LADDEC®
clinical review

LADDEC® structure similar to autograft bone structure (42X SEM)

LADDEC® retains natural collagen framework (7000X SEM)
Bone graft substitutes: a comparative qualitative histologic review of current osteoconductive grafting materials.

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This paper investigated the osteogenic potential of 6 osteoconductive grafting materials derived from human, bovine, and synthetic sources: HTR, BOP, Biogran, LADDEC®, Dembone, and Osteograf. Twenty-eight New Zealand rabbits were used in this study. The active group consisted of 24 animals and the control group consisted of 4 animals. The median condyle of each tibia was drilled with a 5-mm-diameter bur to form 8 mm-deep cavities. A control group included 8 osseous cavities, with 1 hole in each tibia. These cavities were washed and left unfilled. In the active group, each grafting material filled 8 osseous cavities in 8 tibiae of different animals. Half of the active and control osseous cavities were studied with scanning electron microscopy. It was concluded that LADDEC® bovine bone granules possessed the best potential for an osteoconductive grafting material, followed by the bioglass crystals of Biogran and the hydroxyapatite particles of Osteograf, respectively. The least potential for rapid bone formation was demonstrated by the copolymer of HTR and BOP, and Dembone allograft bone particles did not reveal active bone healing.

Effects of LADDEC® on the formation of calcified bone matrix in rat calvariae cells culture.

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Osteoblasts from 21 day-old fetal rats calvaria were isolated using a collagenase digestion procedure. Cells were cultured in the presence of LADDEC® (a highly purified bovine xenograft) and Bio-Oss (natural bone mineral). Optical microscopic observations showed that osteoblasts attached on the plastic culture dishes and formed close contact with biomaterial particles. By day 5, the osteoblasts formed a confluent monolayer. A cytozymatic method showed intense alkaline phosphatase (ALP) staining around and between the substrate granules of the two materials. By day 14, inverted phase microscopic observations showed that osteoblasts formed bone nodules around and between substrate particles. In addition, at this time, the Von Kossa staining was positive. Using a conventional assay, ALP specific activity was higher in the presence of LADDEC® than in the presence of Bio-Oss. A quantitative morphological method using image analysis showed that the proportion of mineralized bone formed around biomaterial particles in relation to total bone was increased with LADDEC® (15% more than with Bio-Oss). Ultrastructural observations by TEM showed the presence of an electron dense collagen-free layer at the biomaterial/bone interface and a collagenous matrix deposited at the periphery, indicating the bioactivity of the biomaterials. These results indicated that LADDEC® increased the expression of ALP in osteoblast cultures and facilitated the formation of multiple cell layers, providing a culture environment suitable for mineralization.
Biomaterials for bone filling: comparisons between autograft, hydroxyapatite and one highly purified bovine xenograft.
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Bone grafts are becoming increasingly common in orthopaedics, neurosurgery and periodontology. Twenty one New Zealand rabbits were used in the present study comparing several materials usable as bone substitutes. A 4.5 mm hole was drilled in the inner femoral condyles. Holes were filled with either an autograft (from the opposite condyle), an hydroxylapatite (Bioapatite), or a highly purified bovine xenograft (T650 Lubboc). Animals were sacrificed at 1, 3 and 6 months post implantation and a quantitative analysis of newly-formed bone volume (BNF/IV) and remaining biomaterials (BMAT/IV) was done. In addition, some holes were left unfilled and served as controls. At 6 months, there was no tendency for spontaneous repair in the control animals. The autografted animals have repaired their trabecular mass and architecture within the first month. Hydroxylapatite appeared unresorbed at six months and only thin and scanty new trabeculae were observed. The xenograft induced woven bone trabeculae formation on the first month. This was associated with resorption of the material by two multinucleated cell populations. At six months, the epiphyseal architecture was restored and the biomaterial has disappeared in most cases. Xenografts appear a promising alternative to autografts and allografts, whose infectious risks and ethical problems should always be borne in mind.

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Comparison of mechanical properties of human, bovine bone and a new processed bone xenograft.
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The study compared the mechanical properties of human bone, fresh bovine bone and a new highly purified bone xenograft: T650 (Lubroc-LADDEC®). Destructive, compressive tests were performed to determine Young's modulus and ultimate strength, with a constant deformation rate of 0.025 mm min⁻¹. The stress-strain curves obtained from all the non-human specimens especially the T650, did not differ significantly from those observed with human bone. Human and fresh bovine samples presented a significantly different Young's modulus. The T650 samples, depending upon their trabecular texture (dense or medium) also differed significantly from each other (132.9 +/− 52.3 versus 80.0 +/− 37.3 MPa, P < 0.05). Their moduli were similar to those of bovine and human cancellous bone, respectively (117.49 +/− 61.53 versus 77.36 +/− 54.96, P < 0.05). The ultimate strength of T650 dense (9.6 +/− 3.7 MPa) was similar to bovine (8.5 +/− 4.2 MPa) and human bone (8.78 +/− 5.2 MPa): the T650 medium (5.9 +/− 2.8 MPa) was significantly different from the other specimens.
Type I collagen in xenogenic bone material regulates attachment and spreading of osteoblasts over the beta1 integrin subunit.

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Xenogenic bone biomaterials have been proposed as an alternative to autografts or allografts in human bone restoring or in complement of prosthetic surgery. When appropriate treatments were applied, immunological, inflammatory, bacteriological or virological adverse responses can be prevented. However, these treatments may interact with type I collagen, the major component of the organic bone matrix. Type I collagen can bind osteoblasts via specific cell surface receptors, the integrins. In this work, two different xenogenic biomaterials were studied. Both biomaterials have a bovine bone origin. They displayed similar architectural organization with connected plates and rods and similar surface topography and roughness. They differed by the presence or not of collagen type I. The first one was characterized by preservation of the type I collagen matrix associated with spindle-shaped hydroxyapatite crystals and the second was solely composed by heat-modified apatite crystals. Osteoblast-like cells (Saos-2) were cultured on both biomaterials and examined in scanning and transmission electron microscopy after 7 and 14 days. Both biomaterials were cytocompatible as demonstrated by good ultrastructural cell preservation. (1) At the surface of the collagen containing biomaterial, cells were elongated in shape and oriented according to the trabecular architecture and to the superficial collagen network. After 14 days of culture, cells were confluent and the biomaterial surface was hidden by the cell sheet. The beta 1 integrin subunit was detected by immunogold in transmission electron microscopy in close relationship with the superficial collagen fibres of the biomaterial and with the outer cell surface. When cultures were carried out in presence of anti beta 1 integrin subunit, cells were packed and piled up with lack of specific orientation. (2) At the surface of the deproteinized biomaterial, cells were globular without specific disposition and often partially attached to the surface. After 14 days of culture, large areas of the biomaterial surface remained uncovered. Anti beta 1 subunits conjugated with gold particles were detected around the cells but with no specific association with the deproteinized biomaterial. These results strongly suggest that presence of type I collagen fibres in the matrix of a bone biomaterial is of major interest to determine cell attachment, spreading and orientation via interaction between type I collagen and beta 1 integrin subunit of osteoblasts.

Shape and orientation of osteoblast-like cells (Saos-2) are influenced by collagen fibers in xenogenic bone biomaterial.

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The surface topography of a substratum has been shown to influence the growth and morphology of cells in culture. In this study, human osteoblast-like cells (Saos-2) were cultured on two types of xenogenic biomaterials obtained from bovine bone. Both biomaterials were similar in architectural organization and surface topography, but they differed in matrix components. The first one was characterized by preservation of the mineralized collagen matrix, and the second by complete deproteinization which only preserved the mineral phase. Cells cultured at the surface of both biomaterials were observed using scanning electron microscopy. The beta 1-integrin subunit, known to bind cell and collagen, is the major integrin of the osteoblast. It was localized using immunogold in transmission electron microscopy. At the surface of the collagen-containing matrix, cells exhibited an elongated shape and oriented axis parallel to the underlying collagen bundles. The beta 1-integrin subunit was localized at the outer surface of cells, in close association with collagen and at the contact points between cells and biomaterials. In contrast, at the surface of the single mineral matrix, cells were round shaped with random disposition. Gold particles were found around the cells with no specific relation to the biomaterial. These results strongly suggest that the chemical nature of the surface of a bone biomaterial directly influences adhesion process, shape, and spatial organization of cultured osteoblastic cells.
The treatment of non-contained intrabony defects with guided tissue regeneration: A prospective controlled clinical trial

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The aim of the study is to verify the healing of the non supportive intrabony defects, treated with bovine-derived xenograft (LADDEC®) and resorbable collagen membranes (Mem-Lok®). Numerous treatment modalities, such as the use of guided tissue regeneration (GTR) or the delivery of an enamel matrix derivative (EMD) have been applied to achieve periodontal regeneration with height predictability for the treatment of non-contained intrabony defects. The treatment of non-contained intrabony defects by means enamel matrix derivative has shown scarce results (Iorio Siciliano, 2011; Tonetti et al, 2002), meanwhile the use of a titanium-reinforced expanded polytetrafluoroethylene (ePTFE) membrane has showed a clinical success, but more complications, including bacterial infections and flap dehiscences were recorded (Cortellini, 1993). For these reasons the combination of DBBM and collagen membrane it's a way to treat these defects. The study was designed as a prospective clinical trials. A sample of 7 patients, each with one non-supportive intrabony defect, was recruited from the Department of Periodontology (University of Naples Federico II). The intrabony defects were treated with bovine-derived xenograft (LADDEC®) and resorbable collagen membranes (Mem-Lok®). At baseline were recorded the following clinical periodontal parameters: PD (probing depth), CAL (clinical attachment level), REC (recession). During the intrasurgical phase the CEJ-BD (vertical distance measured from CJ to the bottom of the defect), INFRA (intrabony depth) and WIDTH (horizontal measurement from the alveolar crest to the root surface) were assessed. The following subject inclusion criteria were applied: male and female aged ≥ 18 y, single-rooted and multi-rooted teeth, non supportive intrabony defects (I wall), KG > 2mm. Subjects were excluded on basis of: Relevant medical conditions contraindicating surgical interventions, smokers and ex smokers, Endo-perio disease, mobility of grade 3, FMPS AND FMBS ≥ 15%. The mean age of the patients was 45.6 ± 7.1. At the baseline FMPS and FMBS was 19.1 ± 2.0% and 17.5 ± 2.0%. At baseline the PD was of 8.4 ± 2.0mm, CAL 8.6 ± 1.5mm and REC 0.9 ± 1.1mm. During the surgery CEJ-BD was 10.3 ± 2.7mm, INFRA 6.3 ± 1.7mm and WIDTH 3.9 ± 1.1mm were recorded. After 12 months follow-up period the PD reduction was 4.4 ± 1.1mm, while the Cal gain and recession increase were 4.0 ± 1.2mm and 0.6 ± 1.1mm respectively. From a radiographic point of view the bone fill was 83.4 ± 9.5%. The combination of bovine-derived xenograft (LADDEC®) and resorbable collagen membranes (Mem-Lok®) for the treatment of non supportive intrabony defects showed a greater PD reduction and CAL gain with a minimal REC increase.

Efficacy of platelet-rich-plasma (PRP) and highly purified bovine xenograft (LADDEC®) combination in bone regeneration after cyst enucleation: histological and histomorphometric analysis after 6 months.

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Platelet rich plasma (PRP) is an autologous source of various growth factors, and has been widely used alone and combined with scaffolds in oral and maxillofacial surgery to promote bone healing. Many works have supported the use of PRP with anorganic bovine bone mineral in oral surgery, however today’s debate about its effective usefulness is still open in the scientific community. The objective of this study is to evaluate the efficacy of adding PRP to a new highly purified bovine allograft (LADDEC®) in the bone regeneration of cystic bony defects following cystectomy. Study sample included 20 patients all underwent cystectomy in which the bone defect was filled with PRP and LADDEC®. After 3 months, at re-entry surgery, bone core was taken for histological and histomorphometric analysis. Histological analysis showed a significant presence of bone tissue and vessels. Both, histological and histomorphometric analysis, showed newly formed bone in contact with anorganic bone particles. The mean volume of vital bone was 68% and the mean percentage of vital bone was 48%. The mean percentage of particles of tissue inorganic was 20% of the total volume. All the samples analyzed did not evidence the presence of inflammatory cells. The results of this study showed how the use of LADDEC® in association with PRP allows bone regeneration and has a potential for routine clinical use for regeneration of cystic bony defects.
Bone defects can be repaired through osteoblastic differentiation induced by biomaterials. Since the performance of biomaterials designed for bone repair depends, in part, on the ability of the material to support the adhesion, proliferation and survival of human bone marrow mesenchymal stem cells (hBMSC), a lot of different biomaterial has been investigated in vitro as alternative to autologous bone. In the present investigation, we have studied the effect of a new highly purified bovine allograft characterized by preservation of the type I collagen matrix associated with spindleshaped hydroxypatite crystals (LADDEC®), for the adhesion, differentiation and proliferation of bone marrow-derived mesenchymal stem cells into osteoblasts. The mesenchymal cells were cultured in - MEM medium + 10% fetal bovine serum, Fungizone and ascorbic acid and incubated in humidified atmosphere 95%/5% air/ CO2 at 37°C. The hMSC were harvested (0.05% - 0.02% trypsin/EDTA) and the trypsin effect was then stopped by addition of fresh medium. After resuspending the cells in complete medium, cell count in Bürker chamber was performed. The hMSC (5 x 10⁴) were placed in sterile siliconized tubes with the LADDEC® fragments and transferred into the Nunc plates wells and placed in incubator at 37°C until the end of experimental times (15 and 30 days). The samples were prepared according to electronic microscopy conventional techniques. Subsequently dehydration in increasing gradients of ethanol and Critical Point Drying (CPD Emscope 750) was carried out. The samples were attached onto aluminum stubs with carbon glue and coated with a 100 Å thickness platinum film and observed by SEM (FESEM Hitachi S4000). Elemental analysis of cells cultured on the bone fragments was performed after 30 days using a SEM (Cambridge) X-Ray detector (Inca X-Sight, Oxford Instruments). Semi-quantitative histochemical detection of alkaline phosphatase was carried out using the 86R Sigma-Aldrich kit. To perform the analysis, cells were incubated at room temperature in a solution containing naphthol AS-BI phosphate and stable diazonium salts (Fast Red Violet LB) freshly prepared, buffered to pH 9.5. The sites of activity are highlighted as a red granulation under light microscopy. Morphological and ultrastructural analysis of hBMSC carried out after 15 and 30 days of culture, with Scanning Electron Microscopy, show the LADDEC® substrate capabilities to induce the osteoblastic phenotype. Further evidences of a mineralization process arise from X-Ray microanalysis (Calcium in the microvesicles of plasmalemma) and positivity to alkaline phosphatase reaction. The results demonstrated that highly purified bovine xenograft (LADDEC®) is an excellent scaffold for the differentiation and proliferation of mesenchymal stem cells into osteoblasts.
Soft and hard tissues modifications at immediate transmucosal implants (Laser-Lok® microtextured collar) placed into fresh extraction sites - a six month prospective study with surgical re-entry

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The aim of this prospective study was to assess, after 6 months of healing, the dimensional change of alveolar bony walls at immediate transmucosal implants (Laser-Lok® microtextured collar) placed in fresh extraction sites in conjunction with regenerative procedures. Several studies on the animal and human models have demonstrated that alveolar bone structures are lost following tooth extraction irrespective of the placement of an immediate implant with or without the concomitant regenerative procedure. However the use of an dental implant with Laser-Lok® microtextured collar can be prevent the bone resorption. Titanium oral implants (Single-stage Implant System, BioHorizons) were immediately placed into single-rooted extraction sockets. The implant neck is comprised of a 1mm turned surface, a 0.9mm microgroove with an 8x6μm pitch (connective tissue) and 0.9mm microgroove with a 12x12μm pitch (bone tissue). Peri-implant marginal defects were treated according to the principles of GBR using bovine-derived xenograft (LADDEC®) and resorbable collagen membranes (Mem-Lok®). A total of 13 patients received 13 implants of the BioHorizons System. The following inclusion criteria were applied: Age ≥ 18 years, presence of mandibular or maxillary single-rooted teeth to be extracted because of an endodontic failure, caries or root fracture, integrity of extraction sockets walls, presence of sufficient residual alveolar bone volume to achieve primary implant stability, presence of a tooth mesially and distally to the extraction site, full-mouth plaque score (FMPS) and full-mouth plaque score (FMBS) ≤ 25% at baseline, presence at least 2mm of keratinized tissue to allow flap management. Subjects were excluded on the basis of: presence of relevant medical conditions contraindicating surgical intervention, pregnancy or lactation, tobacco smoking, periodontally compromised patients, multi-rooted teeth. The surgical re-entry was performed after 6-months healing period. Thirteen patients (seven male and six female) fulfilling the inclusion criteria were enrolled. The mean age of the patients was 42.23±9.43 years (range 27-56 years). At the baseline, the mean FMPS was 20.15±1.34% and the mean FMBS was 19.38±1.19%. The use of Laser-Lok® microtextured collar may provide more favorable conditions for the attachment of hard and soft tissues and reduce the level of marginal bone resorption.