Orthopaedic implants and infections: current status and future prospects

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Chapter 1: Introduction

Orthopedic implants are used to replace articulating surfaces of damaged joints. The surgical procedure involves removal of the damaged joint and a replacement by an artificial joint (referred to as prosthesis). Prostheses are mainly used for hip, knee, shoulder and elbow replacements. The most important reasons to choose for an arthroplasty are to provide pain relief, enhance mobility of the patient, and restore the function of the joint[1]. This thesis will primarily focus on total hip arthroplasty (THA), a common joint replacement.

Total hip arthroplasty

In a THA procedure, both the acetabulum and femoral head are replaced, unlike hemi-arthroplasty, where only the femoral head is replaced[2]. THA is performed e.g. on elderly patients with displaced femoral neck fractures[3] and is also an effective treatment for patients with a severely arthritic hip[4].

Cemented versus non-cemented implants

Orthopedic implants are available in a variety of materials. The majority of implants used today are made of cobalt-chrome alloy, stainless steel, or titanium[5]. The implants can be secured to the bone via two different methods: cemented or non-cemented. The first method uses cement, consisting of polymethylmethacrylate[6], which is applied before positioning the prosthesis. The cement will set and the implant will be secured to the patient's bone. For the non-cemented technique, the implant will be secured press-fit, without an additional compound for anchoring the implant. An uncemented prosthesis has roughened porous surfaces which allow bone ingrowth from the patient. For THA, a combination of the two techniques is often used with a cemented femoral stem and an uncemented cup[7]. Both cemented and cementless implants can achieve improvement for clinical and functional outcomes. Cementless implants are more suitable for younger patients owing to the quality of their bone stock. Furthermore, in case of revision arthroplasty, cementless implants are less difficult to remove than cemented implants. The advantages of using cement are less post-operative thigh pain due the firm fixation, and a reduced long-term revision rate from loosening of the prosthesis compared to cementless implants[8, 9].

Aseptic loosening and chronic infection

The most problematic complications that can occur after THA are aseptic loosening and chronic infection[10]. Aseptic loosening accounts for approximately 75% of total prosthetic hip revisions[11]. Aseptic loosening involves loosening of the implant without an infection. The underlying mechanism is a point of discussion, but is probably the result of a combination of several factors. An example of such a factor is an immune reaction to small particles from the implant. Debris from THA can come from three sources: polyethylene debris from the acetabular component, polymethylmethacrate debris from cemented implants, and metal debris[12]. The interactions of the particles with immune cells (e.g. macrophages) result in the release of inflammatory mediators. These mediators may lead to a disturbed balance in bone remodeling, which can result in osteolysis. Osteolysis can lead to implant loosening or bone fracture[13]. Aseptic loosening can also be a consequence of too much stress on the implant due to a highly active behavior of the patient, which can damage the cement mantle[14]. Although infection is not a cause of aseptic loosening, the widespread use of antibiotic loaded bone cement for THA has been associated with a decrease in prosthetic revisions due to aseptic loosening[11]. Theoretically, the use of antibiotics should not influence the incidence of aseptic loosening, however, this observation suggests false positive diagnoses. The patients which were diagnosed incorrectly, might suffer from a low-grade infection, which are easily missed with routine diagnostics. Consequently, these patients might not get an optimal treatment. A major difficulty in finding a solution for this problem is that is no gold standard for finding and defining an infection and the causing organism[15]. Microbiological cultures are not specific enough[16] and have a chance of a

false positive result due to contaminations or a false negative result due to obtaining too few biopsy samples or too few may also result in false negative results. As there is not yet a perfect solution for diagnosing a prosthetic joint infection, the best method would probably be combining several diagnostic tests[15, 16]. An infection of the prosthetic implant is one of the most challenging complications of total joint arthroplasty. Prosthetic infections can be divided in acute and chronic infections. Acute infections present themselves within a few months postoperatively and are typically manifested as an acute onset of fever, joint pain, and a warm, red skin at the implant site. Acute infections can often be treated by irrigation of the implant and surrounding tissue, and debridement with retention of components[17]. In comparison, patients with a chronic infection after primary THA often present with persistent joint pain or implant loosening. Chronic infected implants are difficult to treat and the infection cannot be eradicated without some form of resection arthroplasty[17]. Chronic infections occur in approximately 1-2% of primary total hip arthroplasties[18-20]. Although this percentage may not seem alarmingly high, the annual number of primary arthroplasty procedures will continue to grow due to an aging population. For example, the estimated annual number of total hip replacements in the Netherlands by the year 2020 will increase by 44% (17,401 in 1997) to 25,090 operations[21]. In order to limit the amount of hip arthroplasties and minimize the economic impact, more research for prophylaxis and treatment of infection is needed.

Focus of this thesis

For this thesis, we are curious to know the current status of treatment and prevention of infected implants. Furthermore, we are interested in the latest animal models regarding infections in the orthopedic field, and novel techniques which can be used to improve current treatment strategies or reduce the amount of infections.

Chapter 2: Detecting infections, microorganisms, and prophylaxis and treatment of infections

Prophylaxis

Although orthopedic surgeons thoroughly perform thoroughly their procedures during surgery and patients are strictly managed before and after surgery, infections keep occurring. Therefore, other prophylactic measures are taken to prevent infections. Systemic antibiotics are effective as prophylaxis in reducing acute and chronic implant-related infections in patients following total joint replacement[22, 23]. Another method of preventing infections after primary joint replacement is adding antibiotics to the bone cement. The use of antibiotic-impregnated cement reduces infection and revision rates for primary total hip arthroplasty[24]. Furthermore, when antibiotics are given both systemically and locally, less revisions due to infections are needed than when the antibiotics provided in either of these forms[25, 26].

Blood tests

When suspecting an infected prosthesis, blood tests are performed for examining indicators of infection. C-reactive protein is an acute-phase enzyme that is produced in the liver in response to inflammation, infection, neoplastic disorder, and surgery. Furthermore, the erythrocyte sedimentation rate is elevated after e.g. an infection or in case of an inflammatory disease. When both C-reactive protein and the erythrocyte sedimentations levels are elevated, together with an increased white blood cell count, it is likely that an infection is present.

X-rays

In addition to blood tests, radiographs are often taken. The images are compared with the pictures taken directly after implantation. The radiographs are examined for progressive widening of the radiolucent zone, migration of a cemented component, or change in alignment. However, radiographs are often neither sensitive nor specific enough to diagnose infection[27].

Joint fluid culture

Furthermore, after suspecting an infection, samples of the joint fluid are taken and cultured for microbiological examination. This method can be used to identify the type of microorganism involved and determine the antibiotic susceptibility to guide antibiotic treatment[28, 29]. To minimize the risk of a false negative result, the patient should not use antibiotics a few weeks prior to aspiration[30].

Microorganisms

The most commonly cultured microorganisms from the site of infection are gram-positive bacteria. Coagulase-negative staphylococci and *Staphylococcus aureus* (*S. aureus*) are the most frequently found bacteria. Streptococci, gram-negative bacilli, enterococci and anaerobic bacteria are also encountered, however, in a lesser degree[31]. Fungal infections after THA are rare, with the Candida species being the most frequent pathogen according to literature[32].

Once the bacteria are attached to and grow on the surface of an implant, they begin to produce a highly hydrated matrix of extracellular polymers. This matrix together with the embedded microorganisms are known as a biofilm[33]. Within the biofilm, the bacteria are protected from antimicrobial treatment and the host immune system[34-36]. Furthermore, the bacteria will enter a stationary phase of growth, probably because of the lack of nutrients and oxygen. A consequence is that biofilm-forming microorganisms are more resistant to growth-dependent antimicrobial agents than their free-living (planktonic) counterparts[37].

Treatment of chronic infected implants

The treatment of a chronic infection at the implant site after THA is a major difficulty. There is no gold standard for the optimal treatment. A very important endpoint is eradicating the infection and preventing a new one, however, the patient's quality of life and the costs should also be taken into consideration. When a prosthesis is chronically infected, revision is required to eradicate the infection. This can be performed via a one- or a two-stage revision. In a one-stage revision, a new prosthesis is implanted immediately after the removal of the original implant. A two-stage revision requires an initial surgery to remove the implant, and a surgery to place a new one. In most two-stage arthroplasties, a spacer is usually implanted before the final prosthesis is given to the patient. The function of the spacer is to minimize muscle shortening and retain soft tissue tension for maintaining functionality of the joint. Furthermore, spacers consist of antibiotic loaded cement, which delivers antibiotics into the joint space. This way, higher local antibiotic concentrations can be achieved than those obtained with systemic antibiotics alone[38]. The decision to perform a one-stage THA revision will depend on certain criteria. When a patient has adequate bone stock, and when the microorganism infecting the joint is known and has low virulence with good antibiotic sensitivity, the patient may benefit more of a one-stage than of a two-stage revision arthroplasty[39, 40]. For a two-stage revision, patients undergo an extra surgery and a longer hospital stay. Furthermore, a two-stage replacement is more expensive[41]. However, a two-stage has the advantage that the surgeon is able to evaluate the progress of infection before reimplantation. Additionally, the use of antibiotic-containing spacers during the interval period helps controlling the infection[20, 42],which contributes to a lower change for reinfection after two-stage THA revisions than after one-stage revisions[43, 44].

Chapter 3: Animal models for Orthopedic implant infection

To minimize the amount of infection after primary THA, more knowledge about methods for prophylaxis and treatment of infection needs to be available. Furthermore, the detection of infection and identification of the causing micro-organism are not optimal yet. In order to achieve this, animal experiments can be very helpful for improving current methods or testing novel ideas. For this purpose, all papers between 2006 and now concerning animal models and orthopedic implant related infections were investigated for animal models, used animal species, regular used and novel evaluation methods for infection, and novel techniques. This search resulted in 14 papers which provided useful information on these topics.

Type of animals
All papers used small rodents for orthopedic implant related infection research, namely rats[45-49], rabbits[50-55], and mice[56-58]. Experimental models using small animals allow easy handling and are more cost-effective than using large animals.

Most current animal models used for orthopedic implant-related infection involve early perioperative infection models. However, prosthetic infections can also develop months or even years after the surgery. Unfortunately, few animal models exist that allow monitoring of these infections.

Periprosthetic infection models
A pre-clinical screening tool to evaluate the efficacy of *in vivo* therapeutic strategies, Bernthal *et al.* developed a mouse model[56]. To develop a post-arthroplasty infection, a Kirschner (K)-wire is placed in the intramedullary canal of the femur before an inoculum of *S. aureus* is pipetted into the joint space. Interestingly, the *S. aureus* strain used in this model emits bioluminescent signals from live bacteria. Furthermore, the mice are genetically engineered so they possess fluorescent neutrophils. These two properties of this model provide the possibility of measuring the infection and inflammation in real-time without requiring euthanasia of the animals. The limitations of the model are that the steps involved in total knee arthroplasty are simplified. Furthermore, the implants were made from stainless steel, other metals or materials were not included in this experiment. Summarizing, this model may be used as a rapid and precise *in vivo* preclinical screening tool to evaluate the efficacy of potential strategies to prevent or treat post-arthroplasty infections.

Antoci *et al.* developed a periprosthetic infection model using Wistar rats which is simple, cost-effective, and reproducible[49]. The periprostetic infection is established by implanting titanium rods intramedullary in the femur, which are injected with *S. aureus*. The implant can be modified with antimicrobial or antibiofilm agents. The limitations of this rat model, and of the aforementioned mouse model, are the difference in size and surface characteristics compared to human implants, which may limit the extrapolation of the results. This model can be used as a tool for researching the efficacy of an implant modified with antimicrobial or antibiofilm components in preventing bacterial infection or biofilm formation.

One of the few hematogenous periprosthetic infection models caused by methicillin-resistant *S. aureus* (MRSA) found in recent literature was established by Poultsides *et al.*[54]. New Zealand White rabbits received tantalum implants in the proximal tibia. After 28 days, a community-acquired MRSA strain was injected into the femoral artery to induce a hematogenous infection. The rabbits were euthanized four weeks later. This model resembles hematogenous periprosthetic infections in humans and offers the potential for examining the mechanisms of hematogenous infections. This

model is simple and reproducible. Furthermore, it can be used for testing prophylactic properties of antimicrobial or antibiofilm agents.

Inducing bacterial infections using *S. aureus*

For inducing infections, the bacteria strain *Staphylococcus aureus* was used for all current discussed studies, because this microorganism is most often isolated from implant associated infections[31]. Interestingly, some studies used a bioluminescent strain of *S. aureus*[56-58], which provides the possibility of real-time *in vivo* imaging of the bacterial infection. The bacterial growth throughout the course of infection can be monitored and quantified without sacrificing animals.

Imaging techniques

Several studies use radiographs for examining the development and progression of bone infection, to verify whether the implant shows signs of loosening, or to evaluate formation of new bone[45, 46, 48, 49, 54]. In addition to X-rays images, a high resolution 3D imaging technique such as micro computed tomography (µCT) is sometimes utilized for analysis of bone morphology or signs that are characteristic for infection[45, 48, 49]

Bacterial culturing and quantification

Culturing samples of periprosthetic tissue or bacteria attached to the implant itself are standard methods used for microbiological diagnosis of prosthetic joint infection. After explantation, the extend of surface colonization was examined in many studies. This is achieved by rolling the implant over blood agar plates after explantation. The plates are then checked for positive infection, or CFU is calculated after a 24-48 hour incubation time[45, 46, 48-51, 53]. Furthermore, the implant is then often placed in tryptic soy broth (TSB), where-after positive infection is demonstrated by judging the clearness of the medium[46], or the amount of adherent bacteria on the implant is determined by serial diluting the TSB, culturing the broth on blood agar plates and calculating the amount of CFU[45, 47, 49]. In some studies, the bacteria are detached from the implants before culturing by sonicating the implant first[57, 58]. To quantify the amount of bacteria in the bone surrounding the implant, the bone is homogenized, suspended in TSB, and cultured on blood agar plates, where after the CFU per gram tissue is calculated[47, 48, 50, 51, 53-55]. In one study, a PCR was performed on the homogenized bone for bacterial 16s rRNA quantification[54].

Blood tests

Only one study performed complete blood counts for leukocyte concentration and proportion of neutrophilic leukocytes after locally infecting the animals. Furthermore, this was also the only study which cultured blood for confirming the infection was local and not hematogenous[53]. The only study with a hematogenous infection model examined the leukocyte concentration and erythrocyte sedimentation rate before and after infecting the animals for signs of infection[54].

Visualization of bacteria and biofilms

Various methods for examining histology are available, however, not all examined studies used histological stainings. Schmidmaier *et al.* embedded their undecalcified samples in methylmethacrylate (MMA) and performed a Masson-Goldner staining for signs of bone infection, and a Gram staining for detecting Gram-positive bacteria[46]. Poultsides *et al.* stained sections of MMA embedded samples with basic fuchsin and methylene for signs of infection, and stained with Gram stain[54]. For assessing a periprosthetic infection histologically, Alt and colleagues embedded the undecalcified bone samples including the implanted material in acrylic resin and stained the samples with toluidine-blue, and hematoxylin and eosin (H&E) stains[48, 52, 55]. Beside using acrylic resin or MMA for embedding, paraffin can also be used for embedding samples. However, to be able to make sections, the bone has to be decalcified for this method. In some studies, for examining the amount

of neutrophils, the paraffin-embedded samples were stained with H&E[54, 58]. Furthermore, in one study, the paraffin samples were stained for gram-positive bacteria with a Gram staining[56].

Besides visualizing the amount of bacteria adherent on the implant, biofilm formation can be confirmed by using other techniques. Alt *et al.* analyzed bacterial biofilm formation on implants using scanning electron microscopy (SEM)[48]. Furthermore, in some other studies, the biofilm formation on implants was detected by using variable-pressure SEM (VP-SEM)[56-58]. VP-SEM allows visualization of samples without the need for coating, so artifacts like dehydration or shrinkage will not occur.

For detection of bacteria on paraffin sections, Alt *et al.* used fluorescence *in situ* hybridization (FISH)[48]. In this study, FISH was used to identify *S. aureus* by detecting prokaryotic DNA with two different probes: one that targeted all prokaryotic 16s DNA and one that targeted *S. aureus* specific DNA. This study showed that detection of bacteria in implant-associated bone infections in bone tissue by using FISH on paraffin sections is possible.

Quantifying neutrophil recruitment

Besides an H&E staining for examining neutrophil recruitment to measure the degree of inflammation, some studies used a more precise technique. Bernthal *et al.* used in a mouse model of post-arthroplasty *S. aureus* infection genetically engineered mice that possess fluorescent neutrophils[56], which were also used in a study by Pribaz *et al.*[57]. These mice provide the possibility for *in vivo* whole animal imaging to quantitatively measure inflammation in real-time, without requiring euthanasia of the animal. Another method used for quantifying the amount of neutrophils is measuring the myeloperoxidase (MPO) activity on homogenized tissue[58]. The MPO activity closely approximates the number of neutrophils.

Sarda-Mantel *et al.* evaluated the use of scintigraphy for detection of prosthetic joint infections[51]. To be able to discriminate between infected and uninfected prosthetic joint,s an antimicrobial peptide which specifically targets bacteria was used. This peptide was labeled with a radioactive compound which emits gamma radiation. A camera which can measure gamma radiation wash used for imaging. This study showed that using scintigraphy was helpful in ruling out infection when there was none. However, further studies are needed to examine the feasibility of using this technique in animal models and for discriminating between prosthetic joint infection and aseptic loosening in patients.

Chapter 4: Discussion

The current methods for prophylaxis and treatment of infections of orthopedic implants are not adequate yet, which is a growing problem due to the growing number of arthroplasty procedures[21]. The treatment of an infection could be improved when the difficulties associated with detecting whether an implant is infected and specifying the responsible microorganism are overcome.
In this thesis, the current status of treatment and prevention of infected orthopedic implants was examined. Furthermore, the latest animal models and techniques used in these experiments were summarized.
An interesting difference between patients and animal experiments is the use of blood tests. As most animal experiments involve local bacterial infections, blood tests are not often performed because the result would be negative, as these animals do not develop a hematogenous infection. Furthermore, blood tests cannot be used to measure the extend of the infection, nor to identify the causing agent. A more sensitive method is culturing the fluid and tissue surrounding the implant, and specifically detecting bacteria by using PCR or FISH. However, this requires biopsies which can be a burden for the patient.
Imaging techniques can be used for evaluating signs of infection in human patients, however, the most frequent used techniques, radiographs and CT-scans, are not 100% accurate. A possible alternative can be the use of scintigraphy by labeling the bacteria with a radioactive peptide. This technique is already used for other medical applications like detection of breast cancer[59], however, further studies are needed to examine the feasibility of using this technique for discrimination between prosthetic joint infection and aseptic loosening in patients.
A huge disadvantage of treating infected implants is the presence of a biofilm which renders the effects of antibiotics useless. Currently, no solutions are available for this problem, except for replacing the implant. Ideally, the formation of a biofilm is prevented by selectively eliminating all bacteria surrounding the implant, however, the current techniques are not sufficient. A possible solution can be applying a coating on the implant surface, with antimicrobial and antibiofilm properties. Ideally, the coating should be degradable so the high levels of antibiotics are temporary, as high concentrations can be disadvantageous for bone formation.
Summarizing, new techniques are needed for easily detecting an infection of an orthopedic implant, and for specifying the organism responsible for the infection, so infection can be prevented and treated more accurately. For solving this problem, various animal models are currently available to test new antibiofilm or antibiotic strategies, or to test novel techniques.

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