Orthoss®, the natural choice in bone regeneration. The excellent biofunctionality makes Orthoss® the ideal bone graft substitute. Bone regeneration materials from Geistlich have been used successfully in more than three million patients.

Chondro-Gide®, the leading natural collagen matrix in cartilage regeneration. This standardised, easy to handle matrix can be used to treat cartilage defects using both AMIC® and ACI. The product includes a sterile Aluminium Template, ideal for creating an accurate impression of the defect.
Introduction

“I don’t like implanting necrosis”

was the response of a long term user of Orthoss® when we asked why he does not use allografts as a bone graft substitute. What is the motivation for such a statement and is there truth in this conclusion?

The use of allografts has increased in the past decades. With this, new and more stringent quality measures had to be established to warrant safety. As a result, the legislation had to be adapted and has become ever more complex. To help you understand the benefits and risks associated with the use of allograft, as well as the implications of the new European Tissue and Cells Directive on allograft safety and therefore on bone banks, you will find an expert opinion on this topic in our newsletter. The article was drafted by Signifix, an independent Life Science company.

Signifix is focused on professional Regulatory Affairs and Quality Assurance consultancy services. Their specific expertise is in complex medical devices or combination between medical devices and human tissues and cells and/or active ingredients. The company has extensive experience in FDA approval processes, FDA pre-approval processes, CE marking, local authorisations as well as other international approvals. One of Signifix core competences is in the area of human tissue based products such as regenerative medicine products, bone allografts and demineralised bone matrix.

This is substantiated by the fact that they have successfully filed several FDA 510(k) applications for various orthopaedic bone graft implants including allografts. Furthermore, Signifix led the implementation of appropriate quality systems, procedures and safety requirements at various tissue establishments according to the European Tissue and Cells Directive (EUTCD) regulation. This also included obtaining license approvals for these establishments, as well as performing audits of human tissue processors and distributors in both the USA (American Association of Tissue Banks standards) and the EU (EUTCD standards).

Orthoss® offers an alternative to allograft as a natural bone graft substitute. Over 20 years of clinical experience and several hundred publications have shown that Orthoss® is an ideal bone graft substitute which is safe and efficient. What have we done to make this product safe?

The provided overview – Safety Standards of Orthoss® – not only elaborates on the testing involved during development and production process but also includes expert opinions and statements from legislative bodies and the Red Cross, about the safety of Orthoss®.

Why is Orthoss® an ideal bone graft substitute and why is the biofunctionality of Orthoss® superior to that of commercially available solvent dehydrated allografts? The research analysis department at Geistlich Pharma AG has compared the morphology of these two products to answer these questions for you. The results are summarised on pages 11–13.

We kindly thank Dr. Andrea Camera and Dr. Gabriele Cattaneo from Pietra Ligure as well as Dr. Mario Spinelli and Dr. Paolo Gabellieri from Livorno and Cecina for providing excellent case reports which illustrate the use, properties and efficacy of Orthoss® in critical size defects. The first case describes the reconstruction of a tibial bone defect with 40 cm³ Orthoss® in TKA revision surgery. In the second case Orthoss® was supplemented with concentrated bone marrow aspirate, as a composite bone graft solution, and successfully used in an open tibial fracture.

Geistlich Surgery is a specialist in the regeneration of bone and cartilage. The natural matrix structures possess optimal characteristics for bone and cartilage regeneration with a high biologic tolerance and are trusted by surgeons around the globe.

Geistlich Surgery
September 2010
Is the European Tissue and Cells Directive the Holy Grail for Safe Allograft Bone?

Jeroen Pieper, Richard van der Linden, Eliane Schutte.
Signifix BV, Bilthoven, The Netherlands

Introduction
The use of bone grafts in reconstructive orthopedic procedures has markedly increased over the past decades (1–4). Various options are at the surgeon’s disposal. Autograft remains the “golden standard” (2, 26). It contains all the essential elements to optimally support and enhance new bone formation. In addition, its use diminishes the risk for infectious disease transmission and there is no immune response. Depending on the surgical procedure, however, its availability may be limited. Moreover, harvesting of autograft can be associated with significant donor site pain and even morbidity. Allograft bone is often used as an alternative (1–4).

This paper reviews the benefits, risks for disease transmission and concerns associated with the use of allograft bone. In addition, the implications of the new European Tissue and Cells Directive on bone allograft safety and the bone banks from which they originate are addressed.

Allograft Bone
Allograft bone, by definition, is bone harvested from one individual and implanted into another of the same species (2). It was the first readily available alternative to autograft and is indicated for orthopedic procedures such as impaction grafting, defect filling, (revision) arthroplasty, and spinal surgery (2, 4, 26, 28). Allograft is used to provide structural support during bone healing and to act as scaffold for the ingrowth and formation of new bone. This osteoconductive nature combined with the original trabecular porosity of allografts makes them a suitable alternative to autograft for the repair and remodeling of bone defects. Allograft bone can be delivered fresh frozen or freeze dried and is available in various shapes and sizes including chips, blocks, wedges, dowels, screws and structural cages. Benefits of allograft bone include its availability, decreased operative time and blood loss as well as its established performance.

The major concern with the use of allograft bone, however, is the risk for the transmission of infectious diseases from the donor. Furthermore, as a donor derived material, variations are to be expected in biomechanical and bone regenerating performance.

Brief History of Allograft Bone and Bone Banks
The first depicted musculoskeletal transplant shows the legend of Cosmos and Damian dating to the 3rd century (4, 28). The Saints are depicted in a 15th century painting performing a posthumous miracle by replacing a dissected limb of a church member with the lower extremity of a deceased Moor.

One of the first modern uses of allograft bone was reported in 1887 on a successful transplantation of a tibial graft from one child to another (28). Significant progress in bone banking came mid 20th century as a result of the military need to treat war injuries combined with the development of new bone processing methods such as freezing, freeze-drying, demineralization, and irradiation. The first tissue bank was established in 1949 in the US. This was followed by a rapidly expanding international network of bone banks to meet growing demands with a focus on civilian need. In Europe, allograft bones were commonly procured, processed and supplied by local internal hospital bone banks (11). In many countries tissue and bone banks also cooperate with intermediary organ centers which take care of procurement and allocation of tissues. Examples include the “Transplantation Services Authority” in the UK, and the “Etablissement Français des Greffes” in France.

Picture of an oil painting attributed to the Master of Los Balbases, Burgos, Spain, 1495. The miracle represents the replacement of an ulcerated leg of a Christian verger by an undiseased leg of a dead Moor by the Saints Cosmos and Damian.
Brief History of Tissue Legislation
National legal frameworks related to tissue transplantation have been in place prior to the implementation of the European Tissue and Cells Directives. Bone banks, however, were essentially autonomous and self-regulated on quality and safety aspects. They established their own protocols and procedures based on different developing standards from various scientific organizations such as the American Association of Tissue Banks (AATB), Centers for Disease Control and Prevention, the European Association of Tissue Banks, the European Association of Musculo Skeletal Transplantation (EAMST), the Council of Europe Guide to Safety and Quality Assurance for Organs, as well as guidelines from national competent authorities such as the Human Tissue Authority (UK) or the Paul-Ehrlich Institute (Germany). European initiatives for the harmonization of legislations of Member States related to the removal, grafting and transplantation of human tissue started as early as 1978 (12). Various working groups within the European Council developed standards and policies related to ethical, organizational, legal and technical aspects. This resulted in the publication of the Guide to safety and quality assurance for organs, tissues and cells in 2002. It is this Guide which became the major reference for the European Union Tissue and Cells Directive (EUTCD) as prepared by the Directorate General for Health and Consumer Affairs (also known as DG Sanco).

European Union Tissue and Cells Directives
The European Union Tissue and Cells Directives (EUTCD) provide a harmonized framework for the regulation of the quality and safety of human tissues and cells across Europe. It is aimed to safeguard public health, to prevent the transmission of infectious diseases and to facilitate exchange of human tissues by ensuring the same high quality and safety across the EU. The EUTCD is comprised of the parent Directive 2004/23/EC of 31 March 2004 and the two implementing technical Directives 2006/17/EC of 8 February 2006 and 2006/86/EC of 24 October 2006 which accompanied it. Specifically, these Directives set the standards for the quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. In addition, they provide a system for the traceability of tissues from donation to patient and the requirements for tissue establishment accreditation and licensing for aforementioned tissue activities. Directive 2004/23/EC came into force in April 2006 and was transposed into national law by the majority of the Member States in 2007–2008. National law, however, allowed for the inclusion of additional local requirements such as for example on additional serological tests, making it a non-uniform interpretation of the Directive. Italy, Belgium and Denmark, for example, are part of a minority Member State group who have implemented Nucleic Acid Testing (NAT) for HIV-1, Hepatitis B Virus and Hepatitis C Virus for donor release next to the minimum serological testing requirements of the 2006/17/EC Directive.

Implications of the EUTCD for bone banks
A direct implication of the EUTCD for bone banks is the requirement to establish procedures and ensure quality assurance and control for aspects like donation, procurement, processing, storage and distribution. Furthermore, a traceability system needs to be in place from donor to patient and back. While these EUTCD requirements did not necessarily alter the basic operating principles for most bone banks, it resulted in substantially increased organizational requirements and complexity (8, 11). In addition, the EUTCD requires all bone banks to be licensed and controlled by the national competent authority. As a result, various small local bone banks which existed within individual hospitals were replaced by central and regional tissue establishments which serve the need for hospitals in the region. Not all bone and tissue banks were faced with additional organizational complexity. In France, for example, tissue banks already required inspection and accreditation by AFSAPS prior to the implementation of the EUTCD, making it a more natural transition for the majority of these establishments (7). Denmark was one of the first countries to transpose and implement the EUTCD. The Directive stimulated extensive reorganization of bone banks in this country (9). The majority of the bone banks transferred their activities, at least partially, to public blood banks or departments of clinical immunology. This is attributed to the experience with managing quality systems and ability to implement the required serological testing regime. It is noteworthy that some surgeons fabricated parts of the safety documentation to avoid what was seen as unnecessary donor questioning. As part of the EUTCD a questionnaire related to the donor’s risk behavior is mandatory. Risks for disease transfer were considered by these surgeons to be relatively low, not justifying the extra documentation workload. Moreover, the risk behavioral questionnaire and sexual relationship related questions in particular were seen to infringe on the trust between surgeon and patient prior to surgery.
The presence of a quality system is paramount to ensure the quality and safety of allograft bone. Requirements for such a system cover aspects like the organizational structure; responsibilities, qualifications and training; risk management; good manufacturing practices and standard operating procedures; validation (equipment, processes, disinfection, sterilization); environmental monitoring; audits and inspections. Additional national requirements may be applicable. In Germany, for example, allograft bone is considered a pharmaceutical according to the German Medicines Act requiring bone banks to have a pharmaceutical manufacturing license (11).

The Responsible Person is per the EUTCD responsible for donor release. In Italy, Belgium and France these Responsible Persons are directly affiliated with tissue banks. Companies involved in tissue activities and selling allograft in these countries are required to have agreements in place with these tissue banks. It is further noteworthy that Italy is one of the EU countries with the most stringent Donor File requirements.

In Germany and Switzerland, femoral head derived allograft may follow the following route. After consent and screening, femoral heads are procured by surgeons at a hospital, tested and shipped to a commercial establishment for cleaning and sterilization. The processed allograft is subsequently returned to the same hospital for allograft implantation. While both the hospital and processor perform human tissue activities and as such require a Tissue Establishment license, the responsibility and regulatory requirements in this example are particularly demanding for the surgeon and its affiliated hospital.

Processing of allograft bone

The processing of allograft bone is not a transparent process as bone banks typically employ proprietary methods (6, 26). Examples are the Allowash (Lifenet), RICA (Allosource) and Tutoplast (Tutogen) processes. In general, bones are stripped from soft tissue and frozen. Washing and extraction steps may include the use of sodium hydroxide, detergents and organic solvents such as ethanol and acetone. They are aimed to remove bone marrow and lipids, induce cell lysis, and generally clean the tissue. In addition, these solvents contribute to the inactivation of coated viruses such as HIV and the hepatitis viruses. This is typically followed by extractions in hydrogen peroxide and peracetic acid which further reduce viral and microbiological contaminations as well as residual antigenicity. As a next step the allografts can be washed in solutions containing antibiotic and antiviral agents. Finally, allograft bone is dried allowing terminal sterilization.

Aseptic processing does not provide a sterile allograft bone but rather limits the accumulation of bacteria, fungi and spores. Sterilization eliminates these microbial pathogens typically to a sterility assurance level of $10^{-6}$ (one in a million risk of a bacteria being present). Terminal sterilization, however, should not be considered as the single process to achieve sterile and safe grafts. It is the combination of donor screening, serological testing, processing and terminal sterilization which ensures a high level of allograft bone safety. Potential sterilization and inactivation methods include gamma irradiation, chemical sterilization such as the peracetic acid-ethanol treatment and heat inactivation (15, 17, 30). AATB (2002) and EAMST (2005) have recommended a minimum gamma irradiation dose of 25 kGy for bone tissue (14, 27). Gamma irradiation, however, is known to adversely affect the biomechanical properties of bone allograft in a dose dependent manner. Processors may even sterilize allograft bone at doses as up to 50 kGy. Chemical inactivation may be impeded by the limited graft permeability and the presence of residual solvents, whereas human viruses and bacterial spores may have considerable thermal resistance (29).

Transmission and testing of pathogens

Risks for the transmission of viral and microbial pathogens remain one of the main concerns with the use of allograft bone (4,5,10). It is the quality of the donor bone which is the primary parameter determining the safety and quality of the final allograft. While the applied practices of donor screening, testing, allograft processing and sterilization have been successful in substantially reducing the risk for viral and bacterial infections, risks still exist.

Viral pathogens

Three cases of Human Immunodeficiency Virus (HIV) infection were reported in 1992 related to tissue recovered in 1985. As the donor was only recently infected, serological testing was negative on HIV-antibody formation (18, 20). Li et al (19) reported a case of HIV infection dated in 1996 following transplantation of allogeneic bone. Two cases of Hepatitis C Virus (HCV) transmission were reported in 1992 (23, 24). In one of them, the recipient became infected following transplantation of a non-disinfected femoral head from a donor who became infected with HCV as a result of a plasma transfusion in 1985 (21). In 2002, HCV was transmitted to four recipients of musculoskeletal allografts from a seronegative donor (11, 31). Tissue banks standard only screen a limited number of known viruses. These include HIV 1 and 2 (anti-HIV-1,2), Hepatitis B Virus (HBsAg, anti-HBc), HCV (anti-HCV-Ab) and Syphilis (Treponema pallidum bacteria). Human T-lymphotropic -1 Virus (HTLV-I) testing is performed for donors originating from endemic countries. This leaves the opportunity for the transmission of unknown or unscreened pathogens. Examples are emerging diseases such as the West Nile Virus causing encephalitis as well as zoonosis which refers to infectious diseases such as the Avian Influenza virus H5N1 which can be transferred from animals to humans.
Testing
Both Enzyme-Linked Immuno Sorbent Assay (ELISA) and the Nucleic Acid Testing (NAT) are used for serological testing of viruses. ELISA analyses the presence of specific immunoglobulins as a result of the cell mediated immune response, whereas NAT directly measures viral RNA/DNA. False positive results during serological testing can be attributed to the presence of hemolysis and the post mortem time of blood sampling while false negatives can be due to hemodilution and the delay between withdrawal of the blood sample and testing. False negative results for NAT can potentially result from small changes in the genome of the virus. NAT testing reduces the window phase (the time from infection until it can be detected with a test) for seroconversion and is thus favored for the early detection of infectious diseases. In addition it is a more powerful assay with higher sensitivity. As a result and as previously indicated, there is a tendency for several countries to implement NAT for donor testing which contributes to testing inconsistencies at the European level. In this respect, AATB testing requirements which include NAT testing for HIV-1 and HCV, reduce allograft risk by reducing the window phase and improving sensitivity for testing of these viruses when compared the antibody testing requirements of the 2006/17/EC Directive alone.

Non-viral pathogens
Tissue transplants are primarily contaminated by pathogens originating from the donor (11, 15, 17, 26). Microbial pathogens are generally transported into the bone during bacteriaemia, i.e. the presence of viable bacteria in the blood allowing them to be transported to other tissues. Traumatic deaths in particular provide a risk factor for contamination. Secondary contamination may occur during tissue procurement, processing and final implantation of the bone allograft. Pathogenic microorganisms such as bacteria and fungi can potentially induce post-operative infections, bone healing complications, aseptic shock and even death (18, 22). Microbial contamination rates as high as 92% have been reported (16). Studies have shown that bone allograft harvested from morgues (within 24 hrs after cardiac arrest) revealed significantly higher infection rates (48%) than those harvested from multi-organ procurements (directly after explantation of vascularized organs) (11%) (10). This can be explained by the lead time between time of death and harvesting. In spite of refrigerated conditions, the slowly decreasing body’s temperature allows for the rapid proliferation of micro-organisms within several hours. In addition, the pathogenicity of bone grafts procured from the morgue (63%) is much higher those procured in the operation theater (32%). With respect to the latter, it is simply the case of aseptic surgical techniques, gowning requirements and air controlled operating rooms, risks for allograft contaminations exists.

The number of people present during procurement has also been shown to influence these risks. Further, the duration of the procurement also needs to be considered as an average increase in bone graft contamination of 95% per hour has been calculated (10). Infection rates for serious to deep infections of up to 17.5% have been reported following head allografts obtained from living donors (11, 17, 18). It is not always possible to correlate recipient infection with a contaminated allograft bone. Only a minority of the surgeons routinely swab allograft for bacterial culture evaluation prior to surgery (16). Observed infections can originate from the allograft bone, intra-operative contamination or endogenous factors related to the patient and its related comorbidities like diabetes.

There have been no reports on the transmission of infectious agents (prions) causing the chronic degenerative nervous diseases Transmissible Spongiform Encephalopathies (TSE), including variants of Creutzfeldt-Jakob disease in humans. Bone is classified as a tissue with no detectable infectivity for TSE. Further, donor screening specifically includes a risk assessment for the transmission of diseases caused by prions.

Adverse events
Mroz et al (22) reviewed musculoskeletal tissue recalls by the FDA between 1994 and 2007. A total of over 59,000 musculoskeletal allograft specimens were recalled in this period accounting for 96.5% of all recalled allograft tissues. Recalls were primarily related to improper donor evaluation, graft contamination, recipient infection and positive serology. It should be noted, however, that the majority of the recalls (approx. 28,000 grafts) originated from improper donor recovery from the procurement bank Biomedical Tissue Services (BTS).

In 2006, employees of BTS, New Jersey, USA, were convicted for illegally harvesting donor bone and other cadaver tissues (6, 22). Consent forms were forged, bone was harvested under unsanitary conditions, not tested according to applicable regulations, and illegally sold to medical companies for further processing. The facility was not AATB accredited. Despite the presence of well established AATB tissue banking standards and a rigorous oversight system at that time, suspect grafts have been implanted into patients. One patient was infected with HBV as a result of allograft transplantation from which the bone originated from BTS.

In December 2008, AFSSAPS issued an alert letter to national Competent Authorities for the EUTCD related to a recall of bone-derived products from which the bone donations originated from the Bulgarian tissue bank Os- teo Centra Bulgaria. Critical and major deficiencies were found related to procurement activities. There were serious concerns regarding traceability and validity of blood samples labeling and donor records. It is these aforementioned adverse events which makes allograft bones susceptible to the public opinion.
Conclusion
It is highly unlikely that the depicted Saints Cosmos and Damian appropriately performed donor screening and testing to minimize patient risks, which made the first allograft transplantation a true miracle indeed. To date, however, the EUTCD with its harmonized standards and quality requirements for tissue establishments unequivocally adds in ensuring the highest quality and safety of bone allografts.

Risks for the transmission of infectious diseases are considered to be extremely low. They are, however, inherently associated with donor derived materials and as such still exist. Transmission of viral and non-viral infectious pathogens thus continues to be the most serious concern. It is therefore the orthopedic surgeon’s responsibility to inform patients on both the risks and benefits associated with the use of allograft bone. This not only requires a fundamental understanding on bone grafting in general. Knowledge and awareness on bone banking processes and their validations as well a familiarization with the bone bank and processor from which the allografts originate are equally important.

References
7. Caton J, Eyraud S. Five year follow up of a bone bank with more than 25,000 implanted grafts. SOFCOT, November 2003.
Orthoss® is an inorganic, natural, nanocrystalline carbonated hydroxyapatite intended for bone regeneration in aseptic indications. This includes the filling of bone voids following trauma, reconstruction in orthopaedics and in spinal surgery. The Orthoss® matrix has a macro- and microporous structure which is similar to human cancellous bone. It’s interconnecting pore structure and high inner surface area, provide an optimal osteoconductive matrix which is structurally integrated into the surrounding bone and incorporated into the physiological remodelling process.

This high degree of similarity to human bone is based on the natural origin and the patented, highly effective purification process which removes proteins and inactivates viruses and other pathogens and preserves its natural mineral structure and high porosity. These factors form the basis for the excellent biofunctionality of Orthoss®.

As a result of the excellent biofunctionality, Orthoss® is an ideal bone graft substitute which can be used alone or during composite bone grafting using autogenous bone or bone marrow aspirate when treating large defects. It was developed for the specific needs of orthopaedic surgery and has been in clinical use for over 20 years. Since 1985, more than 5 million patients have been treated using the natural bone graft substitutes of Geistlich in both the orthopaedic and dental field.

Orthoss® fully complies with the stringent safety requirements for medical devices in Europe, the USA and other countries. Among the numerous guidelines and standards that Geistlich Pharma AG complies with, the ISO 22442 is the most important and regulates medical devices which utilise animal tissue and its derivatives.

Selection and processing of the raw material
The high level of safety of Orthoss® is based on the following aspects during production:

1) Defined origin of raw materials
The raw materials used are processed from selected and certified slaughterhouses in Australia. Australia is regarded as a BSE-free country. During production, only extremity bones are used. In recent publications (WHO, 2000 and EMEA/410/01 Rev 2, 2003) bone tissue was classified as tissue with no detected BSE infectivity (category C).

2) Comprehensive traceability of the source material
Our restricted source of tissue allows an excellent control of the sourcing process and traceability. All precautions are taken to ensure that the animals from which the material is sourced from are free of BSE. The origin of the animal is verified, ensuring the Australian sourcing (born and raised).

3) Animal health tests
Ante and post-mortem inspections are required by the Australian Quarantine and Inspection Service (AQIS) before declaring the animal as fit for human consumption. Geistlich collects bone material only from this source.

4) Processing only in certified slaughterhouses
The slaughterhouses used for sourcing the bone material are AQIS approved, which forces them to closely follow stringent regulations. Necessary precautions are taken to avoid the risk of cross-contamination of the bone material with other tissue/organs.

5) Monitoring of processing
Every single step in processing is monitored by independent controllers at every time point during slaughtering and processing of the raw material.

6) Protein removal and inactivation
Orthoss® is highly purified in a patented multi-stage purification process which is highly effective in removing proteins. Heat treatment, several chemical purification steps, including a strong alkaline treatment over a prolonged period, and finally gamma-sterilisation are used. These methods are recognised as being effective in inactivating prions and viruses.

7) Quality controls
Every batch of Orthoss® is tested for purity using highly sensitive (ppm range) and validated methods for demonstrating the absence of proteins.
Tests for assessing the absence of protein material
Inhouse studies as well as three scientific studies [Wenz et. al. 2001; Benke et. al. 2001; P. Jenö, 2001] were conducted with Orthoss® to detect the presence of proteins. In these studies, a total of eleven different methods for the detection of proteins in the ppm range were used. In none of these studies, proteins could be detected in Orthoss®.

- Lowry protein assay (detection of proteins)
- Hydroxyproline content (detection of collagens)
- Amines and amino acids (detection of amines, amino acids)
- Biuret protein test (detection of proteins)
- Ninhydrin test (detection of proteins, peptides, amino acids)
- HPLC (detection of proteins, peptides)
- SDS-PAGE
  - Western Blot (immunological detection of proteins)
  - Coomassie staining (detection of proteins)
  - Silver staining (detection of proteins)
- MALDI-TOF (detection of proteins)
- Immunohistological test (detection of proteins).

Expert's report on safety with regard to BSE
An expert opinion was obtained concerning the safety of Orthoss® with regard to the risk of BSE transmission.

After evaluating the production process, the acknowledged prion specialist and BSE expert Dr. Bruno Oesch confirmed the effective inactivation of prions, the causative agent of BSE by Geistlich's proprietary production process. According to Dr. Oesch and assuming an extremely unlikely and unfavourable case within a purely hypothetical risk analysis, the probability of BSE transmission is negligible (1:40,000,000,000).

A risk analysis according to the model used by the German Federal Institute for Medicinal Products and Medical Devices (BfArM) underlines furthermore the high degree of safety of Orthoss®. The requirements with regard to BSE transmission are exceeded by far for Orthoss®.

Virus safety
The European Standard EN 12442-3:2000 Annex A provides the option to perform a literature search for demonstrating the capacity of a process to remove or inactivate potential viral contaminants. An expert opinion on virus safety of Orthoss® according to this EN standard was obtained from Dr. Hannelore Willkommen. This report states that

“The conditions of the three production stages provide strong evidence for the virus safety of Orthoss®. This conclusion needs not to be substantiated by experimental studies. The virus safety of Orthoss® meets the current requirements. Theoretical considerations and data from the literature justify this conclusion”.

Blood donation
Orthoss® patients have been incorrectly excluded from donating blood with reference to animal transplants. However, international and national authorities and institutions confirm that patients treated with Orthoss® may donate blood without hindrance.

According to the definition of the American FDA health authorities, Orthoss® is not to be considered as an animal transplant material (xenotransplant). According to the definition of the FDA, blood donation after implantation of Orthoss® has to be considered as harmless.

The Swiss Red Cross (SRK) confirms that Orthoss® is not an animal implant and points out that implantation has no influence on the ability to donate blood.

The Australian Red Cross has updated their guidelines for the selection of blood donors to the extent that individuals who have Orthoss® implanted will no longer be deferred from blood donation.

The exclusion of patients who have been treated with Orthoss® is thus unjustified and contradicts the recommendations of the FDA and the blood transfusion service of the SRK.

Certificates of international authorities
EDQM-certificate for medical devices of animal origin
Orthoss® is one of the first medical products to conform to the stringent requirements of the EDQM (European Directorate for Medical Quality). This certificate confirms that the sourcing of the raw material used for Orthoss®, as well as the manufacturing process, fulfill the safety requirements of the European Pharmacopoeia.

CE-certificate and FDA-registration
The production and strict control procedures as well as the clinical documentation were reviewed by the responsible regulatory authority. Orthoss® fulfills the relevant provisions of European Directive 93/42/EEC (Class III) and is CE-marked since 1996. Orthoss® is approved for use in patients and is certified as a medical device in all countries of the European Community. Orthoss® also received a 510(k) premarket notification as a medical device by the U.S. Food and Drug Administration in 2002 and was reregistered in 2009.
The rapidity, extent and quality of new bone formation is strongly influenced by the biofunctionality of the scaffolds used in bone regeneration. The internal structural properties such as porosity, pore geometry, pore size and pore size distribution, the inner surface area and the morphology of the scaffold are important parameters in this process.

Beside the favourable chemical composition, osteoconductive properties are promoted by an interconnecting macroporosity, a large inner surface area as well as suitable shaped pores.

The interconnectivity between the pores is a prerequisite for the formation of a vascular network as well as the migration, attachment and differentiation of osteoblastic progenitor cells throughout the defect.

The inner surface area of an interconnected pore system and the wettability are measurements determining the amount of blood, proteins and growth factors which can be absorbed and adsorbed throughout the whole matrix of the biomaterial structure. In bone regeneration, perfect impregnation of the porous bone matrix with bone marrow aspirate, bone marrow aspirate concentrate, blood or cell-culture medium is expected. The formation of a wet surface layer is necessary for a good interfacial contact between the implant and the biological environment. This can only be achieved by a fast and complete wetting of the biomaterial. Incomplete scaffold impregnation might impair cell growth and proliferation (Stähli 2010).

The following table shows a comparison of the composition, morphology, porosity and hydrophilicity of Orthoss® and mineralised solvent dehydrated allograft (Research Analysis Department, Geistlich Pharma AG, Wolhusen, Switzerland):

<table>
<thead>
<tr>
<th>Material &amp; Origin</th>
<th>Orthoss®</th>
<th>Mineralised Solvent Dehydrated Allograft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovine cancellous bone, extremity bones from mature animals. Selected &amp; certified slaughterhouses in Australia (BSE free).</td>
<td>Mineralised solvent dehydrated bone allograft.</td>
</tr>
<tr>
<td></td>
<td>Patented stepwise chemical processing followed by heat treatment to effectively inactivate prions and viruses.</td>
<td>Production process removes fats, inactivates or removes viruses, prions and bacteria but leaves collagen and organic tissue in varying amounts.</td>
</tr>
<tr>
<td></td>
<td>Anorganic, natural nanocrystalline carbonated hydroxyapatite.</td>
<td>Natural crystalline carbonated hydroxyapatite with 34 wt% organic soft tissue.</td>
</tr>
<tr>
<td>BET specific (real) surface area [m²/g]</td>
<td>80.3 ± 1.2 m²/g</td>
<td>0.62 m²/g</td>
</tr>
<tr>
<td></td>
<td>The inner surface area of Orthoss®® is over a 100 times larger than mineralised solvent-dehydrated allograft, resulting in superior osteoconductive properties.</td>
<td>The small surface area results from collagen and organic tissue practically completely blocking the pore system.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pore analysis</th>
<th>32 Vol.-%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity: 77 ± 2 Vol.-%</td>
<td>2 Vol.-%</td>
</tr>
<tr>
<td>Pore Size &amp; Distribution:</td>
<td></td>
</tr>
<tr>
<td>&lt;30nm</td>
<td>26 Vol.-%</td>
</tr>
<tr>
<td>30nm–10µm</td>
<td></td>
</tr>
<tr>
<td>&gt;10µm</td>
<td></td>
</tr>
<tr>
<td>30 Vol.-% (~10nm)</td>
<td>42 Vol.-% (~100µm)</td>
</tr>
</tbody>
</table>
Orthoss® and Allograft Comparison

<table>
<thead>
<tr>
<th>Pore analysis</th>
<th>Orthoss®</th>
<th>Mineralised Solvent Dehydrated Allograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>The porosity of Orthoss® is almost 3x larger compared to the solvent dehydrated allograft. The unique bimodal pore structure creates an ideal scaffold for vascularisation and osseointegration.</td>
<td></td>
<td>Almost no nano pores (10–30nm) are found. This is considered to be responsible for the poor capillarity and wettablity which thereby negatively influences the efficiency and biocompatibility.</td>
</tr>
</tbody>
</table>

![Graph showing pore analysis](image)

<table>
<thead>
<tr>
<th>SEM analysis</th>
<th>96x</th>
<th>97x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano pores creating excellent capillarity and resulting in spontaneous and complete penetration of fluid.</td>
<td></td>
<td>Collagen fibres are visible covering the entire fine structures resulting in a slow and incomplete hydration.</td>
</tr>
</tbody>
</table>

![SEM images](image)
Orthoss® is a natural bone graft substitute with an inorganic bone matrix which is similar to human cancellous bone. With the interconnecting pore structure and high inner surface area, Orthoss® is an optimal osteoconductive matrix which is structurally integrated into the surrounding bone and incorporated into the physiological remodelling process.

As a result of the excellent biofunctionality, Orthoss® is an ideal bone graft substitute which can be used alone or during composite bone grafting using autogenous bone or bone marrow aspirate when treating large defects.

The following summarises the advantages of using Orthoss® as a bone graft substitute:

**Orthoss® - Advantages**

- Orthoss® exhibits an excellent biofunctionality with:
  - a morphology similar to that of human bone
  - an interconnecting pore system
  - a distinct high porosity and large inner surface area comparable to human bones
  - a unique bimodal pore structure
- exceptional osteoconductivity and osseointegration
- Orthoss® is highly biocompatible with outstanding interfacial contact between Orthoss® and the biological surrounding.
- Orthoss® is incorporated into the physiological remodelling process and therefore has a volume maintaining effect during the bone healing process.
- Orthoss® combined with 25% autologous bone is sufficient to accelerate new bone formation in the treatment of critical sized defects, thereby limiting the amount of harvested bone and reducing potential complications (Thorwarth at al. 2006).
- Orthoss® distinguishes itself as an ideal carrier matrix for bone marrow cell concentrate.
- The Orthoss® blocks and granules possess good wettabiliy and excellent handling properties deriving from the high porosity and large internal surface area. The blocks are easily formed to the required shape with a suitable instrument, e.g. a scalpel.
- Orthoss® offers a very good price per volume ratio.
- The bone regeneration materials from Geistlich have been used in over 5 million patients successfully.
- Over 20 years of clinical experience substantiate the high safety and efficacy of Orthoss®.

<table>
<thead>
<tr>
<th>Orthoss®</th>
<th>Mineralised Solvent Dehydrated Allograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handling</td>
<td>The morphology of Orthoss® enables complete and spontaneous wetting without vacuum application. The implant can be directly mixed with blood or bone marrow.</td>
</tr>
<tr>
<td>Advantages</td>
<td>Excellent biofunctionality, biocompatibility and handling properties. Exceptional osteoconductivity and integration into bone.</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Stability of Orthoss® is not as high as that of the solvent dehydrated allograft as collagen and organic material have been removed.</td>
</tr>
</tbody>
</table>

SEM examination shows uncharacterisable residue on the implant surface. The impact on biocompatibility is not determinable.

A remaining risk for the transmission of infectious diseases from the donor exists.

As a donor derived material, variations are to be expected in biomechanical and bone regenerating performance.

Orthoss® and Allograft Comparison
Product developments

Orthoss® with New and Improved Vial Cap

Orthoss® is an excellent bone graft substitute, but handling the vial can damage surgical gloves or, when using instrumentation to open the vial, result in glass fragments. This is one of the feedbacks obtained from a surgeon who has been using Orthoss® for many years. Geistlich Surgery has recognised the deficits of the aluminium cap on the Orthoss® vials and has developed a new, optimised polyethylene cap.

Orthoss® is now available in an optimised packaging. The vial cap has been significantly improved so that opening the Orthoss® glass bottles is now rapid, easy and safe.

Optimised polyethylene cap
This product development was implemented as a result of the requirements of many of our customers to further improve the handling of Orthoss®.

The new cap has been injection moulded from polyethylene. The design allows easy opening when wearing surgical gloves. No sharp edges are left behind and no instrumentation is required to open the vial.

Over 20 years of clinical experience substantiate the high safety and efficacy of Orthoss®. The safety of using Orthoss® has now been increased even further with this new vial cap, which is finally available for all Orthoss® granules.

The new Orthoss® cap – simple, quick and safe to open.
Case Report
Reconstruction of tibial bone defect in TKA revision

Dr. Andrea Camera and Dr. Gabriele Cattaneo
Ospedale Santa Corona, Pietra Ligure, Italy

Introduction
Total knee arthroplasty (TKA) is being performed with increasing intensity. Various complications following total knee arthroplasty are becoming more frequent as the number of implantations increase. The average life-span of knee TKA implants is given between 10 to 15 years before a revision surgery is indicated. Revision surgery may be performed for a number of reasons with aseptic loosening being the most frequent cause of implant failure.

Extensive osteolysis is a challenging problem in revision knee surgery and has to be addressed using bone grafting options. An autologous bone graft remains the gold standard but has some limitations such as insufficient amount or quality of available autologous material, prolonged operation time, preparation of the donor site as well as postoperative morbidity of the donor site.

Orthoss® is an optimal osteoconductive matrix which is similar to human cancellous bone. It is structurally integrated into the surrounding bone and incorporated into the physiological remodelling process. The Orthoss® matrix offers a volume maintaining effect during the bone healing process unlike most synthetic materials where rapid resorption results in mechanical destabilisation.

Patient history and diagnosis
An 83-year old patient was presented with pain and poor function (Tegner score 55) in the left knee 5 years after primary TKA. The preoperative radiograph showed severe aseptic loosening of the tibial component due to polyethylene debris (figure 1). No particular errors were found in the positioning of the components.

Surgery and follow-up
Through an extended medial para-patellar approach, extensive debridement was performed of the tissues, which showed a major inflammatory reaction, typical of disease due to debris.

The femoral and tibial components were removed along with the polyethylene component, which was completely worn.

Pulsed lavage was performed with normal saline. A large hollow defect was found in the proximal metaphysis of the tibia (Figure 1). The defect was reconstructed with 40cm³ Orthoss® (2 x 7g with granules 2–4 mm) supplemented with peripheral blood (Figure 2). A Zimmer NexGen® LCCK (Legacy Constrained Condylar Knee) semi-constrained revision prosthesis was implanted with distal diaphyseal gripping extensions in the femur and tibia.

Post-operative rehabilitation included partial weight bearing on crutches of 30% for 15 days and 50% until the follow-up visit after 30 days.

Figure 1 – Polyethylene insert wear resulting in severe tibial osteolysis (description of defect size in right image) and failure of total knee arthroplasty.

Figure 2 – 40 cm³ Orthoss® supplemented with peripheral blood.
Results
During the outpatient follow-up after thirty days, the patient had a functioning knee with a ROM of 0-100° without pain and without signs of inflammation.

Four months post operative follow-up showed initial radiographic osseointegration of the bone substitute at the level of the hollow tibial defect, supported by clinically satisfactory findings of a ROM of 0–110°, a stable knee and absence of pain.

Discussion
The absence of rapid resorption of the bone graft material, the good functional outcome, the patient’s satisfaction and absence of pain on loading encourage us to continue using Orthoss® as a functional and mechanical support to treat severe periprosthetic bone defects in knee revision due to aseptic loosening.

Orthoss® Case Report

Figure 3 – Zimmer NexGen® LCCK semi-constrained revision prosthesis implanted after bone grafting.

Figure 4 – There was no rapid resorption of the bone graft material after 4 months, no implant migration and good function of the knee.

Six month after surgery the patient had a good outcome with a Tegner score of 90, no pain during the maximal flexion and extension (ROM 0-110°), no pain when standing for long periods and no limp during walking.
Case Report
Reconstruction of Critical Size Open Tibial Fracture

Dr. Mario Spinelli1 and Dr. Paolo Gabellieri2
1Asl 6 Spedali Riuniti di Livorno, Italy, 2Asl 6 Ospedale Civile di Cecina, Italy

Introduction
Motor vehicle collisions are a significant cause of morbidity and mortality. High-energy trauma of the lower extremity is a treatment challenge for the orthopaedic and plastic surgeons. External fixation and primary soft-tissue coverage play an important part in severe injury treatment. An external fixator allows for additional fracture corrections and secondary reconstructive procedures, essential in such severe injuries.

Patient history and diagnosis
After a motor vehicle collision a 46 year old woman was taken to our emergency room. On presentation an open tibial fracture 3b type 43-A3 according AO classification, with a lesion of the posterior tibial tendon and bone loss was diagnosed. Wide skin necrosis was present in the anteromedial region of the limb (Figure 1).

Figure 1 – Open tibial fracture 3b type 43-A3 with lesion of the posterior tibial tendon, bone loss and wide skin necrosis
Surgery and follow-up
The operation involved debridement of the open wound, suture of posterior tibial tendon, examination of neurovascular bundle and irrigation with peroxide, iodine solution and sterile saline solution. Primary soft-tissue coverage with local post-injury skin flaps was done. External fixation of distal tibia fracture was performed by placing the unilateral fixator type F4 (Citieffe). The patient received antibiotics according to our protocol for open fractures. After 48 hours we performed a second look; during the following days the wound was dressed and debridement was performed. On the anteromedial part of the lower limb appeared a clear demarcation of flap necrosis.

The imaging studies showed a bone defect and several bone fragments (Figure 3). We therefore planned a second surgery with plastic surgeons.

The external fixator bridge was transformed into an hybrid external fixator with Kirschner wires in the epiphysis, all loose bone fragments were removed and the gap was filled with Orthoss® granules, bone marrow aspirate (Cellect) and growth factors. This construct was isolated from soft tissue by a collagen membrane from Geistlich Pharma AG. Thereafter, plastic surgery was performed to achieve complete skin coverage.

Results
After 3 weeks the patient started walking with crutches with partial weight bearing. After 12 weeks, the external fixator was dynamized and remodelling of the fracture was observed (Figure 5).

After 16 weeks the fixator was removed and full weight-bearing was allowed (Figure 6). A follow-up X-ray 6 weeks after fixator removal is shown in Figure 7.

One year post-op X-ray and clinical evaluation revealed good bone consolidation and function of the lower leg (Figure 8 and 9). The bone graft is fully integrated and re-modeled. A slight equinus deformity is visibly but there is no length discrepancy.

Discussion and conclusion
Orthoss® is an ideal bone graft substitute with excellent osteoconductive properties due to it’s similarity to human bone. It is ideal for use during composite bone grafting using bone marrow aspirate concentrate, here obtained with the Cellect system.

The satisfying result of this case can be attributed to the fact that all elements of classical Tissue Engineering are present, a suitable scaffold, cells and growth factors which are adding an extra osteoinductive component indispensable for successful bone regeneration in critical size defects.
Figure 5 – Dynamization of external fixator and x-ray showed remodeling of bone graft

Figure 6 – 16 Week follow-up prior to removal of the fixator

Figure 7 – 22 Week follow-up (6 weeks after fixator removal)

Figure 8 – AP and lateral X-ray one year post-op

Figure 9 – One year follow-up with an excellent functional and esthetic outcome
Posters and Abstracts

Bone Marrow Concentrate: a novel tool for bone repair!
M. Jäger, M. Herten, E.M. Jelinek, U. Fochtmann, R. Krauspe

Abstract
Background: Recently controversy has arisen regarding the role of mesenchymal stem cell (MSC) in orthopaedic surgery with their potential clinical application in cartilage and bone regeneration. Although autologous bone grafting is still the “gold standard” to heal critical size bony defects, it is associated with significant donor site morbidity. We present clinical and experimental data of autologous bone marrow aspiration concentrate (BMAC) in patients with local bone defects. Materials and Methods: Clinical trial: 44 patients with pseudarthrosis or local bone defects (bone cysts, benign bone tumors, revision endoprosthetic surgery) underwent Jamshidi vacuum aspiration (posterior iliac crest) followed by bone marrow concentration via density gradient centrifugation (Smart prep2®, Harvest Technologies). BMAC was incubated with bovine hydroxyapatite (HA) carrier (Orthoss®, Geistlich) or a collagen membrane (Gelaspon®, Chauvin Ankerpharm). Bone defects were treated with cancellous bone grafting supplemented by BMAC/biomaterial-composit. Bone regeneration was determined by clinical and radiological examinations.

Experimental data: Mononuclear cells were counted and colony forming units (CFU-F/-ALP) determined. In addition, cellular adherence and proliferation on scaffolds was analyzed and the osteogenic potential of BMAC evaluated.

Results: All of the 44 patients showed new bone formation/healing during follow up. There was no severe perioperative complication. However, one patient showed persisting hematoma, and three other individuals had prolonged wound secretions (three required revision surgery). The average concentration factor for BMAC was 5.7 (SD: 1.01). In vitro CFU appeared earlier and were larger suggesting a higher regenerative potential in BMAC. It was shown that BMA Cells adhered on the scaffold, proliferated and displayed osteogenic differentiation with and without DAG supplementation.

Conclusion: Our interim data showed that application of BMAC is easy to handle, a safe procedure and successful in treatment of local bone defects. However, additional supplements (growth factors e.g. BMPs) might be able to improve the clinical outcome of BMAC.

Low-Power Ultrasounds as a Tool to Culture Human Osteoblasts inside Cancellous Hydroxyapatite
L. Fassina, E. Saino, M.G. De Angelis, G. Magenes, F. Benazzo, L. Visai

Abstract
Bone graft substitutes and cancellous biomaterials have been widely used to heal critical-size long bone defects due to trauma, tumor resection, and tissue degeneration. In particular, porous hydroxyapatite is widely used in reconstructive bone surgery owing to its biocompatibility. In addition, the in vitro modification of cancellous hydroxyapatite with osteogenic signals enhances the tissue regeneration in vivo, suggesting that the biomaterial modification could play an important role in tissue engineering. In this study, we have followed a tissue-engineering strategy where ultrasonically stimulated SAOS-2 human osteoblasts proliferated and built their extracellular matrix inside a porous hydroxyapatite scaffold. The ultrasonic stimulus had the following parameters: average power equal to 149mW and frequency of 1.5MHz. In comparison with control conditions, the ultrasonic stimulus increased the cell proliferation and the surface coating with bone proteins (decorin, osteocalcin, osteopontin, type-I collagen, and type-III collagen). The mechanical stimulus aimed at obtaining a better modification of the biomaterial internal surface in terms of cell colonization and coating with bone matrix. The modified biomaterial could be used, in clinical applications, as an implant for bone repair.
Characterization of Platelet Lysate Cultured Mesenchymal Stromal Cells and Their Potential Use in Tissue-Engineered Osteogenic Devices for the Treatment of Bone Defects


**Abstract**

Mesenchymal stromal cells (MSCs), seeded onto a scaffold and associated with platelet-gel, may represent an innovative treatment to improve bone repair. The preparation of MSCs for clinical use requires the fulfillment of Good Manufacturing Practice indications.

The aim of this study was to validate a Good Manufacturing Practice-grade protocol of tissue engineering for bone regeneration, seeding platelet lysate (PL)-cultured MSCs onto an hydroxyapatite clinical-grade scaffold.

Six large-scale experiments were performed. MSC expansions were performed comparing fetal bovine serum 10% and PL 5%. We demonstrated that PL lots contain high levels of growth factors possibly responsible of accelerated growth rate, since the number of colony-forming unit–fibroblast and population doublings were always significantly higher in PL cultures. MSCs were characterized for their phenotype and multilineage differentiation capacity, demonstrating appropriate features for both conditions. Gene expression analysis revealed higher expression of typical osteogenic genes of PL-cultured MSCs, when compared to fetal bovine serum MSCs. Cell transformation was excluded by analysis of karyotype, absence of growth without anchorage, and p53=c-myc gene expression. Scaffolds were precoated with retronectin before MSC seeding, MSC adhesion, distribution, and proliferation were demonstrated through the whole surface of the scaffold by scanning electron microscopy analysis or by immunofluorescence and MSC osteogenic differentiation through quantitative reverse transcriptase–polymerase chain reaction of typical osteogenic genes.

The present report offers a model of an MSC-based bioengineered device, using an hydroxyapatite clinical-grade scaffold (Orthoss®), and supports its potential use in tissue engineering to repair bone defects.
Cell therapy in bone-healing disorders

M. Jäger, P. Hernigou, C. Zilkens, M. Herten, J. Fischer, R. Krauspe
Orthopade. 2010 Apr;39(4):449-62; quiz 463. [Article in German]

Abstract
In addition to stabilizing osteosynthesis and autologous bone transplantation, so-called orthobiologics are playing an increasing role in the treatment of bone-healing disorders. Besides the application of different growth factors, new data in the literature suggest that cell therapeutic agents promote local bone regeneration. Due to ethical and biological considerations, clinical application of progenitor cells for the musculoskeletal system is limited to autologous postpartum stem cells. Here in particular, cell therapy with autologous progenitor cells in one surgical session has delivered first promising results. Based on a review of the literature and on our own experience with 75 patients, this article reviews the rationale and characteristics of the clinical application of cell therapy for the treatment of bony substance defects. Most clinical trials report successful bone regeneration after the application of mixed cell populations from bone marrow.

The association of human mesenchymal stem cells with BMP-7 improves bone regeneration of critical-size segmental bone defects in athymic rats

G. Burastero, S. Scarfi, C. Ferraris, C. Fresia, N. Sessarego, F. Fruscione, F. Monetti, F. Scarfò, P. Schupbach, M. Podestà, G. Grappiolo, E. Zocchi

Abstract
Critical size segmental bone defects are still a major challenge in reconstructive orthopedic surgery. Transplantation of human mesenchymal stem cells (hMSC) has been proposed as an alternative to autogenous bone graft, as MSC can be expanded in vitro and induced to differentiate into bone-regenerating osteoblasts by several bone morphogenetic proteins (BMP).

The aim of this study was to investigate whether the association of hMSC and BMP-7, with providing the necessary scaffold to fill the bone loss, improved bone regeneration in a rat model of critical size segmental bone defect, compared to treatment with either hMSC or BMP-7 and the matrix. In addition, we tested whether pre-treatment of hMSC with cyclic ADP-ribose (cADPR), an intracellular Ca2+ mobilizer previously shown to accelerate the in vitro expansion of hMSC (Scarfi S et al, Stem Cells, 2008), affected the osteoinductive capacity of the cells in vivo.

X-ray analysis, performed 2, 10 and 16 weeks after transplantation, revealed a significantly higher score in the rats treated with hMSC and BMP-7 compared to controls, receiving either hMSC or BMP-7. Microtomography and histological analysis, performed 16 weeks after transplantation, confirmed the improved bone regeneration in the animals treated with the association of hMSC and BMP-7 compared to controls. Pre-treatment with cADPR to stimulate hMSC proliferation in vitro did not affect the bone regenerating capacity of the cells in vivo.

These results indicate that the association of in vitro expanded hMSC with BMP-7 provide a better osteoinductive graft compared to either hMSC or BMP-7 alone. Moreover, cADPR may be used to stimulate hMSC proliferation in vitro in order to reduce the time required to obtain a transplantable number of cells, with no adverse effect on the bone regenerating capacity of hMSC.
In Vitro electromagnetically stimulated SAOS-2 osteoblasts inside porous hydroxyapatite

L. Fassina, E. Saino, M. S. Sbarra, L. Visai, M.G. De Angelis, G. Magenes, F. Benazzo

Abstract
One of the key challenges in reconstructive bone surgery is to provide living constructs that possess the ability to integrate in the surrounding tissue. Bone graft substitutes, such as autografts, allografts, xenografts, and biomaterials have been widely used to heal critical-size long bone defects due to trauma, tumor resection, congenital deformity, and tissue degeneration.

In particular, porous hydroxyapatite is widely used in reconstructive bone surgery owing to its biocompatibility. In addition, the in vitro modification of hydroxyapatite with osteogenic signals enhances the tissue regeneration in vivo, suggesting that the biomaterial modification could play an important role in tissue engineering.

In this study we have followed a biomimetic strategy where electromagnetically stimulated SAOS-2 human osteoblasts proliferated and built their extracellular matrix inside a porous hydroxyapatite scaffold (Orthoss®). The electromagnetic stimulus had the following parameters: intensity of the magnetic field equal to 2 mT, Amplitude of the induced electric tension equal to 5 mV, frequency of 75 Hz, and pulse duration of 1.3 ms. In comparison with control conditions, the electromagnetic stimulus increased the cell proliferation and the surface coating with bone proteins (decorin, osteocalcin, osteopontin, type-I collagen, and type-III collagen).

The physical stimulus aimed at obtaining a better modification of the biomaterial internal surface in terms of cell colonization and coating with bone matrix.
Safety of autologous bone marrow aspiration concentrate transplantation: initial experiences in 101 patients

C. Hendrich, F. Engelmaier, G. Waertel, R. Krebs, M. Jäger
Orthopedic Reviews 2009; 1:e32

Abstract
The clinical application of cellular based therapies with ex vivo cultivation for the treatment of diseases of the musculoskeletal system has until now been limited. In particular, the advanced laboratory and technical effort necessary, regulatory issues as well as high costs are major obstacles. On the other hand, newly developed cell therapy systems permit intra-operative enrichment and application of mesenchymal and progenitor stem cells from bone marrow aspirate concentrate (BMAC) in one single operative session. The objective of the present clinical surveillance study was to evaluate new bone formation after the application of BMAC as well as to record any possible therapy-specific complications. For this purpose, the clinical-radiological progress of a total of 101 patients with various bone healing disturbances was documented (surveillance study). The study included 37 necrosis of the head of the femur, 32 avascular necroses/bone marrow edema of other localization, 12 non-unions, 20 other defects. The application of BMAC was performed in the presence of osteonecrosis via a local injection as part of a core decompression (n=72) or by the local adsorption of intra-operative cellular bone substitution material (scaffold) incubated with BMAC during osteosynthesis (n=17) or in further surgery (n=12).

After an average of 14 months (2–24 months), the patients were re-examined clinically and radiologically and interviewed. Further surgery was necessary in 2 patients within the follow-up period. These were due to a progression of a collapsed head of the femur with initial necrosis in ARCO Stage III, as well as inadequate new bone formation with secondary loss of correction after peri-prosthetic femoral fracture. The latter healed after repeated osteosynthesis plus BMAC application without any consequences. Other than these 2 patients, no further complications were observed. In particular, no infections, no excessive new bone formation, no induction of tumor formation, as well as no morbidity due to the bone marrow aspiration from the iliac crest were seen.

There were no specific complications within the short follow-up period and a simple intraoperative use of the system for different forms of bone loss could be demonstrated. In the authors’ opinion, the on-site preparation of the bone marrow cells within the operating theater eliminates the specific risk of ex vivo cell proliferation and has a safety advantage in the use of autologous cell therapy for bone regeneration. Additional studies should be completed to determine efficacy.
Application of a new chair-side method for the harvest of mesenchymal stem cells in a patient with nonunion of a fracture of the atrophic mandible - A case report

C. Wongchuensoontorn, N. Liebehenschel, U. Schwarz, R. Schmelzeisen, R. Gutwald, E. Ellis, S. Sauerbier

Abstract

Purpose: This case report describes a new clinical method for chair-side processing of a cell mixture which contains mesenchymal stem cells (MSCs) which was applied for the first time in the treatment of a nonunion of an atrophic fractured mandible.

Methods: Bone marrow was aspirated and a corticocancellous bone graft was harvested from the iliac crest of a 56-year-old woman with medical comorbidities and a fracture of the atrophic mandible. The fracture was stabilized with a reconstruction bone plate, and mononuclear cells including MSCs were concentrated by centrifugation and applied in combination with a particulate bone transplant. A sample of the grafted cells was characterized by flow cytometric analysis and by their ability to differentiate into various cell types.

Results: The fracture healed uneventfully. No complications occurred during the 4-month follow-up. Conclusion: Adding MSCs is a feasible alternative to enhance bone healing. This chair-side method requires little training and no cell laboratory support.
Congress Preview 2010

Geistlich Surgery will be present at a number of events during the remainder of this year. We look forward to meeting you during our symposia and congresses.

09.–11. September 2010
Vienna, Austria – 27. AGA Congress

26.–29. September 2010
Sitges, Spain – 9th World Congress ICRS
Satellite Symposium
Is it worth repairing cartilage in the Talus?
Chairman: Victor Valderrabano, Switzerland
Mesenchymal stem cells and in-situ cartilage regeneration – W. Richter, Germany
Cartilage Repair Strategies in the ankle joint – C. Becher, Germany
Osteochondral lesions of the Talus: Diagnosis and Treatment – V. Valderrabano, Switzerland

08.–09. October 2010
Bochum, Germany – Surgical Course for Cartilage and Meniscus Surgery
Operationskurs – Knorpel- und Meniskuschirurgie
Wissenschaftliche Leitung: Dr. med. Tobias Vogel
Zentrum für Regenerative Medizin und Knorpelchirurgie
Orthopädische Universitätsklinik der Ruhr Universität Bochum
http://orthopaedie.klinikum-bochum.de

26.–29. October 2010
Berlin, Germany – DKOU 2010 (Deutscher Kongress für Orthopädie und Unfallchirurgie)
Lunch Symposium
Zelltherapie bei Knochenheilungsstörungen
Chairman: Marcus Jäger, Germany
Zelltherapie bei avaskulären Nekrosen und Pseudarthrosen – P. Hernigou, France
Zelluläre Trägermaterialien zur ossären Regeneration – M. Jäger, Germany
Knochenersatzstoffe – S. Landgraeber, Germany

08.–11. November 2010
Paris, France – 85. SOFCOT (Société Française de Chirurgie Orthopédique et Traumatologique)

20.–24. November 2010
Rome, Italy – 95° SIO7 (Società Italiana di Ortopedia e Traumatologia)

Further Information
Please consult our web page for further information and invitations to the lunch symposia.

www.geistlich-pharma.com