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A novel *ex vivo* model for investigation of fluid displacements in bone after endoprosthesis implantation

C. GATZKA, E. SCHNEIDER, M. L. KNOTHE TATE*,[1]
AO ASIF Research Institute, Clavadelerstrasse, CH-7270 Davos, Switzerland
E-mail: tate@biomed.ee.ethz.ch

U. KNOTHE
Hospital Centre Biel, Department of Orthopaedic Surgery, Biel, Switzerland

P. NIEDERER
[1]Institute of Biomedical Engineering, University and Swiss Federal Institute of Technology, Zürich, Switzerland

Tissue perfusion and mass transport in the vicinity of implant surfaces prior to integration or bonding may play a crucial role in modulating cellular activities associated with bone remodeling, in particular, at early stages of the integration process. Furthermore, fluid displacements have been postulated to transduct mechanical stress signals to bone cells via loading-dependent flow of interstitial fluid through the lacunocanalicular network of bone. Thus, an understanding and new possibilities for influencing these processes may be of great importance for implant success. An *ex vivo* model was developed and validated for investigation of fluid displacements in bone after endoprosthesis implantation. This model serves to explicate the effects of surgical intervention as well as mechanical loading of the implant–bone construct on load-induced fluid flow in the vicinity of the implant. Using this model, we intend to quantify perfusion and extravascular flow dynamics in the vicinity of implants and define optimal conditions for enhancing molecular transport of osteotropic agents from the implant surface to apposing bone as well as from the blood supply to the implant surface. Furthermore, the elucidation of main transport pathways may help in understanding the distribution of wear particles in bone surrounding implant, a process which has been postulated to cause osteolysis and implant loosening.

1. Introduction

Once implanted, endoprostheses are expected to perform well over a time span of 10–15 years [1, 2]. Given an increasing tendency to implant endoprostheses in younger, more active patients, expectations with regard to implant life span and performance are accordingly high. One prerequisite for improving on implant design and function is a better understanding of the bone–implant interface. In the past, research has focused on the mechanical stability aspects of tissue–implant interfaces and implant designers have honed in on the importance of surface topology for promotion of osseointegration and mechanical stability [3, 4]. More recently, a new generation of bioactive materials (i.e. bone morphogenetic proteins, osteopromotive and/or resorbable polymers/ceramics, osteotropic drug delivery systems) has been tested for tissue replacement and/or site specific drug delivery applications [5, 6]. Such materials may lend themselves particularly well to the promotion of osseointegration at endoprosthetic surfaces. However, although much research has been conducted in the areas of osseointegration as well as interfacial properties of bone ''bound'' to bioactive implants after integration or bonding has taken place, little is known with regard to tissue perfusion and mass transport in the vicinity of implants prior to integration or bonding. Based on previous studies by our group, these processes may play a crucial role in modulating cellular activities associated with bone remodeling, in particular, at the early stages of the integration process [7–11]. Considering that fluid displacements have been postulated to transduct mechanical signals to bone cells via loading-dependent flow of interstitial fluid through the lacunocanalicular network of bone [8, 12], an understanding and new possibilities for influencing these processes may be of great importance. Apart from our work [7–11], these influences have been shown mainly in *in vitro* experiments [13–16]. Although helpful for understanding the cell's response to mechan-

*Author to whom all correspondence should be addressed.

ical loading under specific applied conditions, *in vitro* cell culture results are difficult to interpret with regard to the relationship between applied loads and biological response in a physiological environment. Furthermore, as the mechanical and biological milieu of these cells is not fully known *in vivo,* conditions in *in vitro* culture experiments represent an approximation.

The objective of this article is to introduce a novel *ex vivo* model for investigation of fluid displacements in bone after endoprosthesis implantation. This model should serve to explicate the effects of surgical intervention as well as mechanical loading of the implant–bone construct on load-induced fluid flow in the vicinity of the implant.

2. Materials and methods

Our goal was to establish a model for investigation of fluid displacements in bone after endoprosthesis implantation. To achieve this goal, an implant and loading system were designed in order to mimic loads occurring *in situ* at the bone–implant interface immediately after endoprosthesis implantation. An established *ex vivo* perfusion model of the sheep forelimb provided the basis for studying transport processes and fluid flow in loaded bone [7, 8].

2.1. Surgical procedures

All surgical procedures were carried out such as to replicate the clinical situation as closely as possible. An intravenous bolus of 5 ml (5000 IE) heparin (Fresenius®) was administered 5 min prior to euthanasia by barbiturate overdose (Vetanarcol® 20 ml). The left and right forelimb of the sheep were amputated at the level of the elbow joint and perfused with 0.9% NaCl solution, according to the protocol described previously [7, 8]. In order to visualize the proximal metacarpus without disturbing bone perfusion, the joint was opened via a limited medial approach. Thereafter, the intramedullary cavity was opened by drilling through the articular surface of the proximal metacarpus. The implant ''bed'' was prepared further by rasping through the subchondral and metaphyseal bone, creating a space to fit the implant. Then, a custom designed titanium prosthesis (Fig. 1) was press fitted into the proximal medial compartment of the metacarpus. Because of interanimal variations, titanium prostheses in five sizes (diameter 8–12 mm) were developed to ensure a press fit into the proximal medial compartment (Figs. 1 and 2). Polyethylene onlets of three different heights (1–3 mm) were designed to fit onto the prosthesis (Figs. 1 and 2), extending 0.5 to 1 mm over the articular surface. Finally, after insertion of the prosthesis and placement of the polyethylene onlets, joint stability was regained by closing the surgical approach. These experiments were carried out within the context of regulations and guidelines for animal experimentation set by the Canton of Grisons, Switzerland.

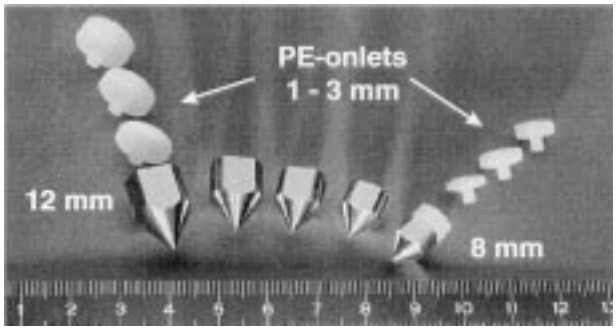


Figure 1 Custom-designed titanium prosthesis with PE-onlet.

2.2. Mechanical loading of the forelimb

After the system of perfusion was established and the prosthesis was implanted, the forelimb was inserted into the loading machine (RUMUL, Russenberger & Müller, Kesselstrasse 10, 8200, Schaffhausen, CH) via the radius and the hoof, analogous to the mechanism of load transfer *in vivo* (Fig. 3). The loading system was developed to simulate loads occurring *in vivo* during typical daily activities, as measured using strain gauges during normal physiological activities, whereby, typically, strains of 0.005% (500 $\mu\varepsilon$) were measured on the anterior aspect of the metacarpus mid-diaphysis during normal gait cycles without impact loading [10]. To reproduce this situation *ex vivo,* a strain controlled system was utilized, in which cyclic loads applied via the testing machine at a rate of 1.0 Hz were monitored via a strain gauge (CEA-06-125UN-120, Micro Measurements Div., Measurements group, Raleigh, North Carolina, USA). Analogous to the *in vivo* measurements, the strain gauge was glued to the anterior mid-diaphyseal surface of the metacarpus. Strain was measured and recorded for each loading cycle using an amplifier (P 3500, Instruments Div., Measurements group, Raleigh, North Carolina, USA) and analog plotter. In each experiment, one limb, chosen randomly, was loaded mechanically and the contralateral limb served as the unloaded control. Other than loading, all other experimental parameters were identical between pairs.

2.3. Tracer and histology methods for visualization of fluid displacemets

Fluorescent dyes (procion red, labeled dextrans) were used as load-induced flow ''indicators'' within the subchondral, metaphyseal and diaphyseal bone of the proximal metacarpus, both in the loaded and unloaded case. Procion red (0.08%, Sigma, St. Louis, MO, USA) was prepared and infused prior to loading according to protocols developed by our group [7, 8]. The efficacy of this marker for tracing interstitial fluid movement has been proven [9]. Immediately after each experiment was completed the metacarpus was excized and prepared for histological processing and microscopy. Two to three 150 μm thick cross-sections were cut out of the metacarpus at three different heights (Fig. 2) using a water-cooled diamond wafer saw (Leitz, Germany). Sections were made using the articular surface as a reference point, whereby the first section was taken 3 mm below the articular surface. The second and third sections were taken 6 and 12 mm below the articular surface, respectively. All cross-sections were then ground between glass plates to *c*. 60–80 μm and coverslipped

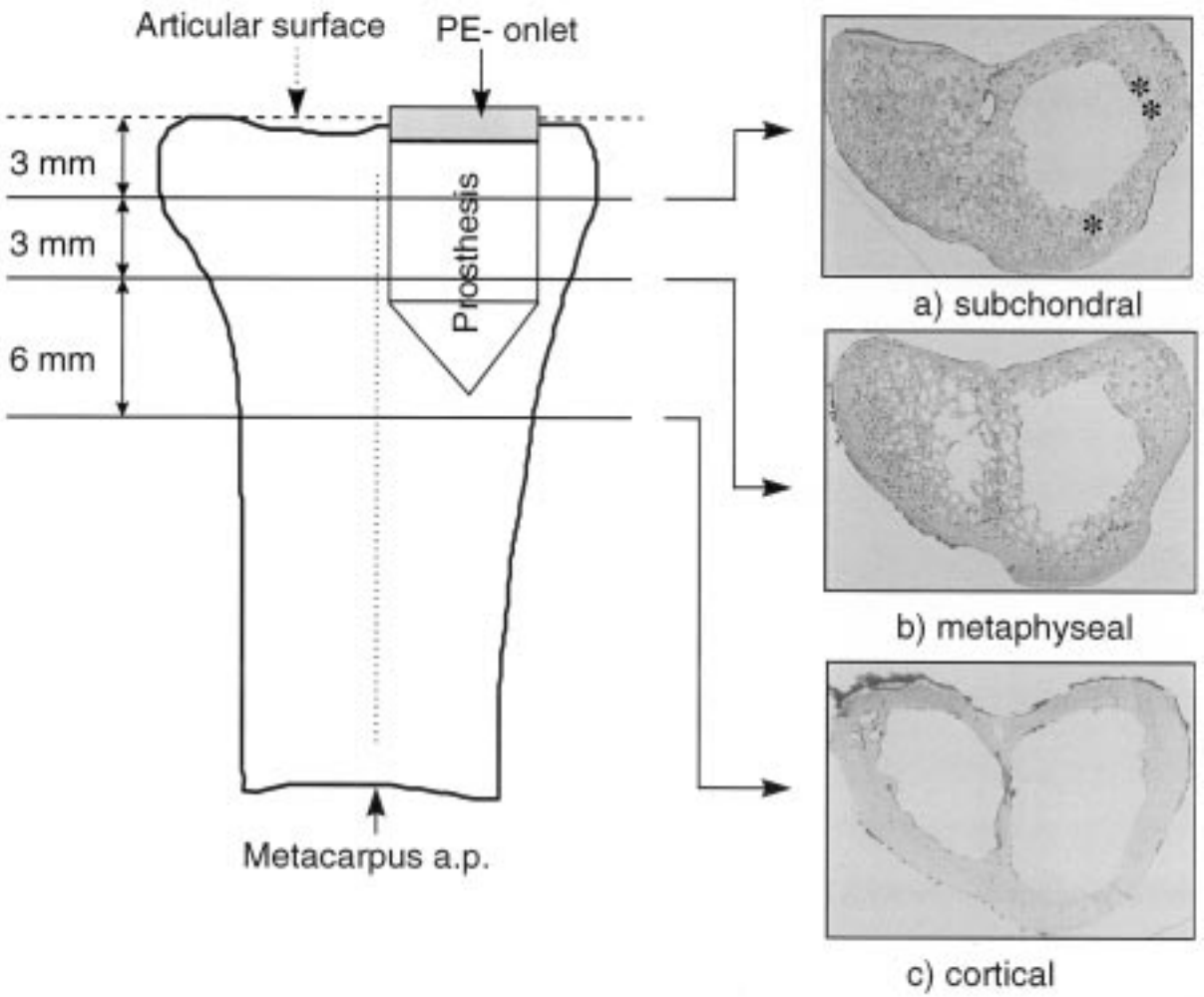


Figure 2 Schematic drawing of the metacarpus showing sites from which histological sections were taken (a–c). The regions from which the micrographs in Fig. 5a (*) and b (**) were taken are indicated.

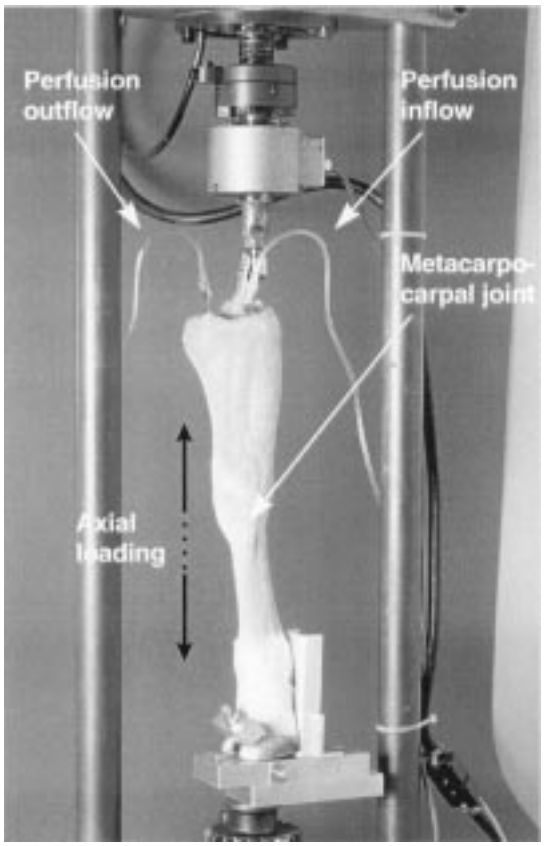


Figure 3 Experimental set-up with sheep forelimb.

with mounting medium. Thin sections were observed under the microscope (Zeiss, Germany) using selective green light excitation (excitation maximum 565 nm, emission maximum 619 nm) achieved with a mercury lamp and filter set, and documented photographically. For high-resolution micrographs, sections were documented and quantitative evaluation was carried out with the aid of a TCS 4D confocal, three channel oil immersion microscope (Leica, Germany).

3. Results

We were successful in developing an *ex vivo* model of the Swiss Alpine sheep forelimb to simulate the condition of bone immediately after endoprosthesis implantation. The perfusion system (after [8]) functioned well, with minimal fluid loss, in this adapted model and was validated by comparing results with those using the previously introduced *ex vivo* perfusion model [7, 8]. Analogous to that model, the sheep forelimb was perfused with 0.9% NaCl solution at the beginning of the experiment. Within minutes after introduction of procion red solution to the system via the nutritive artery, i.e. the a. mediana, the forelimb was completely perfused with the red tracer. The fluid entering the nutritive artery as well as that exiting the main, cephalic vein was dark pinkish red. All subcutaneous tissue exhibited the same color. Similar results have been obtained *in vivo* after

systemic procion red injection, thus validating that our *ex vivo* model mimics the *in vivo* situation [7, 8]. In general, the perfusion of the forelimb, as observed macroscopically over the course of the experiment, was more rapid in the loaded than in the non-loaded limb.

The prosthesis design allowed for a press fit in the subchondral and metaphyseal regions of the metacarpus. The insertion of the custom-designed prosthesis was technically demanding for several reasons. First, the metacarpocarpal joint is extremely tight. In order to preserve joint stability and perfusion, a limited medial approach was used, allowing only a small opening for prosthesis implantation. Secondly, the choice of an appropriately sized implant to achieve a true press fit required ample experience. Undersized implants did not allow for a press fit and oversized implants risked fracture to the bone during implantation. Finally, with experience, implant sizing became routine and press fit conditions in subchondral and metaphyseal regions of the metacarpus were achieved without complications.

The cyclic loading system was designed to simulate loads occurring *in vivo* during typical daily activities [10]. Physiological load transfer was attained by connection of the forelimb to the loading machine, proximally, over a metal bar fixed with polymethylmethacrylate (PMMA) into the radius and, distally, via fixation of the hoof onto a slide block. Primarily axial loading was achieved by adjusting the position of the slide block with respect to the proximal fixation of the radius (Fig. 3). The loading machine specifications allowed for loading of the forelimb up to 20 kN at frequencies from 1 to 20 Hz. Loading cycles and strain magnitudes were documented for each experiment. Strain on the anterior aspect of the middiaphysis was measured and controlled during each loading cycle (Fig. 4) using a strain gauge. The extension of the prosthesis/onlet above the articular surface, via the onlet, (Fig. 2) guaranteed a direct load transfer from the carpal bones of the metacarpocarapal joint via the prosthesis into the proximal metacarpus. The loading system was validated by comparing cycle versus strain plots taken experimentally with those measured *in vivo* within the context of another study [10]; *ex vivo* strain magnitudes were comparable to those measured during normal walking activity.

Fluid displacements in close vicinity to the bone–implant interface could be studied using this novel *ex vivo* system, together with tracer techniques developed by our group [7–11]. The perfusion and loading set-up could be run for at least 4 h. By defining histological sections at three different heights with respect to the articular surface, we were able to study tracer distribution in regions with predominantly cancellous bone (Fig. 2a), mixed cancellous and cortical bone (Fig. 2b), as well as predominantly cortical bone (Fig. 2c). We were particularly interested in investigating tracer transport and fluid flow and how they were affected by the prosthesis press fit, with and without mechanical loading. Furthermore, areas of mainly lamellar bone, which were not apposing the implant directly, were of interest (Fig. 5). With our histology techniques we were able to

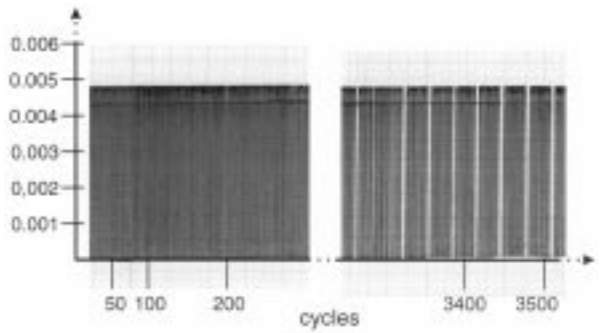
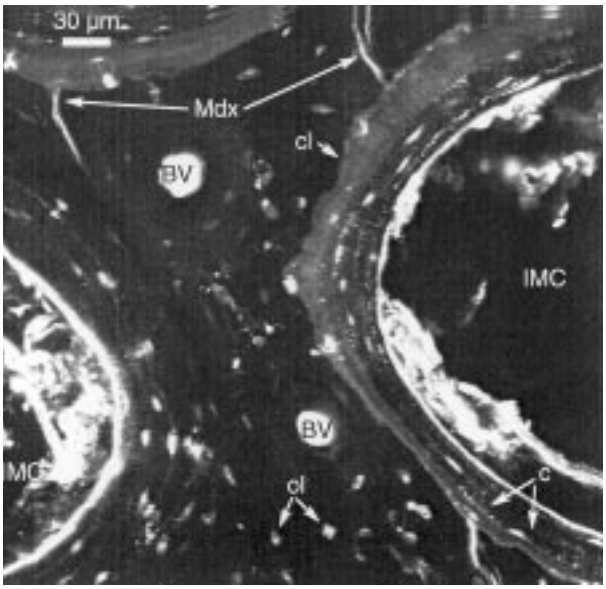
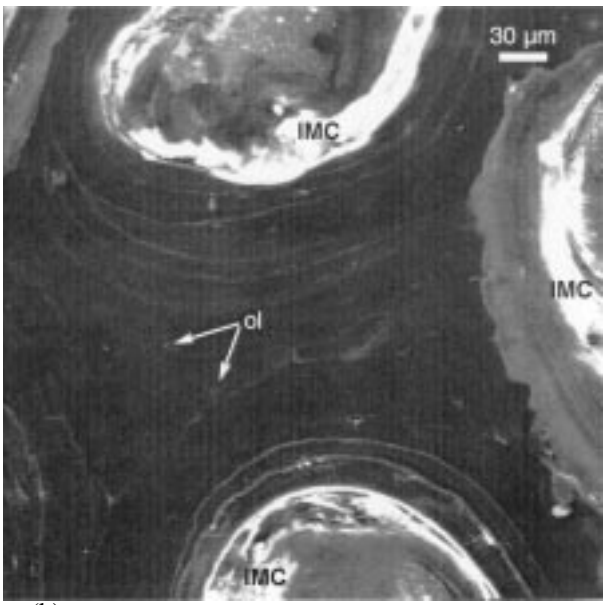


Figure 4 Strain versus cycle plot at 1.0 Hz. Sections show early and late stages of loading. White spaces are artefacts from the plotter pen.



(a)



(b)

Figure 5 Confocal micrograph of the sheep metacarpus cross section; after perfusion with procion red and concomitant mechanical loading. (a) The anterior region of the section is depicted. The osteocyte lacunae (ol) show the presence of the fluorescent tracer. Tracer is also apparent in the intramedullary cavity (IMC), blood vessels (BV) and canaliculi (c). Microdamage (Mdx) and cement lines (cl) are visible as well. (b) The medial region of the same section is shown. In this region, there is far less tracer present in the lacunocanalicular system. Canaliculi are not stained in this region.

differentiate between regions in bone where the tracer has clearly penetrated the bone from those regions where no penetration has taken place (Fig. 5).

4. Discussion

To study fluid displacements under controlled mechanical loads in the close vicinity of the bone–implant interface, a novel *ex vivo* model was developed and validated based on a previously described perfusion model [7, 8]. A loading system was designed to simulate loads occurring *in vivo* during typical daily activities. By injecting fluorescent tracer substances such as procion red and labeled dextrans into the a. mediana of the metacarpus, a system of perfusion was established and tracer transport could be visualized at tissue, cellular and subcellular levels. These tracers served as ''flow indicators'' through the tissue, providing valuable information with regard to load-induced fluid displacements at the bone–implant interface.

The loading and perfusion system has several advantages. Perfusion time, number of loading cycles and loading frequency can be adjusted to determine correlations between tracer distribution and loading regimes. Using this experimental system, it is possible to study the effects of prosthesis design, surface topology and anchoring techniques on bone perfusion and fluid displacements. Finally, the system lends itself for the investigation of pharmacokinetics under physiological conditions, e.g. with regard to osteotropic agent delivery from surface coatings on implants which are subjected to mechanical loads *in situ*. This model is particularly applicable for the study of particle and fluid migration at the bone–implant and cement–implant interfaces, which have been postulated to contribute to osteolysis and implant loosening [17–20]. In addition, distribution pathways of polyethylene debris could be monitored using this model.

The model was developed based on an *ex vivo* model to study transport processes and fluid flow in loaded bone [7, 8]. The sheep metacarpus was chosen for this experimental model for several reasons. First, the relatively simple geometry of the metacarpus allows for easy analogy to physiological loading conditions and computer model simulations. Second, sheep bone is morphologically similar to human bone. Thirdly, the metacarpus is infused by one main artery; this ensures perfusion of tracer throughout the soft and bony tissues of the forelimb. Fourthly, the metacarpus is subjected primarily to axial loads *in situ* and thus lends itself particularly well for the study of fluid displacements incurred under axial loading conditions. Finally, there are two specimens per animal, allowing for paired studies, in which both sides are perfused and in which an implant is inserted. While one leg is cyclically loaded, the contralateral limb serves as a control.

The fluorescent tracers used in this study allow for fluid flow and mass transport phenomena to be elucidated in bone tissue subjected to mechanical loads [8]. The injection of procion red allows for the assessment of molecular transport of small substances, e.g. smaller nutritional amino acids (MW: 300–400 Da), between the blood supply and the osteocytes [9]. Labeled dextrans (MW: 20 kDa) were chosen as ersatz molecules for e.g. bone morphogenetic proteins (MW: 20–40 kDa). It has been shown that diffusive transport mechanisms may suffice to supply small molecules, such as amino acids, to the osteocytes within time periods required for metabolism. However, diffusion alone is not sufficient for the transport of larger molecules, such as proteins, to osteocytes [9]. Since proteins serve as carriers of small molecules and ions, it is likely that transport via load-induced fluid flow is crucial for maintenance of metabolic activity as well as for activation or suppression of remodeling processes, which are critical at early stages of implant integration.

5. Conclusion

A novel *ex vivo* model for investigation of fluid displacements in bone after endoprosthesis implantation has been introduced. This model serves to explicate the effects of surgical intervention as well as mechanical loading of the implant–bone construct on load-induced fluid flow in the vicinity of the implant. We intend to quantify perfusion and extravascular flow dynamics at the bone–implant interface and define optimal conditions for enhancing molecular transport of osteotropic agents from the implant surface to apposing bone as well as from the blood supply to the implant surface. Furthermore, the elucidation of main transport pathways may help in understanding the distribution of wear particles in bone surrounding implant, a process which has been postulated to cause osteolysis and implant loosening.

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