Calorimetric examination of hip pseudo-capsule after secondary hip surgeries

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Abstract

During revision hip surgeries, a soft pseudo-capsule is formed around the artificial joint. The roles of the pseudo-capsule are to protect and to stabilize the joint. Based on intraoperative observation, we have found that different types of pseudo-capsules have different thickness and elasticity, compared to the primary joint capsule. The purpose of the study was to evaluate and identify the different types of pseudo-capsules, based on thermal characteristics and histological examinations. The pseudo-capsule samples were collected during aseptic hip revision ($n = 3$), septic hip revision ($n = 2$) and during revision surgery after antibiotic-impregnated spacer implantation ($n = 3$). The thermal characteristics (maximal denaturation temperature, T_m , and calorimetric enthalpy change, ΔH_{cal}) were determined from the measurements made by SETARAM Micro DSC-II calorimeter between 37° and 90°C with 0.3 K min⁻¹ heating rate. The denaturation scans of different pseudo-capsule samples have clearly demonstrated specific, representative changes, comparing samples with aseptic and septic origins. These changes were confirmed by histological evaluations; therefore, DSC could be a useful tool in the evaluation of hip pseudo-capsules with different origins, providing further help in the differential diagnostics of septic conditions.

Keywords Hip replacement · Revision · Pseudo-capsule · Sepsis · Collagen · Calorimetry

Introduction

During the recent decades, there has been a continuous worldwide growth in the number of hip replacements. By the twenty-first century, due to the developments in surgical techniques, hip replacements based on both orthopedic and trauma indications became safer and available for more patients. The surgical procedures utilize more types of prosthesis and, importantly, better surgical approaches [1–3].

The different types of surgical approaches allow preserving the joint capsule (capsulotomy), instead of removal (capsulectomy) during hip arthroplasties. The preservation of joint capsule could provide more primary stability, therefore further decreasing the rate of hip dislocations during the postoperative 6–12 weeks.

Months or years after the primary hip replacements utilizing capsulectomy, a pseudo-capsule develops around the implants. The pseudo-capsule found during secondary hip surgeries has different characteristics, compared to the primary joint capsule [4–6]. Furthermore, the pseudo-membrane macroscopically seems to be scar tissue, but the elasticity and thickness may be different in case of aseptic and septic hip conditions.

The differential scanning calorimetry has been found useful in the differential diagnosis of various musculoskeletal diseases, including degenerative, inflammatory and septic conditions [7–13].

Therefore, the purpose of this study was to evaluate and identify the different types of pseudo-capsules, based on thermal characteristics and histological examinations.

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Materials and methods

Sample collection

The $4 \times 4 \times 20$ -mm-sized sample of the anterior part of the pseudo-capsule was resected during secondary hip replacements, which was performed using DAA (direct anterior approach). Then, samples were properly stored for further measurements, as described in details elsewhere [14]. The pseudo-capsule samples were obtained from patients who underwent revision surgery: (1) due to aseptic loosening ($n = 3$), (2) due to septic loosening ($n = 2$) or (3) after antibiotic-impregnated spacer insertion ($n = 3$). These samples were considered as waste from the point of surgery. In case of septic loosening, increased levels of inflammatory parameters, such as leukocytes and CRP (C-reactive proteins), were detected. In each of the cases, the presence of bacteria was confirmed by microbiological culturing, samples collected from multiple points from the surface of the removed implant and the medullar cavity.

The study protocol has been prepared in accordance with the ethical guidelines of the 2003 Declaration of Helsinki. After receiving permission from the Local Ethics Committee, patients or nearest relatives provided a signed informed consent after they were informed clearly about details of the study and joint capsule sampling.

Histological processing

The samples from the pseudo-capsules were fixed in 4% formaldehyde for at least 2 days. After fixation, two representative sections from each sample were cut. Then, the tissues were dehydrated and embedded in paraffin according to the standard histopathological processing and 4- μ m-thick sections were cut by microtome. The slides were stained with hematoxylin–eosin.

Thermal analysis

We have investigated the thermal denaturation of pseudo-capsule samples by a SETARAM Micro DSC-II calorimeter. The analysis was made between 0 and 100 °C with a heating rate of 0.3 K min^{−1}. Conventional Hastelloy batch vessels ($V_{\max} = 1$ mL) were used for the experiment. The average sample size was $4 \times 4 \times 20$ mm. Normal saline was used as a reference. The reference and sample vessels were equilibrated with a precision of ± 0.1 mg; this way we did not have to do any correction between vessels' heat capacity. With the help of a two-point setting SETARAM peak integration, calorimetric enthalpy change was calculated from the area under the heat absorption curve; then, the results (denaturation or melting temperature (T_m = temperature, where 50%

of the heated sample will be denatured) and calorimetric enthalpy change (ΔH_{cal}) data of samples) were compared.

Results

Histological evaluation

The main characteristics of the samples were analyzed, such as collagen and fat ratio, absence or presence and severity of inflammation, absence or presence of destructed bone fragments and additional changes.

The samples from the patients with aseptic loosening were invariably cell-poor collagen-rich tissues without the presence of inflammation. In some cases, embedded bone fragments or pigment was seen (Fig. 1).

The samples from the patients with septic loosening have shown moderate hyper-cellularity with fibroblasts, macrophages and focal hyper-cellular inflammatory areas which located frequently in the perivascular area. In these foci and in the interstitium, mainly lymphocytes, macrophages and scattered neutrophils are seen (Fig. 2a–b).

In the histological samples from those patients who underwent antibiotic-impregnated spacer insertion again, mainly cell-poor collagenous tissue is visible; in one sample, increased fat content was detected. No significant inflammation was exhibited (Fig. 3).

DSC measurements

The result of denaturation of hip pseudo-capsules is shown in Fig. 4. The obtained curves show that the half width ($T_{1/2}$) of transitions is decreasing in case if the antibiotic-impregnated spacer was implanted. These changes indicate a more cooperative structure of the pseudo-capsule, likely due to the preventive effect of the constantly released antibiotic. The $T_{1/2}$ decreases further in septic loosening, probably as a consequence of structural changes in response to perivascular inflammation.

To compare the effects of aseptic and septic loosening on the structural changes of pseudo-capsule, the

Fig. 1 Histological image of pseudo-capsule in aseptic revision surgery (stained with hematoxylin–eosin)

Fig. 2 Histological image: **a** pseudo-capsule in septic revision surgery with perivascular inflammation and **b** pseudo-capsule in aseptic revision surgery with hyper-cellularity (staining as in Fig. 1)

Fig. 3 Pseudo-capsule in revision surgery with antibiotic spacer (staining as in Fig. 1)

calorimetric enthalpy seems to be the practical measure of the severity of problem.

The results shown in Table 1 clearly demonstrate the dependence of denaturation temperature and calorimetric enthalpy on the severity of loosening. Applying antibiotic-impregnated spacer, the decrease in ΔH_{cal} is about 20%, while in case of septic loosening it is $\sim 50\%$, indicating extended loosening of the structure of pseudo-capsules. The number of collected samples is not enough to make verified statistical conclusion, but based on thermal parameters the severity of different types of loosening can be well separated.

Discussion

We have previously shown that differential scanning calorimetry provides useful data in the analyses of capsules from patients with different types of hip diseases. The denaturation curves indicated a significantly different,

Fig. 4 DSC scans of hip pseudo-capsules. Symbols: solid line: aseptic loosening, dotted line: effect of antibiotic-impregnated spacer and dashed line: septic loosening. The curves are averages (Table 1), and endotherm deflection is directed downwards

Table 1 Maximal denaturation temperature (T_m) and mass-normalized calorimetric enthalpy (ΔH_{cal}) of hip pseudo-capsules (data show average values, rounded to two places)

Sample	Thermal parameters	
	$T_m/^\circ\text{C}$	$\Delta H_{cal}/\text{J g}^{-1}$
Aseptic loosening ($n = 3$)	60.85	4.28
Antibiotic-impregnated spacer ($n = 3$)	60.02	3.40
Septic loosening ($n = 2$)	61.53	2.33

individual pattern for each type of degenerative or inflammatory hip diseases [13]. Recently, Dande et al. have demonstrated that thermal analyses of synovial fluid of the knee joint could help in the diagnostics of septic conditions [14].

In case of revision hip arthroplasties, detection and confirmation of bacterial presence can be difficult. The patients' symptoms, the time elapsed from the primary surgery, results of imaging and laboratory investigations may contribute only in part to reach the right diagnosis. Moreover, the intraoperative diagnosis of joint infections could also be challenging and the microbiological confirmation of bacterial presence may take days, occasionally weeks [15, 16].

Here, we found that the thermal denaturation scans of different pseudo-capsule samples have clearly demonstrated specific, representative changes, comparing samples with aseptic to septic origins. It could be explained by the conformational changes of the tissue collagens in response to bacterial toxins and the release of inflammatory

mediators in septic conditions [7, 9]. Moreover, these changes were confirmed by histological evaluations as well.

Meanwhile, the pseudo-capsule samples collected during aseptic revision surgeries or after gentamycin-impregnated spacer implantation showed similar thermoanalytical characteristics and histological results, in both cases due to the absence of bacterial infection.

This study has a potential limitation because of the relatively small number of samples; therefore, we did not perform standard deviation calculation (and this way statistical analysis) because the number of samples (n) was smaller, than five. The mean values were analyzed to evaluate the effect of septic conditions on the measured thermal parameters. However, despite this limitation, we believe that the differences observed in the thermal characteristics and the shape of DSC scans provide valuable and scientifically relevant data in the analysis of hip pseudo-capsules.

Conclusions

DSC could be a useful tool in the evaluation of hip pseudo-capsules with different origins, providing further help in the differential diagnostics of septic complications.

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