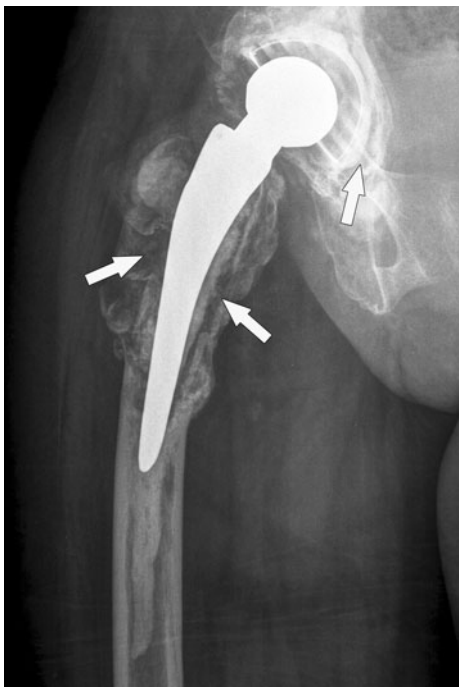

Fig. 1 Conventional X-ray of a loosened total hip arthroplasty. The *arrows* show the osteolytic areas

Treatment

Aseptic loosening of implants is the most common complication of hip replacement and represents an increasing problem because of still rising numbers of primary arthroplasties. An average rate of aseptic loosening of total hip arthroplasty is 23.1% for cemented procedures [14]. The surgical management of this problem is well known. A revision hip prosthesis implantation with or without cement is typically recommended. The average survival of these revision prostheses is 92% at 10 years [15].

Periprosthetic infection following total hip replacement can be a catastrophic complication for the patient. When possible, the preferred treatment approach is an insertion of another prosthesis. The treatments available include single-stage exchange, and two-stage exchange [16]. A single-stage procedure, debridement with component retention, is an attractive solution. Success rates in the literature vary widely (18–90%) according to the patient selection criteria [17]. A two-stage reimplantation with an antibiotic-impregnated spacer is a much safer and widely used method; the average survival of this technique is over 83% [18–20].

Aim of the study

Our hypothesis was that, during the septic and aseptic loosening of total hip arthroplasty, there is a clear pathological abnormality in the tissue elements building up the interfacial membrane, which is formed in both forms of loosening. Besides examining the aseptic interfacial membrane with differential scanning calorimetry (DSC), we planned to carry out investigations of membrane destruction caused by septic complications. A calorimetric examination of this type has not yet been carried out on international level.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the aseptic and septic interfacial membrane. With our measurements, we wanted to confirm significant differences between calorimetric results of septic and aseptic origin interfacial membranes.

Earlier examinations have demonstrated that DSC is a useful and well-applicable method for the demonstration of thermal consequences of local and global conformational changes in the organs of the musculoskeletal system. Different authors have demonstrated thermal effects of degenerative

processes in various human tissue samples [21–29]. Than et al. [30, 31] demonstrated clear differences between the various anatomical origins of the cartilage as well as intact and osteoarthritic samples with the changes in total enthalpy and heat capacity, as well as by the shape of DSC scans .

Materials and methods

Sample preparation

The pathologic human samples serving as a basis for research were derived from the tissue fragments taken during operations and considered to be a waste material. Such were the interfacial membrane pieces removed during operations of revision hip arthroplasties in the cases of aseptic loosening and during prosthesis removals in the cases of septic implant loosening. We investigated stem parts of cemented hip arthroplasties only, and so interface membranes were harvested from femoral bone/cement interface. The shape of the samples was cubic with 5-mm length and contained the full thickness of the membrane without any bone tissue. Samples were then put into sterile physiologic saline solution. All the individual samples were stored separately at 4 °C, not longer than 6 h.

Our measurements were carried out on 8 septic and 12 aseptic samples. The six male and 14 female patients were between the age of 65 and 75. The aseptic loosening of the prosthesis was proven using X-ray or CT investigations and scintigraphy. In the cases of septic complications, we proved the loosening of the prosthesis with radiography, scintigraphy, CT imaging and aspiration.

DSC measurements

The pieces of different samples have been prepared and measured within 6 h of removal. The thermal denaturation of different parts of human 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. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (samples plus buffer) on average. Typical sample wet masses for calorimetric experiments were in-between 100 and 200 mg. Sterile physiologic saline 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 for 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 using two-point setting SETARAM peak integration. The data treatment after ASCII conversion was done by OriginPro 7.5.

Statistical analysis

The data in this study were evaluated with the Student paired t test. Significance was set at $p < 0.05$.

Results and discussion

According to our knowledge, this study is the first in the line of interfacial membrane research that used a thermal analytical method. The aim of our study was to compare the thermal parameters of the septic and aseptic interfacial membrane, and to identify any discrepancy or conformity of the results from different origins of the prosthesis loosening.

The thermal parameters of the septic and aseptic interfacial membranes are absolutely different from those shown in Table 1. We proved significant difference between the calorimetric enthalpy of septic and aseptic samples: $p = 0.005$. There was no significant difference between the average main melting temperature of different groups: $p = 0.40$.

The aseptic samples showed a single endotherm denaturation with a wide $T_{1/2}$, while the septic ones exhibited two endotherms with a smaller $T_{1/2}$ Fig. 2. It could be the sign of more severe damage of the tissues, reduced synthesis of type 1 collagen, decreased fibroblast proliferation and appearance of an aggressive septic granulomatous tissue. These consequences are supported by the thermodynamic data:

- The calorimetric enthalpy in the aseptic group was on average ~2 J/g (normalised on wet sample mass), while in the septic group, it was significantly higher (~3.2 J/g).
- The average main melting temperature was 62.2 °C in the aseptic group and ~60 °C in case of septic one.
- We observed a more pronounced heat capacity change between native and denatured states in case of septic probes, which could be explained by the structural alterations caused by different biological and biochemical processes during prosthesis loosening.

With our investigations, we could demonstrate that DSC is a useful and well-applicable method for the investigation

Table 1 The characteristic thermal parameters of the denaturation of the septic and aseptic interfacial membrane samples

Sample	T_m/°C Mean ± SD	ΔH_{cal}/J/g^{-1} Mean ± SD
Aseptic ($n = 12$)	62.2 ± 0.2	2.13 ± 0.04
Septic ($n = 8$)	60.2 ± 0.3	3.22 ± 0.04

T_m melting temperature, ΔH_{cal} calorimetric enthalpy

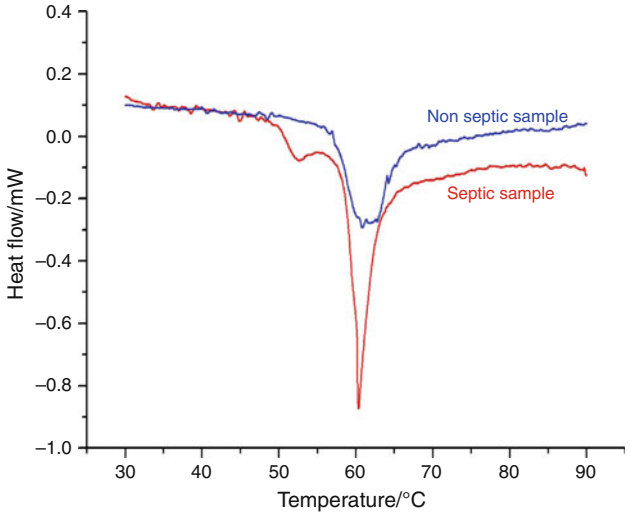

Fig. 2 Thermal denaturation scans of septic and aseptic interfacial membrane

of the interfacial membranes developed during septic and aseptic loosening of hip arthroplasty. The pathological structural changes of the interfacial membrane can satisfactorily follow this method. These investigations could help us make correct diagnosis in the problematic cases of loosened total hip arthroplasty. To verify the septic feature of a given sample, calorimetry could serve as one of the quickest procedures available.

Acknowledgements The SETARAM Micro DSC-II was purchased thanks to a grant (CO-272) from the Hungarian Scientific Research Fund (Dénes Lőrinczy).

References

1. Goodman SB, Ma T. Cellular chemotaxis induced by wear particles from joint replacements. Biomaterials. 2010;31:5045–50.
2. Glant TT, Jacobs JJ, Mikecz K, Yao J, Chubinskaja S, Williams JM, Urban RL, Shanbhag AS, Lee SH, Sumner DR. Particulate-induced, prostaglandin- and cytokine-mediated bone resorption in an experimental system and in failed joint replacements. Am J Ther. 1996;3:27–41.
3. Yao J, Glant TT, Lark MW, Mikecz K, Jacobs JJ, Hutchinson NI, Hoerrner LA, Kuettner KE, Galante JO. The potential role of fibroblasts in periprosthetic osteolysis: fibroblast response to titanium particles. J Bone Miner Res. 1995;10:1417–27.
4. Koreny T, Tunyogi-Csapó M, Gál I, Vermes C, Jacobs JJ, Glant TT. The role of fibroblasts and fibroblast-derived factors in periprosthetic osteolysis. Arthritis Rheum. 2006;54:3221–32.
5. Tunyogi-Csapo M, Koreny T, Vermes C, Galante JO, Jacobs JJ, Glant TT. Role of fibroblasts and fibroblast-derived growth factors in periprosthetic angiogenesis. J Orthop Res. 2007;25:1378–88.
6. Lochner K, Fritsche A, Jonitz A, Hansmann D, Mueller P, Mueller-Hilke B, Bader R. The potential role of human osteoblasts for periprosthetic osteolysis following exposure to wear particles. Int J Mol Med. 2011;28:1055–63.
7. Huss RS, Huddleston JI, Goodman SB, Butcher EC, Zabel BA. Synovial tissue-infiltrating natural killer cells in osteoarthritis and periprosthetic inflammation. Arthritis Rheum. 2010;62:3799–805.
8. Miller TT. Imaging of hip arthroplasty. Semin Musculoskelet Radiol. 2006;10:30–46.
9. Cahir JG, Toms AP, Marshall TJ, Wimhurst J, Nolan J. CT and MRI of hip arthroplasty. Clin Radiol. 2007;62:1163–71.
10. Fabbri N, Rustemi E, Masetti C, Kreshak J, Gambarotti M, Vanel D, Toni A, Mercuri M. Severe osteolysis and soft tissue mass around total hip arthroplasty: description of four cases and review of the literature with respect to clinico-radiographic and pathologic differential diagnosis. Eur J Radiol. 2011;77:43–50.
11. Bedair H, Ting N, Jacovides C, Saxena A, Moric M, Parvizi J, Della Valle CJ. The Mark Coventry award: diagnosis of early postoperative TKA infection using synovial fluid analysis. Clin Orthop Relat Res. 2011;469:34–40.
12. Buttaro MA, Tanoira I, Comba F, Piccaluga F. Combining C-reactive protein and interleukin-6 may be useful to detect periprosthetic hip infection. Clin Orthop Relat Res. 2010;468:3263–7.
13. Meermans G, Haddad FS. Is there a role for tissue biopsy in the diagnosis of periprosthetic infection? Clin Orthop Relat Res. 2010;468:1410–7.
14. Wirtz DC, Niethard FU. Etiology, diagnosis and therapy of aseptic hip prosthesis loosening: a status assessment. Z Orthop Ihre Grenzgeb. 1997;135:270–80.
15. Jaiswal A, Gilbert RE, Sunil Kumar KH, Carrothers AD, Kuiper JH, Richardson JB. Function and survival after revision of hip resurfacing. Hip Int. 2011;21:610–5.
16. Oussedik SI, Dodd MB, Haddad FS. Outcomes of revision total hip replacement for infection after grading according to a standard protocol. J Bone Joint Surg Br. 2010;92:1222–6.
17. Klouche S, Lhotellier L, Mamoudy P. Infected total hip arthroplasty treated by an irrigation-debridement/component retention protocol. A prospective study in a 12-case series with minimum 2 years' follow-up. Orthop Traumatol Surg Res. 2011 Mar 7. Epub.
18. Sharma AK, Gioe TJ, Nelson TE. Two-stage exchange for infected resurfacing arthroplasty: use of a novel cement spacer technique. J Arthroplasty. 2011;26:976–8.
19. Engesæter LB, Dale H, Schrama JC, Hallan G, Lie SA. Surgical procedures in the treatment of 784 infected THAs reported to the Norwegian arthroplasty register. Acta Orthop. 2011;82:530–7.
20. Hofmann AA. Two-stage exchange is better than direct exchange in the infected THA. Orthopedics. 1999;22:918–22.
21. Gazsó I, Kránicz J, Bellyei Á, Lőrinczy D. DSC analysis of the abnormalities of human leg skeletal muscles. A preliminary study. Thermochimica Acta. 2003;402:117–22.
22. 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.
23. Pintér C, Bognár G, Horváth B, Sydo T, Ligeti E, Pulai J, Lőrinczy D. DSC analysis of human fat tissue in idiopathic avascular necrosis of the femoral head– a preliminary study. J Thermal Anal Calorim. 2009;95:781–5.
24. Benkő L, Danis J, Hubmann R, Kasza G, Gömöri É, Rőth E, Lőrinczy D. DSC examination of the esophagus after implication of special stents, designed for the management of acute esophagus variceal bleeding. An experimental study. J Thermal Anal Calorim. 2009;95:763–8.
25. Bognár G, Szabó I, Pintér C, Ligeti E, Lőrinczy D. Changes in thermal denaturation properties of the long head of the biceps during lifetime. J Thermal Anal Calorim. 2010;102:65–8.
26. Wiegand N, Vámhidy L, Lőrinczy D. Differential scanning calorimetric examination of ruptured lower limb tendons in human. J Thermal Anal Calorim. 2010;101:487–92.
27. Sohár G, Pallagi E, Szabó-Révész P, Tóth K. New thermogravimetric protocol for the investigation of normal and damaged human hyaline cartilage. J Thermal Anal Calorim. 2007;89:853–6.

28. Tóth K, Sohár G, Aigner Z, Greksa F, Szabó-Révész P. Novel calorimetric properties of human cartilage samples in rheumatoid arthritis. J. Thermal Anal Calorim. 2009;5:813–5.
29. Tóth K, Sohár G, Pallagi E, Szabó-Révész P. Further characterization of degenerated human cartilage with differential scanning calorimetry. Thermochimica Acta. 2007;464:75–7.
30. Than P, Lőrinczy D. Differential scanning calorimetric examination of the osteoarthritic hyaline cartilage in rabbits. Thermochimica Acta. 2003;404:149–53.
31. Than P, Vermes C, Schäffer B, Lőrinczy D. Differential scanning calorimetric examination of the human hyaline cartilage: a preliminary study. Thermochimica Acta. 2000;346:147–51.