



Treatment of a post-extractive socket with a lyophilized equine bone paste and implant rehabilitation: clinical, histological and histomorphometric outcome.

Trajtimi i nje xhepi post-ekstraktiv me nje paste kocke kali te lipolizuar dhe me rehabilitim implantar: rezultate klinike, histologjike dhe histomorfo-metrike.

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ABSTRACT

Objective: Anorganic bovine bone has been well studied and proved to be effective for socket preservation because of its slow resorption over time. Non-antigenic, enzyme-treated equine bone has emerged as a possible alternative biomaterial and its effectiveness investigated in different bone regeneration indications, but at present no evidence exists about its use in socket regeneration and preservation. The objective of this study is to report a first case about the use of this material in this application, and present a first clinical and histological assessment. **Methods:** A variant of the enzyme-treated equine bone, featuring partially denatured bone collagen, and added with exogenous collagen to create a lyophilized dry paste, was used to graft a post-extractive socket in a patient needed tooth 34 to be extracted. No protective membrane was placed, and no specific flap preparation was performed. After 3 months, an osseointegrated implant was placed at the grafted site and a bone biopsy was collected for histologic and morphometric assessment. **Results:** The patient healed uneventfully and was rehabilitated successfully. Socket bone levels were maintained on a short-term basis. Histological analysis of the bone biopsy showed that the graft material had undergone nearly complete remodelling with no signs of inflammation. Newly formed bone, residual biomaterial and medullar spaces were, respectively, 36.4%; 12.1% and 51.5%.

Conclusions: The lyophilized equine bone paste allowed for successful short-term socket preservation and for effective bone regeneration. The handling properties of the graft were quite satisfactory. Studies in a controlled clinical setting aimed to investigate its effectiveness are highly advisable.

Keywords: Post-extractive socket; Bone Regeneration; Equine Bone; Collagen.

INTRODUCTION

Implant supported prosthetic treatment is nowadays a standard approach for the rehabilitation of edentulous patients. As its application is spreading, the demand for long-term successful aesthetic results is increasing. Consequently, an augmented knowledge and awareness of all the factors¹ that contribute to the long term successful management of soft tissues, namely gingiva and papillae, has highlighted the importance of a correct management of the underlying bone levels.^{2,3} This calls for bone grafting also of post-extractive sockets, with two aims: achieving a greater (or, in more severe cases, sufficient) bone volume to place the implant in and, additionally, to preserve the ridge height in order to allow for the better aesthetic outcome.⁴ Autogenous bone has long been regarded the gold standard for bone regeneration given its osteoconductive, osteoinductive and osteogenic properties.⁵ Increased morbidity,⁶ and lack of a sufficient quantity at collection for grafting large or multiple defects are, yet, substantial limits to its application.⁷ As an alternative, homologous bone, heterologous bone, and

ABSTRAKT

Objektivi: Kocka jo organike e gjedheve eshte studiuar mire deri tani dhe eshte provuar qe eshte efektive per ruajtjen e xhepave per shkak te kohes se ngadalte te rezorbimit. Kocka e kalit e deanitegjenuar, e trajtuar me enzima, ka sinjalizuar per nje biomaterial alternativ dhe efektiviteti i tij eshte testuar ne indikime te ndryshme te regjenerimit kockor, por per momentin nuk ekziston asnje evidence per perdorimin ne rigjenerimin dhe ruajtjen e xhepave. Objektivi i ketij studimi eshte te reportoje nje rast te pare te perdorimit te ketij materiali ne kete lloja aplikimi, dhe te paraqese qasjen e pare klinike dhe histologjike.

Metodat: nje variant i kockes se kalit te trajtuar me enzima, duke shfaqur pjeserisht kolagjen kockor te denaturuar, i eshte shtuar kolagjen ekzogjen per te krijuar nje paste te thate lipofile, u perdor per nje graft ne nje xhep post-ekstraktiv ne nje pacient qe kishte nevojte te ekstraktonte 34. Nuk u vendos membrane mbrojtese dhe nuk u be asnje preparim i vecante per lembo. Pas tre muajsh, u vendos nje implant i osteointegruar te vendi i grafitit dhe u be nje biopsi per vleresime histologjike dhe morfometrike. **Rezultatet:** Pacienti u sherua normalisht dhe u rehabilitua me sukses. Nivelet e xhepit kockor u ruajtten ne nje periude afat-shkurter. Analiza histologjike e biopsise se kockes tregoi qe grafiti i ishte nenshtuar rimodelimit te plote pa shenja inflamacioni. Kocka e formuar rishtazi, biomateriali i mbetur dhe hapesirat medulare paraqiteshin respektivisht: 36,4%; 12,1%;51,5% Konkluzionet: pasta lipofile e kockes se kalit lejon per nje ruajtje te sukseshme ne terma afatshkurter dhe per regjenerim kockor efektiv. Karakteristikat e manipulimit te grafitit ishin mjaft te kenaqshme. Studimet ne nje kontroll klinik qe synojne te investigojne efektivitetin e tij jane shume te rekomanduar.

Fjale kyce: Xhep post-ekstraktiv, Rigjenerim kockor, Kocke kali, Kolagjen.

PREZANTIM

Trajtimi protetik mbi implante eshte ne ditet e sotme nje perqasje standarte per rehabilitimin e pacienteve pa dhembe. Nderkohe qe perdorimi i tij perhapet, kerkesa per rezultate estetike te suksesshme qe zgjasin po rritet. Si pasoje, nje njohuri dhe kujdes i rritur i te gjithe faktoreve¹ qe kontribuojne ne nje menaxhim te suksesshem dhe qe zgjat te indeve te buta, perkatuesisht gingives dhe papiles, kane vene ne dukje rendesine e nje menaxhimi korrekt te nivelit te kockes mbeshtetese.^{2,3} Kjo i ben te nevojshme graftet kockore edhe ne alveolen post ekstraksion, me dy qellime: te arrihet nje volum kocke me i madh (ose ne rastet e veshtira i mjaftueshem) per te vendosur implantin, pervec kesaj, per te ruajtur lartesine e kreshtes ne menyre qe te arrihet nje rezultat me i mire estetik.⁴ Kocka autogjene eshte konsideruar standarti i arte per rigjenerimin kockor, fale aftesive te saj osteoinduktive dhe osteogjenike.⁵ Butesia e rritur,⁶ dhe mungesa e sasise se mjaftueshme ne mbledhjen e grafitit defektet e medha ose te shumta, ende, ka limite per aplikimin e kesaj teknike.⁷ Si nje alternative, kocka ho-

alloplastic materials, have been used alone or in combination.⁸⁻¹⁷ Concerning xenografts, anorganic bovine bone displays good osteoconductive properties but may also have a low resorption capacity.¹⁸ Low resorption has always been associated with better volume preserving properties.¹⁹ An alternative xenograft, achieved by eliminate antigens from equine bone using degrading enzymes, has been introduced for use as a scaffold in bone regeneration of different bone defects.²⁰⁻²³ The enzymatic process used to eliminate species-specific antigen from the original bone preserves the bone apatite crystal in its native state, a condition that could favour cell interaction with the biomaterial. This xenograft may be added with exogenous collagen, in order to allow better handling, haemostatic effect and, possibly, an improved bone-regeneration process given the well-known biological properties of this molecule.²⁴⁻³¹

Indeed, when osteoclasts were cultured over a similar equine, enzyme-treated, even if bone collagen-preserving bone substitutes,³² their adhesion and activity was significantly higher than that found for osteoclasts grown over deproteinized bovine bone.³³

Regenerated sites augmented with equine bone alone showed no differences as far as the expression of some markers of bone regeneration (NOS1, NOS2 and VEGF) were concerned, in comparison to sites grafted with the same material added with autogenous bone.³⁴ Similar results were achieved when sinuses were grafted with a variant of this material featuring bone collagen partially denatured by means of an additional short-term autoclaving process.^{35,36}

To the author's knowledge, no cases has ever been published concerning the use of this xenograft variant in the treatment of regeneration and volume preservation of post-extractive sockets. The present paper reports therefore a case of a patient undergoing a lower premolar extraction, and was concomitantly treated by grafting a lyophilized equine bone paste, made of this xenograft granules added with equine collagen, in the post-extractive socket. A bone biopsy was collected for histological and histomorphometric assessment to investigate the extent, quality and quantity of bone regeneration.

MATERIALS AND METHODS

Dental implant surgery

The patient was a healthy 42-year-old woman who presented with a compromised tooth at positions 34 due to a peri-implantitis she had already forced the surgeon to remove a previous implant placed in position 35 (Figures 1 a-b). A two-step treatment plan was therefore developed that called for atraumatic extraction of the compromised element and socket regeneration and preservation by grafting a bone substitute. Implant placement was planned at three to six months from bone grafting, according to the radiographic appearance of the graft at intermediate (monthly) controls. The patient provided her informed consent.

At first surgery, antibiotic prophylaxis (Amoxicillin/Clavulanic acid, Augmentin, Glaxo-SmithKline, Verona, Italy), 2 g, 1 hour before surgery and then every 12 hours for 7-9 days, was initiated and the patient was subjected to mouth rinses with Chlorhexidine 0.2% (Corsodyl, Glaxo-SmithKline). Also Nimesulide (Aulin, Roche, Milano, Italy) 100 mg was administered, 1 hour before surgery and then twice a day for 5 days. Local anaesthetic was administered by means of an infiltration with 1% Articaine with adrenaline 1:100000.

No flaps were prepared and the 34 element was extracted atraumatically (Figure 2 a-c). The socket was immediately grafted with a lyophilized equine bone paste (Bio-Gen Putty, Bioteck, Arcugnano, Vicenza, Italy). This graft is a 60:40 bone-collagen w/w mixture of equine bone powder/equine cancellous bone granules, sized 0.5-1.0 mm, made non-antigenic by enzymatic treatment, featuring bone collagen partially denatured by fast autoclaving, and collagen extracted from equine Achille's tendons. The appearance of the graft is a dry white cylinder, sized

mogjene, kocka heterogjene, dhe materialet haloplastike, jane perdorur te vetme ose te kombinuara.⁸⁻¹⁷

Ne lidhje me ksenograftet, kocka inorganike e gjethit siguron osteokonduktivitet te mire, por mund te kete edhe nje kapacitet te ulet rezorbimi.¹⁸ Rezorbimi i ulet ka qene gjithmone i lidhur me ruajtjen e volumit.¹⁹ Nje ksenograft alternativ, arrihet duke eliminuar antigenet nga kocka e kalit duke perdorur enzima degraduese, eshte prezantuar per perdorim sin je skele ne rigjenerim kockor ne defekte te ndryshme te kockes.²⁰⁻²³ Procesi enzimatik i perdorur per te eliminuar antigenet specific te specieve nga ato origjinale. Nje kocke ruan kristalet e apatitit ne gjendjen e tyre natyrale, kusht ky qe favorizon nderveprimin e qelizave me biomaterialin. Ky ksenograft mund te shtohet me kolagjen te jashtem, ne menyre qe te lejoje nje manovrim me te mire, efekt hemostatik dhe , mundesisht, nje process rigjenerimi kockor te permiresuar duke marre parasysh aftesite e mira biologjike te kesaj molekule.²⁴⁻³¹

Ne te vertete , kur osteoklastet jane ne nje kulture kali , te trajtuar me enzima, edhe ne se kolagjeni ruan zevendesimin kockor,³² adezioni dhe aktiviteti i tyre eshte ndjeshem me i larte sesa a ii gjetur per rritjen e osteoklasteve mbi kocken e deproteinuar te gjetheve.³³

Sitet rigjenerative e rritura vetem me kocken e gjetheve nuk kishin ndryshime per sa i perket shprehjes te disa markuesve te rigjenerimit kockor (NOS1, NOS2 dhe VEGF), ne krahasim me sitet me grafe me te njejtin material te shtuara me kocke autogjene.³⁴ Rezultate te ngjashme arrihen kur ne sinuse vendosen graftet me nje variant te ketij materiali sebashku me kolagjen kockor te denatruar me ane ten je procesi autoklavimi.^{35,36}

Ne baze te autorit, nuk jane publikuar raste te tjera ne lidhje me perdorimin e ketij variant ksenografti ne trajtimin e rigjenerimit dhe ruajtjen e volumit te xhepave post-ekstraktiv. Rasti i tanishem paraqet rastin e nje pacienti qe i eshte nenshtuar ekstraksionit te premolarit te poshtem, dhe eshte trajtuar njekohesisht me graft te liofilizuar nga kocka e kalit, e realizuar nga granulat e ketij ksenografti duke i shtuar kolagjen kali, ne xhepat post-ekstraktive. Nje biopsy e kockes eshte mbledhur per ekzaminim histologjik dhe histomorfometrik per te vleresuar shtrirjen, kualitetin dhe sasine e rigjenerimit kockor.

MATERIALE DHE METODA

Kirurgjia dentare implantare

Pacienti eshte nje femer 42 vjecare e shendetshme e cila ka nje dhemb te kompromentuar ne pozicionin 34 si rezultat in je periimplantiti dhe ka detyruar tashme mjekun ta heqe implantin e vendosur ne pozicionin 35 (Figurat 1 a-b). Eshte ndertuar nje plan trajtimi me dy hapa i cili kerkon ekstraksion atraumatik te elementit te kompromentuar dhe rigjenerim te alveoles dhe zevendesimin kockor nepermjet graftit . Vendosja e implantit eshte programuar 3 deri ne 6 muaj nga vendosja e graftit kockor, ne varesi te paraqitjes radiografike te graftit ne vizitat e ndermjetme mujore. Pacienti dha konsentin e informuar.

Ne kirurgjine e pare , profilaksia me antibiotik (Amoxicillin/Clavulanic acid, Augmentin, Glaxo-SmithKline) 2 g, 1 ore par kirurgjise dhe pastaj cdo 12 ore per 7-9 dite, dhe pacienti eshte keshilluar te shperlaje gpojen me Chlorhexidine 0.2% (Corsodyl, Glaxo-SmithKline). Gjithashtu eshte administruar Nimesulide (Aulin, Roche) 100 mg, 1 ore para nderhyrjes dhe dy here ne dite per 5 dite. Anestezia lokale eshte administruar nepermjet infiltrimit te 1% Articaine me adrenaline 1:100000.

Nuk eshte preparuar lembo dhe elementi 34 eshte hequr ne menyre atraumatike (Figura 2 a-c). Gropa alveolare eshte mbushur me graft ne forme paste te liofilizuar te kockes se kalit (Bio-Gen Putty, Bioteck, Arcugnano, Vicenza, Italy). Grafti eshte ne raportin 60:40 kocke -kolagjen w/w perzierje e kockes se kalit ne forme pudre/granula ne forme rrjete te kockes se kalit, permasat 0.5-1.0 mm, i bere jo antigenik nga trajtimi enzimatik, bashkuar me kolagjen kockor te denatruar pjeserisht nepermjet autoklavimit te shpejte, dhe kolagjen i ekstraktuar nga tendina e Akilit nga kali. Pamje e graftit eshte nje cilindri i bardhe i thate ,

approximately 0.5 (diameter) x 1.0 cm (Figure 3). The graft was placed directly into the socket without hydrating it, displaying a high capability of absorbing blood, provoking immediate haemostasis, and increasing slightly its volume while becoming, at the same time, a mouldable paste. The perfect adherence of the graft to the alveolar walls was then easily achieved by modelling it with round instruments. No membrane was placed to cover the graft, and flap edges were stabilized (but not made to collapse) with a single cross stitch with a silk suture (Figure 4 a-b). Healing was uneventful and suture was removed after 15 days. The patient presented to periodical controls monthly, and the healing of the graft was assessed on the base of the increasing radio-opacity of the graft. According to the surgeon's experience, healing showed to be consistent with possible implant placement after 3 months from the graft (Figure 5).

Antibiotic pre- and post-surgical prophylaxis, pain management therapy and post-surgical instruction were the same as in previous surgery. A full-thickness trapezoidal mucoperiosteal flap was detached to expose the bone ridge and assess, clinically, the quality of bone healing. A bone biopsy was collected at the regenerated site, using a trephine with an external diameter of 3 mm under irrigation, and an osseointegrated titanium implant (3.4 x 13.0 mm, Xive, Dentsply, Germany) was placed. Finally, complete flap closure was achieved (Fig. 6a-e) and implant healing assessed, for prosthetic rehabilitation, 3 months after implant placement (Figure 7).

Histological and Histomorphometric analysis

The bone biopsy was fixed in 4% formalin and decalcified for 21 days in a solution containing Sodium Formiate 0.76 M and Formic Acid 1.6 M (Panreac Quimica, Barcelona, Spain). Subsequently, the sample was dehydrated in graded ethanol, and embedded in paraffin. This procedure allowed a rapid infiltration of the tissue and the achievement of the right softness for cutting, with only minimal artifactual shrinking, thus providing a tissue morphology which is representative of the *in vivo* bone features. 5 µm thickness sections were achieved, mounted on slides and haematoxylin-eosin stained and observed and photographed at 3.5x and 10x magnification. Morphometric measurements were performed on digital photomicrographs collected at both magnifications: first, five randomly-chosen fields at 3.5x magnification were analyzed using the ImageJ 1.33 analysis software (National Institute of Health, Bethesda, USA). For each 3 field we obtained the total sample area (TSA), the total bone area (TBA), the newly formed bone area (LBA) and the residual bone substitute area (RBA). TBA was calculated by summing all the area values corresponding to zones occupied by living bone and residual bone substitute. LBA was calculated by summing all the area values corresponding to zones occupied by living, newly formed bone tissue only. RBA was calculated as the difference between TBA and LBA ($RBA = TBA - LBA$). For each field, then, the ratios TBA/TSA , LBA/TSA , $RB = RBA/TSA$ and $NB = LBA/RBA$ were calculated. Finally, the mean values of each ratio over the five fields were calculated ($N=5$).

Subsequently five different, randomly-chosen, fields at 10x magnification were analyzed. For each field, the living bone area (LBA) and the residual bone substitute area (RBA) were measured as described and the average LBA/ RBA ratio was derived again and compared with the one achieved by the other assessor. Statistical analysis was performed by using GraphPad Prism 4.0 statistical program (GraphPad software, San Diego, CA). All results are given as mean ± SD.

RESULTS

Clinical outcome

The patient did not present any clinical symptoms during follow up controls. At the moment of bone core collection the radiographic appearance of the grafted volume was similar to that of the surrounding, non-regenerated bone and no height loss could be observed (Figure 5). The clinical appearance of the

me madhesi rreth 0.5 (diameter) x 1.0 cm (Figura 3). Grafti është vendosur direkt në gropën alveolare të pahidratuar, duke shfaqur një aftësi për të absorbuar gjak, duke provokuar hamostazë të menjëhershme, dhe duke rritur lehtësisht volumin e tij ndërkohë që bëhet në të njëjtën kohë, në formë paste. Adherenca e perkryer e graftit me muret e alveoles arrihet me lehtësi duke emodeluar atë me instrumente të rrumbullaketa. Nuk është vendosur membranë për të mbuluar graftin, dhe janë stabilizuar anet e lembos (për jo ngjitur) me një suture të vetme kryq mendafshi (Figure 4 a-b). Sherimi u bë i qetë dhe suturat janë hequr pas 15 ditësh. Pacienti është paraqitur për kontrolle periodike rregullisht, dhe sherimi i graftit është vlerësuar mbi bazën e radiopacitetit të rritur të graftit. Bazuar në eksperiencën e kirurgut, sherimi është konsistent me vendosjen e mundshme të implantit pas tre muajsh nga vendosja e graftit (Figura 5).

Antibiotiket pre-dhe post-profilaksise kirurgjikale, terapia për menaxhimin e dhimbjes dhe instruksionet post-kirurgjikale ishin të njëjta si në kirurgjinë e mëparshme. Një lembo e plote, e trashë, trapezoidale, mukoperiostale është shkollitur për të ekspozuar kreshten kockore dhe për të vlerësuar klinikisht cilësinë e sherimit të kockës. Një biopsi e kockës është mbledhur në sitin e rigjeneruar, duke përdorur eskavator me një diametër të jashtëm prej 3 mm nën irrigim, dhe është vendosur një implant titaniu (3.4 x 13.0 mm, Xive, Dentsply, Germany). Në fund, është bërë mbyllja e lembos (Fig. 6a-e) dhe është vlerësuar sherimi i implantit, për rehabilitim protetik, 3 muaj pasi është vendosur implanti (Figure 7).

Analiza histologjike dhe histomorfometrike

Biopsia e kockës është fiksuar në 4% formalinë dhe dekalcifikuar për 21 ditë në një solucion që përmban Sodium Formiate 0.76 M dhe Acid Formik 1.6 M (Panreac Quimica, Barcelona, Spain). Si pasoje, shembulli është dehidruar në etanol, dhe fiksuar në parafinë. Kjo procedurë lejon një infiltrim të shpejtë të indit dhe arrijtjen e një butësie të caktuar për prerje, me një tkurrje minimale, duke siguruar një morfologji indore e cila prezanton strukturën in vivo të kockës. Trashësia e seksionit është 5 µm, e montuar në lame dhe e ngjyrosur me haematoxylin-eosin, e observuar dhe e fotografuar me zmadhim 3.5x dhe 10x. Matjet morfometrike janë realizuar me fotomikrografe digjitale të mbledhura me dy zmadhime: së pari, pesë fusha të zgjedhura rastësisht zmadhimi 3.5x është analizuar duke përdorur analizën software ImageJ 1.33 (National Institute of Health, Bethesda, USA). Për çdo 3 fusha në kemi marrë gjerësinë e plote të shembullit (TSA), gjerësinë e plote të kockës (TBA), gjerësinë e kockës së formuar (LBA) gjerësinë e kockës reziduale të zvendësuar (RBA). TBA është kalkuluar duke mbledhur të gjithë vlerat korresponduese të zonave të zena nga kocka e gjalle dhe kocka reziduale e zvendësuar. LBA është kalkuluar duke mbledhur të gjithë vlerat e zonave korresponduese të zena nga indi i gjalle i formuar së fundi. RBA është kalkuluar si diferencë ndërmjet TBA dhe LBA ($RBA = TBA - LBA$). Për çdo fushë, me pas, janë kalkuluar raportet TBA/TSA , LBA/TSA , $RB = RBA/TSA$ dhe $NB = LBA/RBA$. Në fund, janë kalkuluar vlerat kryesore të çdo raporti për 5 fushat ($N=5$). Si pasoje pesë fusha të ndryshme të zgjedhura rastësisht janë analizuar me zmadhim 10x. Për secilën fushë, zona e kockës së gjalle (LBA) dhe kocka reziduale e zvendësuar (RBA) janë matur dhe përkrahur me raportin mesatar LBA/ RBA ky është krahasuar me një të rritur nga një tjetër vlerësuar. Analiza statistikore është realizuar duke përdorur programin statistikor GraphPad Prism 4.0 (GraphPad software, San Diego, CA). Të gjithë rezultatet janë dhënë nepermjet ± SD.

REZULTATET

Te dhënat klinike

Pacienti nuk paraqiti asnjë simptomë klinike gjatë vizitave të kontrollit. Në momentin e mbledhjes së ekstraktit kockor, pamja radiografike e vëllimit të graftit ishte e ngjashme me kockën përreth të përgjeneruar dhe nuk u observua humbje e lartësisë (Figura 5) Pamja klinike e zonës së regjeneruar kur kreshta

regenerated area when the bone ridge was exposed for implant placement was undistinguishable from the one of the adjacent patient's own bone (Figure 6).

Histological results

A quite extended bone structure could be observed. Bone substitute residual particles could be identified as areas, even not showing marked basophilia, in which bone lacunae were devoid of osteocytes. The bone substitute residual particles were in close contact, with no gaps, with the alive bone tissue (eosin-stained and osteocytes-rich). A small amount of connective tissue was observed. Neither cartilage-like tissue nor inflammatory reactions were observed (Figure 8). Histomorphometric measurements showed that, at the time of biopsy collection, newly formed bone (NB) and residual biomaterial (RB) were 36.4% and 12.1% respectively (the remainder area, 51.5%, consisting of medullar spaces).

DISCUSSION

The clinical outcome of the present case shows that grafting the equine lyophilized bone paste has allowed to achieve a more than satisfactory bone regeneration and, concomitantly, a good socket preservation, at least on the short time from grafting to implant placement. Histological findings showed that the biomaterials grafted were biocompatible, as confirmed by the absence of any inflammatory cell, and by the fact that bone graft particles were always contacting closely newly-formed bone areas. This indicates also a good osseointegration of the bone graft particles with the newly formed bone. The absence of cartilage tissue was consistent with a direct ossification mechanism. These results are consistent with previous published data showing the remodelling of these equine bone substitutes to occur at a physiological rate^{35,36} and allowing for implant insertion in newly-formed bone only, or in newly-formed bone containing only a small amount of residual particles.^{20,23,34} This behaviour, different from the one observed when bovine deproteinized bone had been used, where slow or nearly absent remodelling is observed, confirms what already observed in earlier studies.^{38,39} This could be explained by the different processing anorganic bovine bone and enzyme-treated equine bone are subjected to be made non-antigenic. Anorganic bovine bone, in fact, is thermally-treated at a temperature greater than 600°C that eliminates type I bone collagen, while enzymatic removal of antigens occurs in physiologic conditions (37°C), thus preserving type I bone collagen unaltered. This could possibly explain why osteoclast adhesion is somewhat impaired on thermally-treated bovine bone, while it is not on enzyme-treated bone tissue.^{32,33} Our data show that, three months after the graft surgery, a fair amount of the bone graft had been replaced by autogenous, newly-formed bone. This condition, unlike bone regeneration with anorganic bovine bone where graft particles are still present after years, mimics more closely the native bone state and could represent a clinical benefit since, theoretically, could allow to perform early implant placement and early implant loading. In the author's opinion, in addition to the good clinical and biological results observed, the particular handling properties of the lyophilized paste were quite appreciated. The dry paste could be positioned quite easily into the socket: when dry, it was compact and no granules could spread out from the surgical site. When in contact with blood it became immediately mouldable, and stuck to the receiving cavity thanks to the haemostatic properties of the added collagen. Positioning and consequent graft modelling were therefore easy and rapid. Last, but not least, the absence of a covering membrane and of flap collation did not prevent or hinder soft tissue regeneration. Possibly, the collagen component of the paste might have acted as a membrane, preventing soft tissue to invade the grafted volume. Finally, this case report presents the first documented case, to the author's knowledge, of the use of this kind of enzymatic-treated equine bone, added with exogenous collagen, for

kockore u ekspozua ndaj vendosjes se implantit ishte e padalueshme nga pjesa tjetere e zonave kockore fqinje te pacientit (Figura 6)

Rezultatet histologjike

Mund te verehej nje strukture kockore shume e zgjeruar. Pjesizat e zevendesuesi kockor te mbetur mund te identifikohej, edhe pse nuk tregojne bazofili te rritur, ne te cilat lakunat kockore nuk paraqisnin prani te osteociteve. Grimcat e zevendesuesit kockor te mbetur ishin ne kontakt te ngushte, pa hapesia mes tyre, me kocken e gjalle (ngjyrosje me eozine dhe e pasur me osteocite). U verejt nje sasi e vogel e indit konektival. Nuk u vu re as prani e indit kartiloid as prani e reagimeve me natyre inflamatore (Figura 8) Matjet histomorfometrike treguan qe ne momentin e mbledhjes se materialit per biopsy, kocka e neformuar dhe biomateriali residual ishin ne raportet: 36,4% dhe 12,1% (pjesa e mbetur prej 51,5% konsistente ne hapesia medulare)

DISKUTIM

Te dhenat klinike te rastit ne fjale tregojne qe pasta lipofile e grafitit kockor te kalit lejon arritjen e nje regjenerimi kockor mese te kenaqshem dhe ne te njejten kohe, ruajte te mire te xhepit, te pakten ne nje periudhe te shkurter nga momenti i vendosjes se grafitit deri ne momentin e vendosjes se implantit. Gjetjet histologjike treguan qe biomaterialet e perdorura per graft ishin biokompatibel, sic u konfirmua nga mungesa e qelizave inflamatore dhe nga fakti qe grimcat e grafitit kockor ishin gjithmone te rrethuara nga zona formimi te ri kockor. Mungesa e indit kartilagenoz ishte drejtperdrejt e lidhur me nje mekanizem osifikimi. Rezultatet jane koherente me te dhena te publikuara me pare qe tregojne se rimodelimi i ketyre zevendesuesve kockore ndodh ne nje nivel fiziologjik^{35,36} dhe lejojne vendosjen e implantit ne nje kocke te sapoformuar ose ne nje kocke qe permban sasi te vogla te grimcave reziduale.^{20,23,34} Kjo sjellje, e ndryshme nga ato te vezhguara me kocke gjedhesh te deproteinizuar, ku pak ose paotuhajse aspak rimodelim ka qene prezent, konfirmon ate qe eshte vezhguar ne studime te meparshme.^{38,39} Kjo mund te shpjegohet me procesin e ndryshem te cilit i nenshtrohet kocka e kalit dhe ajo e gjetheve per tu trajtuar me enzime dhe per tu deantigenizuar. Kocka inorganike e gjedheve, ne fakt, trajtohet ne menyre termike ne nje temperature me te madhe se 600 grade celcius qe elimino kolagjenin e tipit te pare, nderkohe qe heqje enzimatike e antigjeneve ndodh ne kushte fiziologjike (37 grade celcius) duke ruajtur keshtu te paalteruar kolagjenin kockor te tipit te pare. Kjo mund te shpjegoje pse adezioni i osteoklasteve demtohet ne kocken e gjedheve te trajtuar me nxehtesi, nderkohe qe nuk eshte prezent ne indin kockor te trajtuar me enzima.^{32,33} Te dhenat tona treguan qe pas tre muajsh nga momenti i vendosjes se grafitit kockor, nje sasi e konsiderueshme e grafitit kockor eshte zevendesuar nga kocke autogene, e sapoformuar. Kjo gjendje, ndryshe nga regjenerimi kockor me kocke inorganike te gjedheve ku grimcat e grafitit jane akoma prezente pas disa viteve, imiton me shume gjendjen e kockes natyrale dhe mund te paraqese nje perfitim klinik, duke qene se, teorikisht, mund te lejoje te vendoset me heret implantit dhe te realizohet ngarkesa imediate. Ne opinionin e autorit, pervec rezultateve te mira klinike dhe biologjike te observuara, karakteristikat e vecanta te manipulimit te pastes lipofile ishin shume te cmueshme. Pasta e thate mund te pozicionohej me shume lehtesi ne xhepin kockor; kur ishte i thate behej kompakte dhe granulat nuk mund te largoheshin nga vendi kirurgjikal. Kur ishte ne kontakt me gjakun behej menjehere e modelueshme, ngecte ne kavitet, fale karakteristikave hemostatike te kolagjenit te shtuar. Pozicionimi dhe modelimi i mjaftueshem i grafitit jane per pasoje te thjeshta dhe te shpejta. Se fundmi, por jo me me pak rendesi, mungesa e nevojës per membrane mbuluese dhe lembo nuk pengonte rigjenerimin e indeve e buta. Mundesisht, ky raportim rasti paraqet rastin e pare te dokumentuar, per aq sa eshte ne dijeni autori, te perdorimit te kesaj lloj

socket regeneration and preservation. Given the present results, further studies in a controlled clinical setting are highly advisable.

CONCLUSIONS

Equine lyophilized bone paste might be an interesting option for the regeneration and preservation of post-extractive sockets, given the promising clinical and histomorphometric results observed in the present case, and the handling properties this material shows. Controlled clinical studies should be planned to further investigate the clinical outcome of this bone graft.

Figures

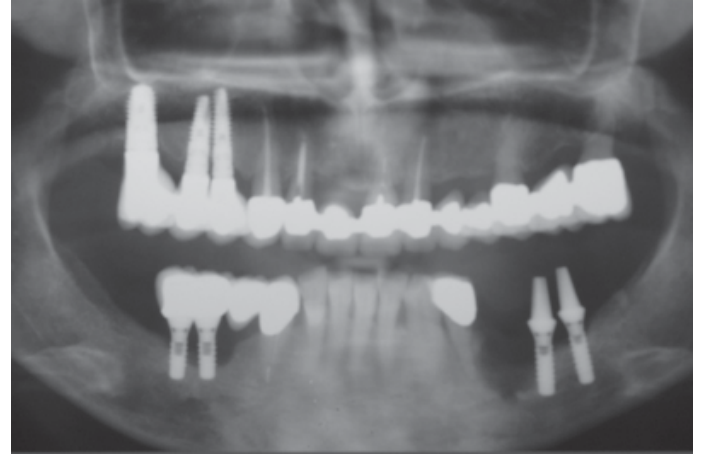
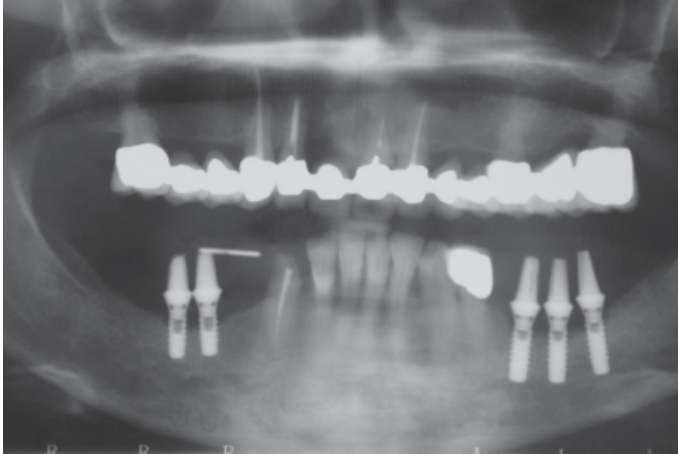


Figure 1a-b. Panoramic radiographs before surgery. a) Periimplantitis affected implant in position 35 that had to be removed (b), causing loss of alveolar support to tooth 34, that was lost and called for extraction. / Radiografite panoramike tregojne perpara kirurgjise: a) Implant i prekur nga periimplantiti ne pozicionin 35 qe duhet te hiqej b) duke shkaktuar humbje te suportit alveolar te dhembit 34, qe ishte i humbur dhe qe u zgjodh ekstraksioni

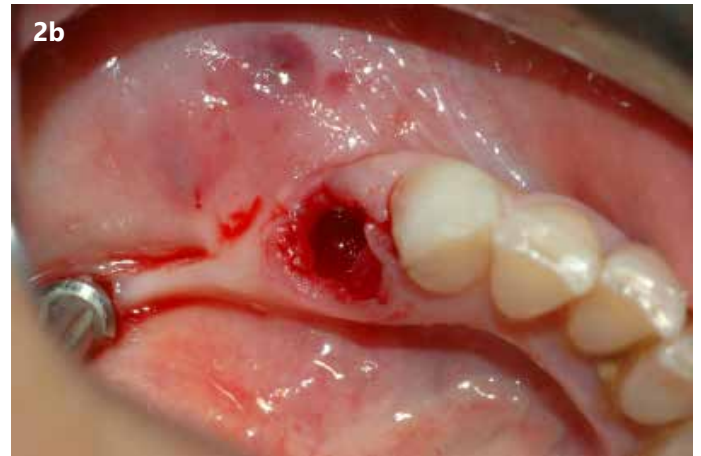


Figure 2 a, b, c. Atraumatic extraction is performed. Ekstraksion atraumatik.



Figure 3. The lyophilized equine bone paste before its use. The paste appears as a dry, compact cylinder. / Pasta kockore lipofile e kalit perpara perdorimit te saj. Pasta duket e thate, kompakte cilindrike.



Figure 4a-b. The socket is filled with the bone paste that, in contact with blood, becomes easily moldable. The flap is stabilized with a single cross stitch, without performing flap preparation or collation. / Ky xhep eshte i mbushur me nje paste kockore qe, ne kontakt me gjakun, behet lehtesisht e modelueshme. Lembo stabilizohet me nje suture kryq te vetme, pa lembo preparuese.



Figure 5. Radiographic healing of the graft 3 months after surgery. Sherimi i grafitit i paraqitur radiografikisht tre muaj pas kirurgjise.

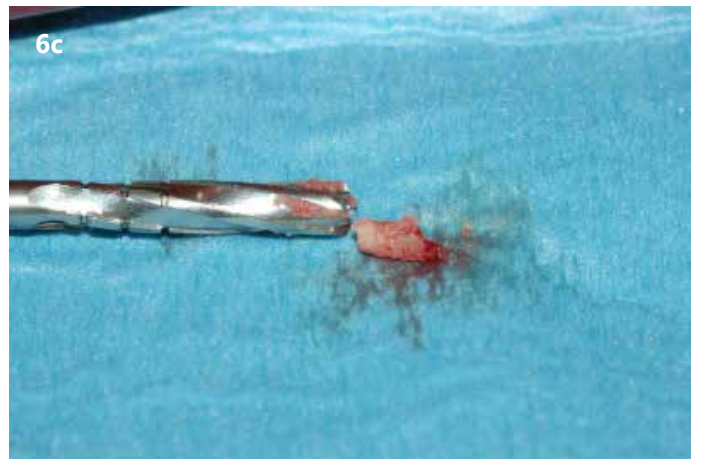
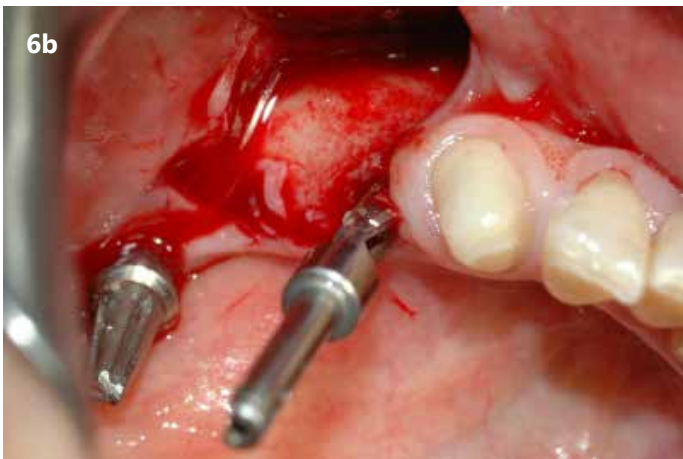


Figure 6a-e. Implant positioning surgery. a) the appearance of the regenerated socket, looking not distinguishable from the adjacent native bone; b) biopsy collection and c) the biopsy; d) implant positioning and e) suturing. / Pozicionimi kirurgjikali implantit. A) shfaqja e xhepit kockor te regjeneruar, qe duket i padallueshem nga kocka natyrale fqinje; b) mbledhja e biopsise, c) vendosja e implantit dhe e) suturimi.

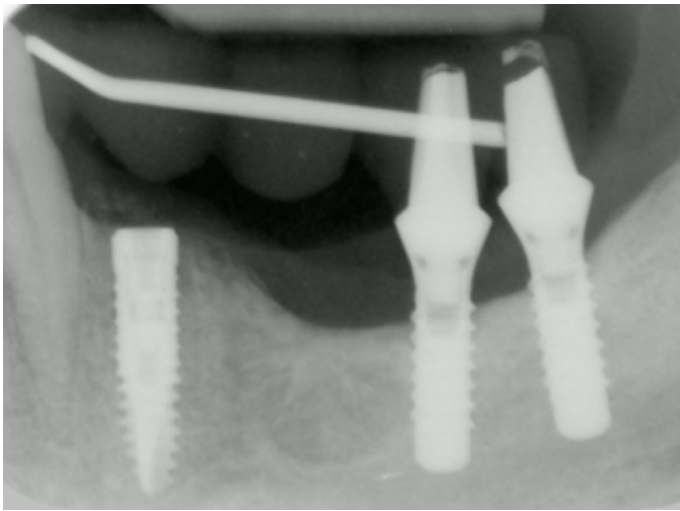


Figure 7. 3 months after implant placement. No signs of peri-implant resorption can be observed. / 3 muaj pas vendosjes se implantit. Nuk ka shenja te rezorbimit peri-implantar.

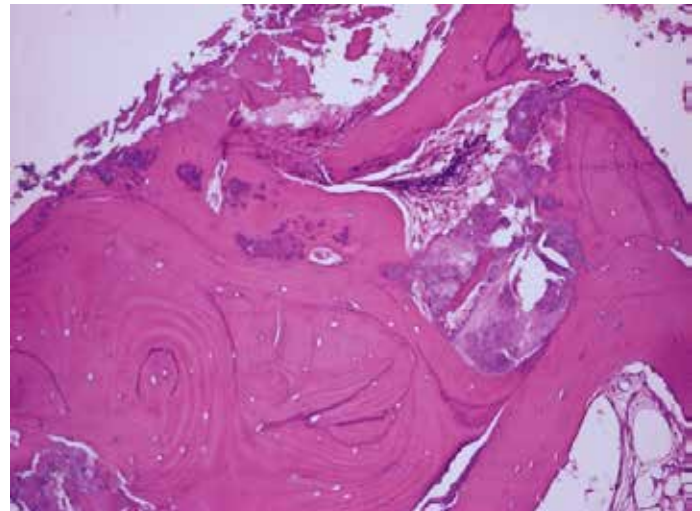


Figure 8. Histologic, 10x. The area of the sample is mainly filled with newly-regenerated bone. Graft particles, still undergoing remodeling, can be observed (dark purple). / Pamje histologjike e zmadhur 10X. Zona eshte kryesisht e mbushur me kocke te regjeneruar rishtazi, ende duke l nenshtuar rimodelimit (vjole e erret).

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