Efficacy of bioactive glass as a bone substitute in Masquelet therapy in the treatment of tibial and femoral pseudarthrosis

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1) Summary:

In the treatment of pseudarthrosis, the application of autologous cancellous bone is still considered the gold standard. However, the possibilities for autologous cancellous bone harvesting are limited, and the morbidity of the harvesting is a considerable burden for the patients. Therefore, there is an intensive search for suitable bone substitute materials.
Bioactive glass (S53P4) is an established synthetic biocompatible bone graft substitute which stimulates bone formation and improves blood supply in the affected areas. Furthermore, bioactive glass is established in the therapy of bone inflammation. The therapy of large bone defects consists of a two- or multi-stage procedure and is called Masquelet therapy. In a first procedure the bone is locally conditioned and in a second procedure the bone defect is filled with a combination of autologous bone and tricalcium phosphate. This autologous bone is obtained from RIA (Reaming-Irrigating- and Aspirating) material of the ipsi- or contralateral femur or from iliac crest cancellous bone with tricalcium phosphate. However, this requires another surgical procedure to obtain the bone graft, which is associated with risks and may cause additional problems for the patient. Multiple studies have shown that filling bone defects with bioactive glass leads to new bone formation and bone regeneration. This could lead to the elimination of the need for autologous bone grafting, thus avoiding the need for further surgical intervention. The planned clinical study will compare the effects on osseus consolidation of pseudarthrosis at the tibia and femur between standard Masquelet therapy (G1) and Masquelet therapy using bioactive glass as bone graft substitute (G2). The study will include 50 patients with pseudarthrosis of the femur and tibia in a prospective randomized design. After randomization, 25 patients will be treated with either Masquelet therapy (G1) or Masquelet therapy using bioactive glass as replacement material (G2).
The clinical course will be evaluated preoperatively, 2 days, 6 and 12 weeks and 6,9, 12 and 24 months postoperatively. In addition to clinical and nativradiological standard control examinations, perfusion is examined by CEUS ultrasound (contrast enhanced ultrasound) and dynamic contrast enhanced MRI (DCE -MRI) preoperatively and 12 weeks postoperatively. Furthermore, blood samples are taken to check the infection levels, local blood flow situation and bone growth markers.

2) Introduction / Scientific Basis:

Bone has the ability to regenerate after injury in a complex physiological process. However, this process can be disturbed in extensive injuries and critical blood supply or infectious situations and delayed fracture healing is a serious complication in trauma surgery (1). Depending on the individual patient risk, 5-10% of all fractures lead to pseudarthrosis. The tibia is most frequently affected (2). Pseudarthrosis is not only a common complication but also leads to serious consequences. Pain, immobilization, and loss of the affected limb pose significant limitations for patients (3). The complex pathophysiology of delayed or failed fracture healing requires multifactorial treatment. The so-called Diamond concept (4) consists of the following components:

- Biomechanical stability: angular stable plates, dynamic intramedullary nails.
- Osteogenic cells: mesenchymal stem cells, autologous cancellous bone (iliac crest or RIA)

- Osteoconductive structures: autologous cancellous bone, synthetic bone substitutes
- Growth hormones: bone morphogenic proteins (BMP-2 and BMP-7)
- Circulation: by improving macro- and micro-circulation, local induction of a Masquelet membrane and debridement of fracture ends.

Although autologous bone grafting is the gold standard for filling bone defects, it is associated with great morbidity for the patient and availability is limited (2). Therefore, in the last decades, there has been an intensive search for suitable bone graft substitutes that could lead to new bone formation and reduce the need for autogenous bone grafting in the future. Initial studies have shown that bioactive glasses have an outstanding antibacterial effect, which can be attributed to an increase in local PH and osmotic pressure (5). Surprisingly, during follow-up of these initial studies, scientists found extensive new bone formation in patients treated with bioactive glass. This led to intensive research efforts to investigate bioactive glass as a bone graft substitute. It was found that bioactive glass with the composition of SiO2, Na2O, CaO and P2O2 initiates growth of tissue and stimulates bone growth (5-9). The effect of bioactive glass is based on the release of ions (Si+, Na+, Ca2+) and their reaction with body fluids. After application in situ, these surface reactions lead to the formation of a calcium phosphate layer that bonds with solid materials such as bone (10). Numerous studies have demonstrated the clinical efficacy of this mechanism in oral and maxillofacial surgery (11, 12), spine surgery (13), and trauma surgery (5, 7-9, 13). Furthermore, numerous studies demonstrated the safety of the clinical application of bioactive glass for filling bone defects (5, 7-9, 13). However, there are no clinical studies evaluating the use of bioactive glass in masquelet therapy of pseudarthrosis. Contrast-enhanced ultrasound (CEUS) as well as dynamic contrast-enhanced magnetic resonance imaging can visualize the vascularization of pseudarthrosis in real time. In this regard, CEUS and DCE-MRI can be used preoperatively to differentiate between infectious and noninfectious pseudarthroses (14, 15). Postoperatively, DCE- MRI provides a valid tool for predicting bony consolidation of pseudarthrosis (16). Perioperative cytokine measurement of bone growth markers is a well-established method that provides an early indication of whether local infection is present or healing is likely (17, 18).
Bioactive glass (S53P4) is approved for the treatment of bone defects in pseudarthrosis in Germany.

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3) Target criteria

To compare the clinical effectiveness of bioactive glass as a bone substitute in applied Masquelet therapy with conventional Masquelet therapy in the treatment of large defect pseudarthroses (2-5 cm3) of the tibia and femur.

Clinical and radiological investigation of the osteostimulative and osteoinductive potency of bioactive glass in the treatment of large defect pseudarthrosis of tibia and femur.

To investigate the applicability of bioactive glass in the treatment of defect pseudarthrosis.

To investigate the angiogenic potential of bioactive glass in relation to the Masquelet technique.

Primary endpoints: bony consolidation within one year after surgery in conventional radiographic imaging in two planes (three of four cortices are bony bridged).

Secondary endpoints: Quality of life (SF36), perfusion (CEUS/DCE-MRI), pain (VAS), bony consolidation within two years after surgery on conventional radiographic imaging in two planes (three of four cortices are bony bridged), revisions, bony consolidation on CT one year postoperatively, postoperative infections, cytokine expression patterns.

4) Test procedure

4.1 General description

The current study will compare two groups of patients. One group will receive standard therapy for pseudarthrosis of the femur or tibia in terms of two-stage or multi-stage Masquelet therapy. In the second group, transplantation of autologous cancellous bone is omitted in the second stage of Masquelet therapy and bioactive glass is used instead. Clinical follow-up of pseudarthrosis patients is standardized in our department and no additional examinations are performed as part of the study. As an extension of the clinical follow-up examinations, only blood samples will be taken as part of the study to determine growth factors, infection parameters, and blood flow markers. The total collection volume of 32 ml of whole blood per follow-up is small.

4.2 Effects

Bioactive glass with the composition of SiO_2, Na_2O, CaO and P_2O_2 initiates growth of tissues, stimulating bone growth (5-9). The effect of bioactive glass is based on the release of ions (Si^+, Na^+, Ca^{2+}) and their reaction with body fluids. After application in situ, these surface reactions lead to the formation of a calcium phosphate layer that bonds with solid materials such as bone (10). Numerous studies have demonstrated the clinical efficacy of this mechanism in oral and maxillofacial surgery (11, 12), spine surgery (13), and trauma surgery (5, 7-9, 13). Furthermore, numerous studies demonstrated the safety of the clinical use of bioactive glass for filling bone defects (5, 7-9, 13). If these results are confirmed in the current study, this represents a promising possibility for avoiding the additional intervention required to obtain the bone graft in the future.

4.3 Adverse effects/risks

Bioactive glass (S53P4) is approved for the treatment of bone defects in pseudarthrosis in Germany. Risks are assumed to be the usual surgical risks, but the indication for surgical therapy will be based on purely medical reasons and will be completely independent of the study. Furthermore, the patient may not respond to the therapy, which may result in

persistent pseudarthrosis. However, this risk exists for both standard therapy and Masquelet therapy with bioactive glass as bone substitute. Follow-up examinations are performed according to the same standard that is used for all patients treated in our clinic. Thus, there is no undesirable burden on the patient. Only blood samples are taken in addition to the standard. Possible undesirable effects of this blood collection correspond to the blood collection that is normally performed in the clinic or by the general practitioner. The amount of blood drawn includes 4 tubes, which is equivalent to 32 ml of blood. This has no relevant influence on the total amount of blood. However, possible undesirable side effects may still occur:
Pain at the puncture site, secondary bleeding, hematoma ("bruise"), infection (very rare), vagal reaction with hypotension, nausea and possibly syncope ("fainting"), dizziness and feeling of weakness after blood collection.

5) Study design

Investigator initiated Study
Clinical post-marketing observation study
Prospective randomized pilot clinical trial (RCT)

6) Randomization procedure

Balanced randomization, patients will be alternately assigned to treatment group G1 and G2 according to randomization time point.

7) Inclusion criteria

Patients suffering from pseudarthrosis of the tibia or femur with large bone defect (2-5cm3) and operated by Masquelet technique.

8) Exclusion criteria

Patients under 18 years of age
Patients who do not consent to participate in the study.
Patients who require amputation of the affected limb due to persistent infections or extensive soft tissue defects.

9) Discontinuation criteria

General: preliminary data or external data indicate unexpected risks to patients.

Individual: patients wish to discontinue their participation in the study

10) Study procedure

Patients suffering from pseudarthrosis with a large bone defect (2-5 cm3) will be treated with the standard masquelet technique.
After written and verbal information to the patients, a total of 50 patients will be randomized. All patients will receive a CEUS ultrasound examination and a DCE MRI examination preoperatively to evaluate local perfusion.
Subsequently, in the first step of the Masquelet technique, inset implants are removed, the pseudarthrosis is debrided, and a PMMA cement spacer is inserted after reduction. Either internal or external fixation is performed depending on stability and soft tissue conditions. During the first procedure, tissue samples are taken for microbiological examination. If germs are positive, this procedure is repeated until all microbiological samples are germ-free. Step two of the Masquelet technique is then performed six weeks postoperatively. The PMMA cement is completely removed and the bone defect is filled with a combination of autologous bone and a tricalcium phosphate matrix (G1) or bioactive glass (G2).
Subsequently, all patients receive the same standardized follow-up examinations. Initial clinical and native-radiologic follow-up examinations are documented two days postoperatively, and patients are discharged to outpatient care as soon as soft tissue conditions permit. All patients also receive physical therapy in the outpatient setting. Clinical and radiological follow-up examinations in our outpatient clinic are performed 6 weeks, 3 months, 6 months, 12 months, and 24 months postoperatively (see Table 1). Signs of infection, range of motion of adjacent joints, pain (Visual Analog Scale), and weight-bearing are obtained during the clinical examination. All radiological data are collected as part of the clinical necessity; no additional radiological examinations are performed.
A questionnaire is used to document preoperative and 3,12 and 24 months postoperative information on pain and range of motion, ability to work and quality of life.
General patient data such as occupation, BMI, profession, risk factors, prior medication, prior surgery, and accident data will be documented preoperatively. One tube of special EDTA and three serum tubes of blood (9 ml each) will be collected from all included patients in chronological order on the above mentioned examination days. After this, the blood is centrifuged within two hours and stored at -80°C. After this, the blood samples are analyzed for bone formation and bone resorption substances, infection values and markers for perfusion. Local perfusion is assessed preoperatively and 12 weeks postoperatively by CEUS and DCE-MRI. Patients in both study groups will be divided according to clinical and radiological criteria into Therapy responders and therapy failures. After 12 months and after 24 months, all data will be statistically analyzed. A total of 50 patients will be included for a period of two years, and the follow-up period will also be two years. The total study duration is therefore four years.

	preoperative	2 days postop.	6 weeks postop.	3 months postop.	6 months postop.	9 months postop.	12 months postop.	24 months postop.
Clinical examination	X	X	X	X	X	X	X	X
X-ray	X	X	X	X	X	X	X	X
DCE-MRI				X				
CT							X	
CEUS	X			X				
Questionnaire SF-12	X			X			X	X
Blood sample	X	X	X	X	X	X	X	X

Table 1: All radiographs and CT scans were performed as part of the structured clinical follow-up.

11) Statistical design

Primary and secondary endpoints will be evaluated categorically. The analysis will be non-parametric using Wilcoxon test ($p <= 0.05$). All collected data will be analyzed and presented descriptively.

12) Ethical and legal aspects

12.1 Ethical principles

The study will be conducted in accordance with the Declaration of Helsinki as amended in October 2013.

12.1.1 Patient/subject information/consent form

Patients will be informed about the study in advance, both verbally and in writing. Participation is voluntary and patients will provide written informed consent.

12.2 Legal basis

The study will be conducted in accordance with the Declaration of Helsinki and the

Professional Code of Conduct for Physicians of the State Medical Association of Baden-Württemberg in the respective current versions.

All X-ray examinations performed will be performed as part of routine clinical follow-up examinations. No additional radiographs will be performed as part of the study.

12.2.1 Vote of the Ethics Committee

The study plan will be submitted to the Ethics Committee of the Heidelberg Medical Faculty for review prior to study initiation. The inclusion of subjects/patients will not be started until the written vote of the Ethics Committee has been received.

12.2.2 Data protection/view of original medical records

The nam es of patients and all other confidential information are subject to medical confidentiality and the provisions of the German Federal Data Protection Act (BDSG). Patient data may only be passed on in anonymized form. Third parties will not be given access to original medical records.

12.2.3 Data destruction in case of withdrawal

In the event of withdrawal from the study, all data not already published will be destroyed, unless the patient has given prior written consent for the data already collected to be evaluated.