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The Vital Potential of Filtered Bone Particles Used for Bone Augmentation

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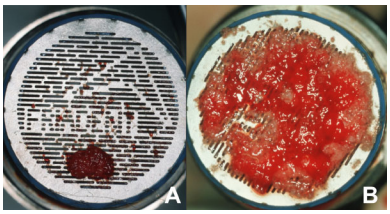
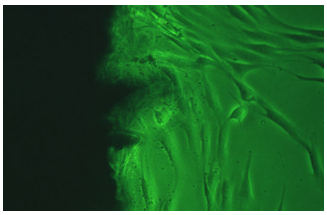
Investigation in vitro

Implant dentistry is firmly established as a component of modern dentistry and implants are frequently used for prosthetic restoration. For this reason there is increasing interest in problematic cases in implant dentistry where the lack of bone matter seems to make it impossible to insert an implant. In the past a number of methods for augmentation and transplanting of material for improving the implant bed have been developed. Examples include transplanting pelvic bone, opening and spreading the existing alveolar ridge (Khoury 1987 and 2000) and procedures for directly and indirectly sinus floor elevation (Boyne 1980, Tatum 1986, Wood 1988, Scipioni 1994, Summers 1994 and 1995, Romanos 1997, Fürst 1999). Allogenous materials such as natural hydroylapatite are also used for augmentation (Haessler 1999). Augmentation with bone particles from the FRIOS® BoneCollector has been clinically proven over a number of years and yields good results. It should be noted here that complete ossification of the particles could be observed after a short time. This fact indicates that the use of filtered bone particles results in better osteoinduction and osteoconduction compared to other augmentation materials (Müller 1985). The good clinical experience with augmentation by bone particles leads to the conclusion that autologous bone material collected with the FRIOS® BoneCollector has a type of "vital potential." This results in the assumption that the bone particles restore the bone in the augmented region and that the filtered bone particles may have a function as a type of control structure with a growth of new bone as a secondary event.
The goal of this study was to verify the vital potential of filtered bone particles for autologous bone transplant.
Bone particles can be harvested during implant surgery procedures by using a bone-collector. The collected particles are used for augmentation purposes in case there occurs a lack of solid bone structure around the implant. By means of histological studies the bone particles were explanted as an in vitro cell culture leading to confluent cultures of ostaoblastic cells. At the end of our investigation it was possible to prove the vital potential of the filtered bone partiles and the osteoblastic origin of the cultivated cells.

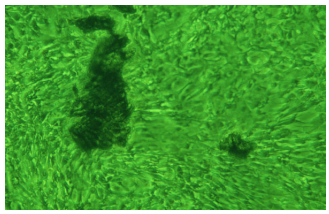
Under the microscope it could be observed that the morphology of the particles seemed to vary. Many appeared as smooth-walled, quadratic structures, others seemed to be amorphous to "jagged" at their edges. After about a week laterally spindle-shaped cells grew out of the bone particles and after further growth they reached the stage of confluence.



FRIOS® BoneCollector (FRIADENT, Mannheim). The bone particles can easily be collected during the procedure of implant surgery. A: collected bone of implant D 4.5 L 10. B: collected bone 3 implants D 4.5 L 13



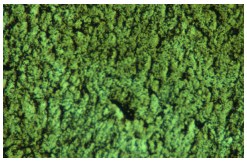
Primary cell culture, bone particle with growing spindular cells. About two weeks after collection and explantation of the bone particles spindular cells can be recognised growing out of them.

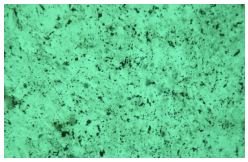


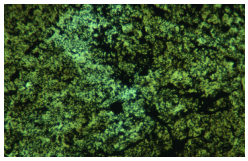
Primary cell culture, bone particle with confluent cells. Another week of breeding and the cells have reached the state of confluation. Now they can be explanted a second time in larger dishes and can be used for the following investigations.

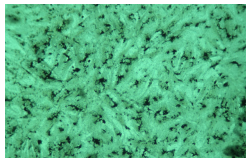
Von-Kossa mineralisation stain

For the von Kossa mineralization staining a mineralization medium based on the standard cell culture medium was started initially. It also included ascorbic acid and glycerophosphate. The cells were stimulated with this medium for approximately two weeks. After this silver nitrate was added and the illumination showed a possible calcium deposit visible as a black structure. It was also added to the nutrient medium during supplementary stimulation with dexamethasone.


Von Kossa stain, positive

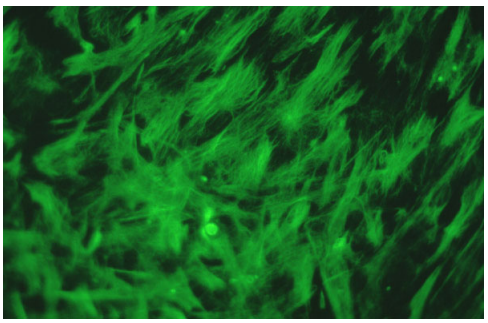

Von Kossa stain, focally positive

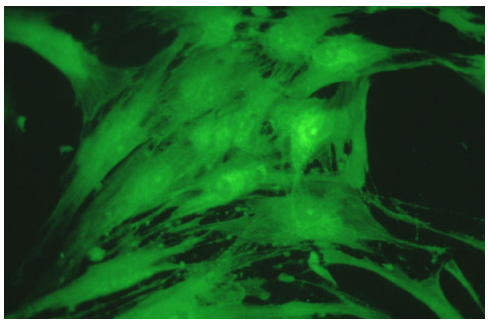

Von Kossa stain, positive after dexamethasone stimulation


Von Kossa stain, focally positive after dexamethasone stimulation

The von Kossa mineralization stains must be evaluated as positive, although here, as in all other test series, less positive, weakly positive and also negative result intensities could be observed. The Figures show different types of positive results. Furthermore the difference between the cells with and without stimulation of dexamethasone can be recognized.

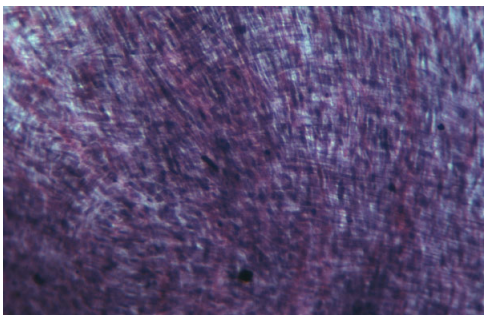
Immunohistochemical confirmation of vimentine and osteocalcine
The mesenchymal origin of the cells was histologically confirmed by the presence of vimentine and osteocalcine, a differentiation marker of mature bone cells (osteoblasts). This was done with indirect immunofluorescence (IIF). In the case of the osteocalcine vitamin D was administered for 14 days. After treatment of the first (rabbit antihuman antibody) and the second antibody with fluorochrome, osteocalcine could be observed as fine granules around the nucleus of the cell and as a filament structure under the fluorescence microscope. The presence of vimentine was confirmed after prior treatment of the cells with methanol and acetone. The antibodies against vimentine were added and the cells were cultured overnight in the refrigerator. As with the procedure with osteocalcine a second fluorochrome-coupled antibody was added to the cells. Vimentine was visible as a filament structure under the fluorescence microscope.


Vimentine


Osteocalcine

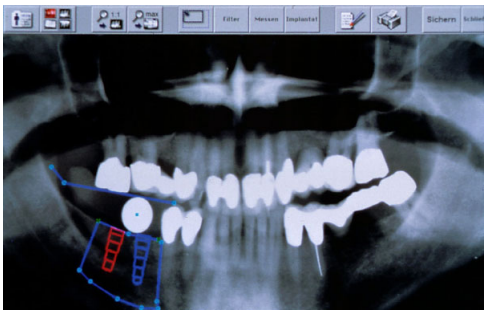
In all test series the immunohistological proves for vimentine and osteocalzine showed positive results.
In the figures the immunohistological stain imposes as granules around the nuclei and in the cytoplasm.

Staining of alcaline phosphatase
The cells were fixed with a suitable fixing medium and flushed with distilled water before adding the staining solution to stain the alkaline phosphatase (AP). The alkaline phosphatase was visible as a red stain after complementary staining with hematoxyline.

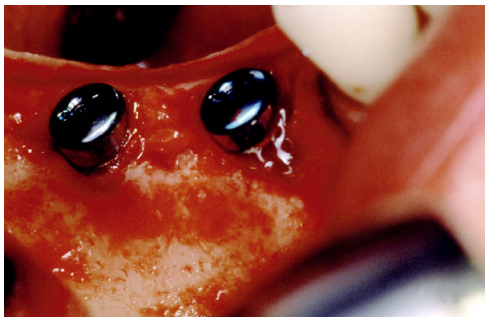

Alcaline phosphatase stain

Investigation in vivo

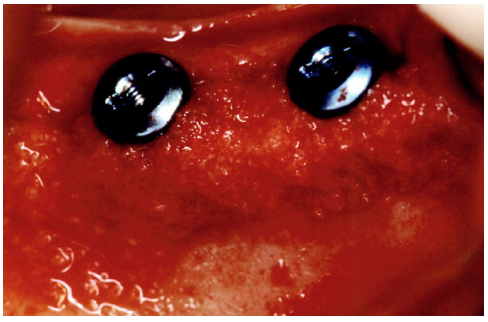
The following pictures of implant surgery and prosthetic reconstruction give an example of how the FRIOS® BoneCollector and the harvested bone particles can be successfully used for augmentation. Once the FRIOS® BoneCollector (FRIADENT GmbH, Mannheim, Germany) is mounted to the suction device of the dental unit, it can be used to harvest the bone particles emerging during the procedure of implant surgery. Special care should be taken in order to avoid the particles from being contaminated by the suction of large amounts of saliva or clotted blood. To avoid this the use of a separate suction unit might be appropriate. The harvested bone particles can easily be removed from the sieve inside the collector by the use of a surgical instrument e.g. a spoon. Before they are used for augmentation they can be stored in a small glass dish. Some patient blood and / or sterile isotonic sodium chloride solution can be added to achieve a smooth consistence and to avoid the particles from drying out. If necessary the bone particles can be added to augmentation materials like ALGIPORE®. This leads to a better osseous integration of the materials, provides an accelerated healing procedure and increases as well the amount of augmentation material available. The use of bone particles in combination with platelet rich plasma with or without additional use of allogenous materials can also be discussed.
According to Haessler and Zöller the use of the bone chips collected with the FRIOS® BoneCollector is appropriate for the augmentation of buccal three dimensional bone defects and for peri-implant defects. It is not primarily necessary to cover the augmented defects with a membrane, although it might be useful when the concerned areas cannot be primarily covered by the muco-periostal flap.



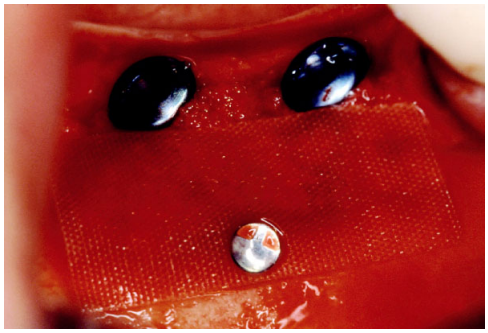
X-ray planning with the FRIACOM® planning software



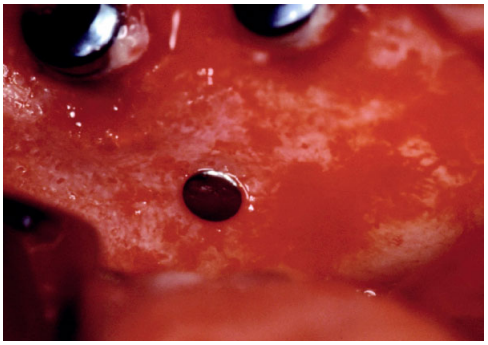
Inserted implants with a lack of solid bone around the buccal implant surfaces



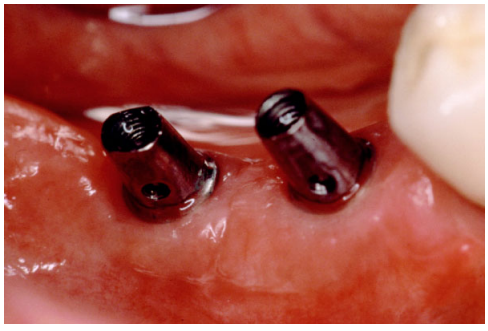
The harvested bone particles have been used for augmentation around the FRILALIT®-2 implant surfaces



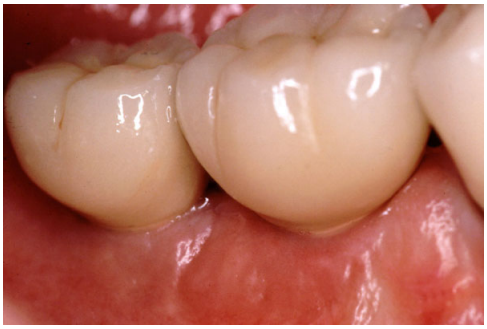
The augmentated particles are stabilized using a membrane and FRIOS® Bone Tack

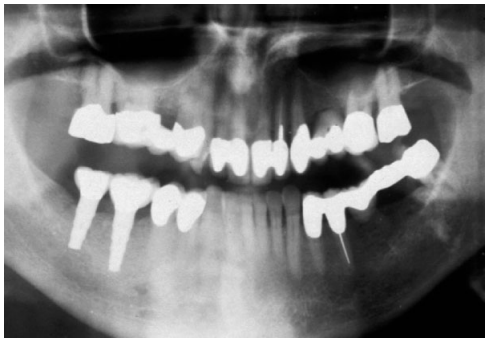


The augmentated implant site after reopening and removal of the membrane. Solid bone has settled around the implants.



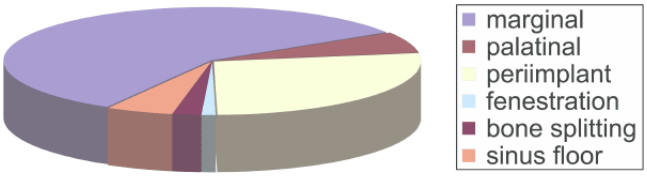
Situation prior to prosthetic delivery



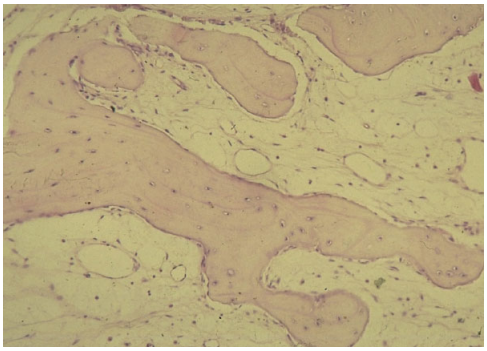


Situation after prosthetic reconstruction

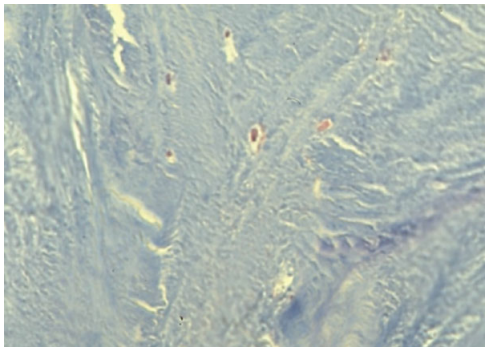
X-ray control after prosthetic reconstruction



During the treatment of 256 patients 566 implants were inserted using the bone-collector. In 57.8% the bone particles were used for marginal defects, in 6.3% for palatinal, in 27.4% for periimplant and in 1.1% for fenestration defects. In 2.1% they were used for bone splitting procedures and in 5.3% for sinus floor augmentation.



Histological finding 2 weeks after surgery: 70 % non-vital matrix 5 % new woven bone new (non mineralized) 25 % mineralized lamella bone.



Histological finding 2 months after surgery: 1% woven bone 99 % lamella bone, small matrix with small cells.

Discussion

The aim of this study was to verify the vital potential of filtered bone particles used for augmentation purposes during implant surgery. The particles were collected during the process of implantation and then explanted as an in-vitro cell culture. After five to seven days primary cells occurred growing around the collected bone particles. Having reached the confluential state of growth, these cells were used for further histological studies in order to determine markers of the assumed osteoblastic phenotype. At the end a vital potential of collected bone particles was positively recognized. The cells growing out of the particles could differentiate to an osteoblast phenotype. This and the vitality of the particles lead to the conclusion that the collected bone particles should have an osteoconductive and an osteoinductive potential.
Collected bone particles can be used as a suitable augmentation material both with immediate implant insertion and late implant insertion. The area of application is extremely variable and can extend over different situations. According to Haessler and Zöller the most frequent use of bone particles is with vestibular defects of the bony implant seat. The second indication is peripilar defects, followed by sinus floor elevations, incongruences between implant and alveole and fenestration defects. Where the defect would require augmentation higher than 4 mm, bone block transplant is a better solution. Within this limit augmentation with bone particles shows very good results. Primary strength of the augmented material can be observed after two weeks (Haessler, Zöller 1995). The suitability of bone particles as augmentation material is also based on the very safe and simple collection method with the BoneCollector. The additional option of mixing the bone particles with conventional materials such as natural hydroxylapatite also offers great flexibility in its application. It can be assumed that the osteogenic properties of the autologous bone also contribute to faster and better bone regeneration than exclusive use of allogenic bone augmentation materials. Possible contamination with saliva does not seem to result in a higher infection rate. However, further microbiological studies are required in this connection (Young 2001).
Because they are easy to use and to harvest during the surgical procedure and because they can be combined with other augmentation materials the bone collector and the collected bone chips are ideal for being used as a standard procedure for augmentation purposes in implant surgery.

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