ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr
OPEN ACCESS

Acta stomatol Croat. 2026;60(2):224-235.
DOI: 10.15644/asc60/2/6

ORIGINAL SCIENTIFIC PAPER
IZVORNI ZNANSTVENI RAD

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Histomorphometric Evaluation of Socket Preservation Healing Using Xenogeneic Bone Substitute Combined with Statin: A Prospective Split-Mouth Clinical Pilot Study

Histomorfometrijska evaluacija cijeljenja u svrhu očuvanja alveole primjenom ksenogenoga koštanoga nadomjestka u kombinaciji sa statinom: prospektivna split-mouth klinička pilot-studija

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Abstract

Objective: Statins exhibit pleiotropic effects on bone metabolism, including osteogenic and angiogenic activity. The aim of this pilot study was to evaluate whether local application of simvastatin combined with a bovine-derived xenogeneic bone substitute enhances histomorphometric outcomes of socket preservation compared with the bone substitute alone after a three-month healing period. **Materials and Methods:** This prospective split-mouth clinical study included nine patients requiring bilateral tooth extraction. One socket was treated with cerabone® plus alone (control), while the contralateral socket received cerabone® plus combined with locally applied simvastatin (1 mg/0.2 mL saline). Bone core biopsies were harvested three months after grafting and analyzed histomorphometrically. The proportions of newly formed bone (NB/TV), residual bone substitute material (BSM/TV), and connective tissue (CT/TV) were quantified. Paired t-tests and Wilcoxon signed-rank tests were used for statistical analysis. **Results:** Histomorphometric analysis demonstrated a similar tissue composition in both groups. Connective tissue represented the predominant component in the control and statin-treated sites (59.1 ± 12.0% and 64.3 ± 9.4%, respectively; p = 0.31). Newly formed bone accounted for 31.0 ± 15.5% in the control group and 26.2 ± 11.6% in the statin group (p = 0.45). Residual bone substitute material showed comparable values between groups (9.9 ± 8.5% vs. 9.5 ± 9.7%; p = 0.91). No statistically significant differences were observed. **Conclusions:** The addition of locally applied simvastatin to a bovine-derived xenogeneic bone substitute did not result in significant histomorphometric differences in socket preservation after three months. At the tested dose and delivery method, simvastatin did not enhance early mineralized tissue formation compared with the bone substitute alone.

Received: January 20, 2026
Accepted: March 12, 2026

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MeSH Terms: Hydroxymethylglutaryl-CoA Reductase Inhibitors; Tooth Socket; Bone Substitutes; Bone Regeneration; Wound Healing
Author Keywords: Statin; Socket preservation; Bone regeneration; Xenograft; Histomorphometry

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Introduction

Bone regeneration is a key objective in oral and maxillofacial surgery, particularly in alveolar ridge preservation and socket grafting before implant placement (1-5). Xenogeneic bovine-derived bone substitutes, such as high-temperature sintered bovine bone combined with hyaluronic acid (e.g., cerabone® plus), provide a stable scaffold for osteoconduction and volume maintenance; however, fast and robust new bone formation remains challenging to achieve (6-8).

To enhance osteogenesis, several pharmacological adjuncts have been explored. Among these, statins—3-hydroxy-3-methyl-coenzyme A (HMG-CoA) reductase inhibitors, initially developed for lipid lowering, have shown pleiotropic effects on bone, including stimulation of osteogenesis and angiogenesis (9-11). Initial screening of over 30,000 compounds identified the statins lovastatin and simvastatin as stimulators of the bone morphogenetic protein-2 (BMP-2) promoter (12). Subsequent *in-vitro* studies demonstrated that statins such as simvastatin up-regulate BMP-2, activate the Ras/PI3K/Akt/ERK pathway and enhance osteoblast differentiation and viability (13).

Among available statins, simvastatin was chosen for this study due to its lipophilic nature, which allows better tissue penetration and cellular uptake compared with hydrophilic statins (e.g., pravastatin) (14,15). Moreover, preclinical studies have demonstrated that local simvastatin application at low milligram concentrations can enhance bone formation in calvarial and mandibular defects without inducing local toxicity or inflammation. These pharmacokinetic and biological characteristics make simvastatin a suitable candidate for local delivery in bone regenerative procedures (16-19). Given these biological properties, the working hypothesis of this study was that locally applied simvastatin combined with a bovine-derived xenograft (Cerabone® Plus) would modulate the early healing microenvironment, potentially enhancing angiogenic and osteogenic activity, and thereby influence early histomorphometric outcomes at 3 months. *In vivo*, local application of statins has been shown to increase bone formation in calvarial or mandibular animal models, with one study demonstrating increased BMP-2, nitric oxide (NO) and bone formation rate following local simvastatin placement in rats (20). Observational human studies also suggest a potential association between statin use and improved bone mineral density or reduced fracture risk, although the results are heterogeneous (21).

From a biomaterials standpoint, combining graft materials with pharmacologic agents to achieve synergistic effects — scaffold (osteoconduction) plus drug (osteoinduction) — remains an attractive strategy in bone regeneration. Recent reviews highlight that while statin-loaded biomaterials have shown promising in preclinical models, "translational barriers" remain: optimal statin dose, delivery kinetics, carrier interaction and human evidence are still lacking (22-24). Recent clinical and translational evidence highlights continued interest in combining biomaterials with pharmacologic agents to enhance bone regeneration. Studies evaluating xenogeneic grafts enriched with bioactive molecules

Uvod

Regeneracija kosti ključni je cilj u oralnoj i maksilofacijalnoj kirurgiji, osobito u očuvanju alveolarnoga grebena i augmentaciji alveole prije ugradnje implantata (1 – 5). Ksenogeni koštani nadomjestci dobiveni iz goveđe kosti, poput visokotemperaturno sinterirane goveđe kosti u kombinaciji s hijaluronskom kiselinom (npr., cerabone® plus), pružaju stabilan skelet za osteokondukciju i održavanje volumena. Međutim, postizanje brze i robusne novonastale kosti i dalje je izazov (6 – 8).

Kako bi se potaknula osteogeneza istraživani su različiti farmakološki adjuvansi. Među njima su statini – inhibitori 3-hidroksi-3-metil-koenzim A (HMG-CoA) reduktaze, na početku razvijeni za snižavanje lipida – pokazali pleiotropne učinke na kost, uključujući stimulaciju osteogeneze i angiogeneze (9 – 11). Početno ispitivanje više od 30 000 spojeva identificiralo je statine lovastatin i simvastatin kao stimulatore promotora koštanoga morfogenetskog proteina-2 (BMP-2) (12). U naknadnim studijama *in vitro* autori su pokazali da statini poput simvastatina povećavaju ekspresiju BMP-a 2, aktiviraju signalni put Ras/PI3K/Akt/ERK te potiču diferencijaciju i vitalnost osteoblasta (13).

Među dostupnim statinima za ovo istraživanje odabran je simvastatin zbog svoje lipofilne prirode koja omogućuje bolju penetraciju u tkivo i stanični unos u usporedbi s hidrofilnim statinima (npr., pravastatin) (14, 15). Nadalje, u pretkliničkim istraživanjima pokazano je da lokalna primjena simvastatina u niskim miligramskim koncentracijama može potaknuti stvaranje kosti u kalvarijalnim i mandibularnim defektima bez izazivanja lokalne toksičnosti ili upale. Te farmakokinetičke i biološke karakteristike čine simvastatin pogodnim za lokalnu primjenu u postupcima regeneracije kosti (16 – 19).

S obzirom na navedena biološka svojstva, radna hipoteza ovog istraživanja bila je da će lokalno primijenjeni simvastatin, u kombinaciji sa ksenograftom dobivenim iz goveđe kosti (Cerabone® Plus), modulirati rani mikrookoliš cijeljenja i potencijalno povećati angiogenu i osteogenu aktivnost te tako utjecati na rane histomorfometrijske ishode poslije tri mjeseca. *In vivo* je pokazano da lokalna primjena statina povećava stvaranje kosti u kalvarijalnim ili mandibularnim životinjskim modelima, pri čemu je u jednoj studiji zabilježeno povećanje BMP-a 2, dušikova oksida (NO) i brzine formiranja kosti nakon lokalne primjene simvastatina kod štakora (20). Promatračke studije na ljudima također sugeriraju moguću povezanost između primjene statina i poboljšane mineralne gustoće kosti ili smanjenoga rizika od prijeloma, iako su rezultati heterogeni (21).

S gledišta biomaterijala, kombiniranje graft materijala (presadak) s farmakološkim agensima radi postizanja sinergijskih učinaka – skelet (osteokondukcija) plus lijek (osteoindukcija) – ostaje atraktivna strategija u regeneraciji kosti. Nedavni pregledi ističu da, iako biomaterijali obogaćeni statinima pokazuju obećavajuće rezultate u pretkliničkim modelima, i dalje postoje *translacijske prepreke*: optimalna doza statina, kinetika oslobađanja, interakcija s nosačem i klinički dokazi kod ljudi još uvijek nisu dovoljno razjašnjeni (22 –

have reported variable effects depending on carrier properties, release kinetics, and defect characteristics (2,3). Similarly, systematic assessments of drug–biomaterial combinations emphasize the importance of dose optimisation and controlled delivery systems for achieving predictable osteogenic responses (23). Despite promising preclinical data, the translational relevance of locally delivered statins remains uncertain due to inconsistent clinical outcomes, heterogeneous dosing strategies, and lack of pharmacokinetic characterisation. Consequently, small-scale clinical pilot studies are needed to explore feasibility, safety, and preliminary biological effects prior to larger controlled trials.

Given this background, the present study aimed to evaluate whether the local application of a statin combined with a bovine-derived xenograft (cerabone® plus) in a split-mouth clinical design would enhance histomorphometric parameters of alveolar bone regeneration compared with the graft alone. Specifically, we assessed residual graft material (BSM/TV %), newly formed bone (NB/TV %) and soft-tissue fraction (CT/TV %) at 3 months. A 3-month healing interval was selected because this reflects the early stage of xenograft integration commonly used for implant site evaluation and corresponds to the standard timeline for biopsy retrieval in clinical studies of socket preservation. For all histomorphometric parameters, TV denotes total tissue volume, comprising mineralised bone, residual graft material, and non-mineralised connective tissue.

Materials and Methods

Study Design

This prospective split-mouth clinical study investigated the histomorphometric characteristics of bone regeneration following preservation with cerabone® plus (Botiss Biomaterials GmbH, Zossen, Germany), with and without the addition of locally applied simvastatin (Simvastatin, Calbiochem, San Diego, CA, USA).

Each patient received two grafts: one placed in a scheduled extraction socket designated as the control site, and the contralateral socket in the opposing quadrant designated as the experimental site, allowing intra-patient comparison under identical biological conditions.

Randomization was performed using a computer-generated random sequence (www.randomizer.org) by an independent investigator not involved in the surgical or analytical procedures. Allocation was concealed until the time of graft preparation.

All procedures were performed in accordance with the Declaration of Helsinki (2013) and approved by the School of Dental Medicine University of Zagreb Ethics Committee (approval no. 05 – PA-30-III-12/2021.). All participants signed written informed consent prior to enrolment.

24). U nedavnim kliničkim i translacijskim dokazima ističe se kontinuirani interes za kombiniranje biomaterijala i farmakoloških agenasa radi poboljšanja regeneracije kosti. U studijama u kojima se procjenjuju ksenogeni graftovi obogaćeni bioaktivnim molekulama pokazani su varijabilno učinci ovisno o svojstvima nosača, kinetici oslobađanja i značajkama defekta (2, 3). Slično tomu, sustavne analize kombinacija lijek-biomaterijal pokazuju važnost optimizacije doze i kontroliranih sustava isporuke za postizanje predvidivih osteogenih odgovora (23).

Unatoč obećavajućim pretkliničkim podatcima, translacijska relevantnost lokalno primijenjenih statina ostaje neizvjesna zbog nekonzistentnih kliničkih ishoda, heterogenih strategija doziranja i nedostatka farmakokinetičke karakterizacije. Zato su, prije većih kontroliranih istraživanja, potrebne male kliničke pilot-studije kako bi se procijenila izvedivost, sigurnost i preliminarni biološki učinci.

S obzirom na navedeno, cilj ovog istraživanja bio je procijeniti hoće li lokalna primjena statina u kombinaciji sa ksenograftom dobivenim iz goveđe kosti (cerabone® plus) u *split-mouth* kliničkom dizajnu poboljšati histomorfometrijske parametre regeneracije alveolarne kosti u usporedbi sa samim graftom. Konkretno, analizirani su rezidualni graft materijal (BSM/TV %), novostvorena kost (NB/TV %) i udio mekoga tkiva (CT/TV %) poslije tri mjeseca. Razdoblje cijeljenja od tri mjeseca odabrano je zato što je to rana faza integracije ksenografta koja se uobičajeno koristi za procjenu implantacijskog mjesta te odgovara standardnom vremenu uzimanja biopsije u kliničkim studijama o očuvanju alveole. Za sve histomorfometrijske parametre, TV označava ukupni volumen tkiva koji uključuje mineraliziranu kost, rezidualni graft materijal i nemineralizirano vezivno tkivo.

Materijali i metode

Dizajn istraživanja

U ovom prospektivnom kliničkom istraživanju sa *split-mouth* dizajnom ispitivale su se histomorfometrijske karakteristike regeneracije kosti nakon očuvanja alveole s cerabone® plusom (Botiss Biomaterials GmbH, Zossen, Njemačka) s dodatkom lokalno primijenjenoga simvastatina i bez tog dodatka (Simvastatin, Calbiochem, San Diego, CA, SAD). Svaki pacijent primio je dva grafta: jedan je postavljen u planiranu alveolu za ekstrakciju (kontrolno mjesto), a drugi u kontralateralnu alveolu u suprotnome kvadrantu (eksperimentalno mjesto), čime je omogućena intraindividualna usporedba u identičnim biološkim uvjetima. Randomizaciju je proveo s pomoću računalno generiranoga slučajnog niza (www.randomizer.org) neovisni istraživač koji nije sudjelovao u kirurškim ili analitičkim postupcima. Raspodjela je bila skrivena do trenutka pripreme grafta. Svi postupci provedeni su u skladu s Helsinškom deklaracijom (2013.) i odobrilo ih je Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu (odobrenje br. 05 – PA-30-III-12/2021.). Svi ispitanici potpisali su informirani pristanak prije uključivanja u istraživanje.

Patient Population and Surgical Procedure

Nine adult patients (five females, four males) aged 29 to 79 years (mean 56 years) requiring extraction of two non-adjacent teeth located in contralateral quadrants of the same jaw, enabling a split-mouth comparison, were enrolled. All participants were systemically healthy (ASA I–II) and had no history of metabolic bone disease, bisphosphonate or corticosteroid therapy, or systemic statin use (Table 1). Moreover, all patients presented with good periodontal health, with no untreated periodontitis, active infection, or suppuration at the time of surgery.

All extraction sites exhibited intact socket morphology at baseline. The buccal plate was preserved in all cases, as were the remaining alveolar walls. No signs of periodontal inflammation were present around the post-extraction alveolus. In cases where tooth extraction was indicated due to a chronic periapical (ostitic) process, the pathological lesion was completely removed together with its surrounding shell, and only the sites with intact bony walls after thorough debridement were included in the study. The average mesio-distal width of the post-extraction alveolus was approximately 10–11 mm, while the bucco-oral width ranged from 11–12 mm, indicating comparable socket dimensions across sites.

Atraumatic tooth extractions were performed under local anaesthesia (articaine 4 % + epinephrine 1:100,000; Septodont, France). Atraumatic extraction was performed using periotomes and fine luxators (Hu-Friedy, Chicago, USA) without raising a flap, in order to preserve socket walls and minimise trauma. Following the socket debridement, the grafting procedure was randomised per side:

- Control site: cerabone® plus granules (0.5–1 mm; Botiss Biomaterials GmbH, Zossen, Germany) hydrated in sterile saline.
- Experimental site: cerabone® plus combined with simvastatin (Simvastatin, Calbiochem, USA) 1 mg in 0.2 mL saline immediately before placement.

The 1 mg simvastatin dose used in this study represents a low local concentration previously shown to be biologically active in preclinical calvarial and mandibular models, while remaining below thresholds associated with local cytotoxicity or inflammatory reactions (15-18).

All grafts were covered with a resorbable collagen membrane (Jason® membrane, Botiss Biomaterials GmbH, Zossen, Germany) and closed primarily with 4-0 monofilament sutures. Postoperative care included amoxicillin 500 mg three times daily for 5 days and 0.12 % chlorhexidine rinses twice daily for 1 week.

Clinical follow-up was performed at 7 days, 1 month, and 3 months postoperatively to assess healing and detect potential complications such as soft tissue dehiscence, membrane exposure, infection, graft loss, or adverse tissue reactions. Healing was uneventful in all cases. At 7 days, soft-tissue closure, absence of dehiscence, and membrane coverage were evaluated and sutures were removed. At 1 month, mucosal maturation and ridge contour stability were assessed. At 3 months, sites were re-evaluated clinically before biopsy retrieval.

Populacija ispitanika i kirurški postupak

U istraživanje je bilo uključeno devet odraslih pacijenata (pet žena, četiri muškarca) u dobi od 29 do 79 godina (prosječno 56 godina) kojima je bila potrebna ekstrakcija dvaju nesusjednih zuba smještenih u kontralateralnim kvadrantima iste čeljusti, što je omogućilo *split-mouth* usporedbu. Svi ispitanici bili su sistemski zdravi (ASA I – II) bez anamneze metaboličkih bolesti kosti, terapije bisfosfonatima ili kortikosteroidima i sistemske primjene statina (tablica 1.). Svi su također imali dobro parodontno zdravlje, bez neliječene parodontne bolesti, aktivne infekcije ili supuracije u vrijeme zahvata.

Sve alveole nakon ekstrakcije imale su očuvanu morfologiju. Bukalna kortikalna ploča bila je očuvana u svim slučajevima, kao i ostali zidovi alveole. Nije bilo znakova parodontne upale. U slučajevima kada je ekstrakcija bila indicirana zbog kroničnoga periapikalnog procesa, patološka lezija bila je u cijelosti uklonjena zajedno s okolnim tkivom, a u studiju su uključena samo mjesta s intaktnim koštanim stijenkama nakon temeljite kiretaže. Prosječna meziodistalna širina alveole bila je približno 10 – 11 mm, a bukooralna širina iznosila 11 – 12 mm, što upućuje na usporedive dimenzije između mjesta.

Atraumatske ekstrakcije učinjene su u lokalnoj anesteziji (artikain 4 % + epinefrin 1 : 100 000; Septodont, Francuska). Ekstrakcije su obavljene periotomima i finim luksatorima (Hu-Friedy, Chicago, SAD) bez podizanja režnja kako bi se očuvali zidovi alveole i smanjila trauma. Nakon debridmana alveole, postupak augmentacije randomiziran je po strani:

- kontrolno mjesto: cerabone® plus granule (0,5 – 1 mm) hidratizirane u sterilnoj fiziološkoj otopini
- eksperimentalno mjesto: cerabone® plus u kombinaciji sa simvastatinom (1 mg u 0,2 mL fiziološke otopine) neposredno prije aplikacije.

Doza simvastatina od 1 mg niska je lokalna koncentracija za koju je u pretkliničkim modelima dokazano da je biološki aktivna, a pritom ispod razine povezane s citotoksičnošću ili upalnim reakcijama (15 – 18).

Svi graftovi prekriveni su resorptivnom kolagenom membranom (Jason® membrane, Botiss Biomaterials GmbH, Njemačka) te primarno zatvoreni monofilamentnim šavovima 4-0. Postoperativna terapija uključivala je amoksicilin 500 mg tri puta na dan tijekom 5 dana te jednotjedno ispiranje 0,12-postotnim klorheksidinom dva puta na dan.

Kliničko praćenje provedeno je poslije sedam dana, jednog mjeseca i tri mjeseca radi procjene cijeljenja i eventualnih komplikacija (dehiscencija, izlaganje membrane, infekcija, gubitak grafta, neželjene reakcije). Cijeljenje je u svim slučajevima bilo uredno.

Biopsy Retrieval and Tissue Processing

At 3 months post-grafting, bone core biopsies (Ø 2 mm, length 8 mm) were harvested from the center of the preserved socket during implant site preparation using a trephine bur under copious sterile saline irrigation. Biopsy retrieval was performed without flap elevation, through the healed mucosa, to minimize additional trauma and maintain the integrity of the regenerated site. The depth of 8 mm corresponded to the planned implant length and ensured sampling of the entire regenerated compartment while avoiding perforation of the native bone floor.

Samples were fixed in 10 % neutral buffered formalin, dehydrated in graded ethanol, and embedded in methyl methacrylate to preserve mineralised architecture.

Trephine bur (Ø 2 mm, Hager & Meisinger GmbH, Neuss, Germany) was used for retrieval. Sections (~50 µm thick) were cut using a microtome (Leica SP1600, Leica Microsystems, Germany), and staining was performed using toluidine blue and Masson's trichrome kits (Sigma-Aldrich, USA). All samples were coded and analysed in a blinded fashion.

Histomorphometric Analysis

The histomorphometric evaluation was performed according to established protocols (24). Digitised slides were obtained using a light microscope (Axio Scope.A1, Zeiss) with a digital camera (Axiocam 105, Zeiss), a motorised stage, and ZEN Core v3.0 software for panoramic scans. Quantitative histomorphometry measured the percentage of NB/TV, BSM/TV, and CT/TV. Analyses were performed using ZEN Core and verified in ImageJ v1.53 (NIH, Bethesda, USA); three systematically selected high-magnification fields per section were averaged per specimen. Fields were selected using a systematic uniform random sampling protocol. The analysed area per field measured 1.2 mm². Interobserver reliability was assessed in 20% of samples and demonstrated excellent agreement (ICC > 0.90).

Statistical Analysis

All statistical analyses were performed at the patient level (n = 9 pairs) in accordance with the split-mouth study design. Continuous variables are presented as mean ± standard deviation (SD). Normality was assessed on the distribution of paired differences (experimental minus control) using the Shapiro–Wilk test. No statistically significant deviations from normality were detected for NB/TV, BSM/TV, or CT/TV ($p > 0.05$ for all comparisons), supporting the use of parametric testing. Primary comparisons between control (Cerabone® Plus) and experimental (Cerabone® Plus + Statin) sites were performed using paired t-tests. Given the small paired sample size and the limited power of normality testing in small datasets, Wilcoxon signed-rank tests were additionally conducted to confirm the robustness of the findings. Non-parametric analyses yielded results consistent with parametric testing for all evaluated outcomes. For each parameter, mean differences, 95% confidence intervals (CIs), and standardized effect sizes (Cohen's d for paired samples) were calculated to quantify the magnitude and precision of treatment effects. Statistical significance was defined as p <

Uzimanje biopsije i obrada tkiva

Tri mjeseca poslije zahvata uzeti su koštani uzorci (Ø 2 mm, duljine 8 mm) iz središta očuvane alveole tijekom pripreme ležišta implantata, s pomoću konusnog svrdla uz obilnu irigaciju sterilnom fiziološkom otopinom. Uzorkovanje je obavljeno bez podizanja režnja kroz zacijeljenu sluznicu da bi se minimizirala dodatna trauma. Dubina od 8 mm odgovarala je planiranoj dužini implantata.

Uzorci su fiksirani u 10-postotnom neutralnom formalinu, dehidrirani u etanolu i uklopljeni u metil-metakrilat.

Za uzimanje uzoraka korišten je trepanski svrdlo (Ø 2 mm, Hager & Meisinger GmbH, Neuss, Njemačka). Rezovi (debljine ~50 µm) izrezani su pomoću mikrotoma (Leica SP1600, Leica Microsystems, Njemačka), a bojenje je provedeno pomoću toluidinskog plavila i Massonovog trikromskog kita (Sigma-Aldrich, SAD). Svi uzorci su kodirani i analizirani na slijepo.

Histomorfometrijska analiza

Analiza je provedena prema standardiziranim protokolima. Digitalizirani preparati dobiveni su svjetlosnim mikroskopom (Axio Scope.A1, Zeiss) uz digitalnu kameru i softver ZEN Core v3.0.

Mjereni su: NB/TV (%) – novostvorena kost, BSM/TV (%) – rezidualni graft materijal i CT/TV (%) – vezivno tkivo. Analize su obavljene u softverima ZEN Core i ImageJ. Tri reprezentativna polja po uzorku analizirana su i prosječno izračunata. Pouzdanost između ispitivača bila je visoka (ICC > 0,90).

Statistička analiza

Sve statističke analize provedene su na razini pacijenta (n = 9 parova) u skladu s dizajnom studije s podijeljenim ustima. Kontinuirane varijable prikazane su kao srednja vrijednost ± standardna devijacija (SD). Normalnost je procijenjena na temelju distribucije parnih razlika (eksperimentalna minus kontrola) pomoću Shapiro-Wilkovog testa. Nisu otkrivena statistički značajna odstupanja od normalnosti za NB/TV, BSM/TV ili CT/TV ($p > 0,05$ za sve usporedbe), što podupire upotrebu parametrijskog testiranja. Primarne usporedbe između kontrolnih (Cerabone® Plus) i eksperimentalnih (Cerabone® Plus + Statin) mjesta provedene su korištenjem parnih t-testova. S obzirom na malu veličinu parnog uzorka i ograničenu snagu testiranja normalnosti u malim skupovima podataka, dodatno su provedeni Wilcoxonovi testovi predznačenog ranga kako bi se potvrdila robusnost nalaza. Neparametrijske analize dale su rezultate u skladu s parametrijskim testiranjem za sve procijenjene ishode. Za svaki parametar izračunate su srednje razlike, 95% intervali pouzdanosti (CI) i standardizirane veličine učinaka (Cohenov d za uparene uzorke) kako bi se kvantificirala veličina i preciznost učinaka liječenja. Stati-

0.05 (two-tailed). As this investigation was designed as an exploratory pilot study, no *a priori* sample size calculation was performed. Therefore, inferential results should be interpreted cautiously and primarily as hypothesis-generating, intended to inform effect-size estimation and sample-size planning for future confirmatory trials. All analyses were conducted using IBM SPSS Statistics version 29.0.1.0 (IBM Corp., Armonk, NY, USA).

Results

Nine patients (four females, five males; mean age 56 ± 17 years, range 28–79 years) completed the study. Each participant contributed two grafted sites (control and experimental), resulting in a total of 18 biopsy specimens for analysis (Table 1). All surgical procedures were uneventful, with no intra- or postoperative complications, inflammation, or graft exposure observed. Postoperative healing was satisfactory in all cases, and all sites demonstrated clinically stable soft-tissue coverage and adequate ridge contour at the time of biopsy retrieval (three months post-grafting). No systemic or local adverse effects related to statin application were reported.

The histomorphometric evaluation demonstrated a consistent and reproducible pattern of tissue distribution in both treatment modalities (Figure 1). No statistically significant

stička značajnost definirana je kao p < 0,05 (dvostrana). Budući da je ovo istraživanje osmišljeno kao eksplorativna pilot studija, nije proveden a priori izračun veličine uzorka. Stoga, inferencijalne rezultate treba tumačiti oprezno i prvenstveno kao generiranje hipoteze, namijenjene informiranju o procjeni veličine učinka i planiranju veličine uzorka za buduća potvrdna ispitivanja. Sve analize provedene su korištenjem IBM SPSS Statistics verzije 29.0.1.0 (IBM Corp., Armonk, NY, SAD).

Rezultati

Devet pacijenata (četiri žene, pet muškaraca; srednja dob 56 ± 17 godina, raspon 28 – 79 godina) završilo je studiju. Svakomu su uzeta dva presatka tkiva (kontrolni i eksperimentalni), što ukupno čini 18 uzoraka biopsije za analizu (tablica 1.). Svi kirurški zahvati bili su bez poteškoća, bez intraoperativnih ili postoperativnih komplikacija, upale ili otkazivanja presatka. Postoperativno cijeljenje bilo je zadovoljavajuće u svim slučajevima, a sva su mjesta pokazala klinički stabilno meko tkivo i adekvatnu pokrivenost presatka u vrijeme uzimanja biopsije (tri mjeseca poslije zahvata). Nisu zabilježeni sustavni ili lokalni štetni učinci povezani s primjenom statina.

Histomorfometrijska analiza pokazala je dosljedan i reproducibilan obrazac raspodjele tkiva u oba modaliteta lije-

Table 1 Demographic and clinical characteristics of the study population and site allocation.
Tablica 1. Demografske i kliničke karakteristike ispitivane populacije i raspodjela mjesta zahvata

No. • Br.	Sex • Spol	Age • Dob	Tooth/Region • Zub/Regija	Control • Kontrola	Tooth/Region • Zub/Regija	Experimental • Eksperimentalno
1	Female • Ženski	64	16	Cerabone® Plus	26	Cerabone® Plus + Statin
2	Female • Ženski	79	16	Cerabone® Plus	26	Cerabone® Plus + Statin
3	Male • Muški	60	16	Cerabone® Plus	26	Cerabone® Plus + Statin
4	Male • Muški	45	15	Cerabone® Plus	26	Cerabone® Plus + Statin
5	Female • Ženski	28	16	Cerabone® Plus	26	Cerabone® Plus + Statin
6	Male • Muški	55	16	Cerabone® Plus	26	Cerabone® Plus + Statin
7	Male • Muški	70	16	Cerabone® Plus	26	Cerabone® Plus + Statin
8	Female • Ženski	45	46	Cerabone® Plus	26	Cerabone® Plus + Statin
9	Male • Muški	29	16	Cerabone® Plus	26	Cerabone® Plus + Statin

Table 2 Histomorphometric outcomes (mean ± SD, 95 % confidence intervals, p-values, and effect sizes) for control (Cerabone® Plus) and experimental (Cerabone® Plus + Statin) sites at three months.
Tablica 2. Histomorfometrijski ishodi (srednja vrijednost ± SD, 95 % interval pouzdanosti, p-vrijednosti i veličina učinka) za kontrolna (Cerabone® Plus) i eksperimentalna (Cerabone® Plus + Statin) mjesta poslije tri mjeseca

Parameter • Parametar	Group • Skupina	Mean ± SD (%) • Srednja vrijednost ± SD (%)	95 % CI of the difference • 95 % CI razlike	*Paired t-test p*-value • p-vrijednost (t-test)	Wilcoxon p-value • p-vrijednost (Wilcoxon)	Cohen's *d* • Cohenov d
Newly formed bone (NB/TV) • Novonastala kost (NB/TV)	Cerabone® Plus	31.0 ± 15.5	[–8.7, 18.4]	0.45	0.594	0.27
	Cerabone® Plus + Statin	26.2 ± 11.6				
Residual bone substitute (BSM/TV) • Ostatni koštani nadomjestak (BSM/TV)	Cerabone® Plus	9.9 ± 8.5	[–6.3, 7.1]	0.91	0.889	0.03
	Cerabone® Plus + Statin	9.5 ± 9.7				
Connective tissue (CT/TV) • Vezivno tkivo (CT/TV)	Cerabone® Plus	59.1 ± 12.0	[–16.2, 5.3]	0.31	0.441	–0.36
	Cerabone® Plus + Statin	64.3 ± 9.4				

Data are presented as mean ± standard deviation (SD), with 95 % confidence intervals (CI) and effect sizes (Cohen's d) calculated for paired comparisons. No statistically significant differences were observed between groups. • Podatci su prikazani kao srednja vrijednost ± standardna devijacija (SD), uz 95 % intervale pouzdanosti (CI) i veličine učinka (Cohenov d) za uparene usporedbe. Nisu utvrđene statistički značajne razlike između skupina.

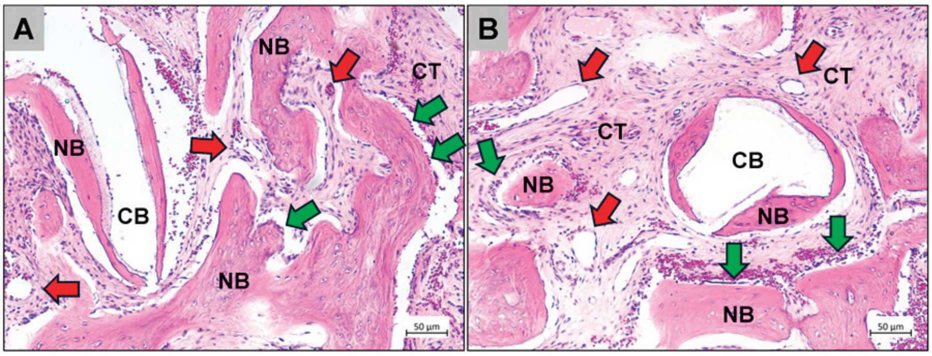

Figure 1 Representative histological sections of the Cerabone® Plus + Statin (A) and Cerabone® Plus (B) sites at three months (HE, 100×; scale bar = 50 µm). Newly formed bone (NB) is apposed to Cerabone® granules (CB), demonstrating osseointegration. Red arrows indicate blood vessels supporting revascularization, and green arrows highlight osteoblast seams at NB/CB interfaces. A gradual transition from connective tissue (CT) to NB reflects progressive tissue maturation.

Slika 1. Reprezentativni histološki presjeci mjesta Cerabone® Plus + Statin (A) i Cerabone® Plus (B) poslije tri mjeseca (HE, 100 ×; mjerna traka = 50 µm); novonastala kost (NB) priliježe uz granule Cerabonea® (CB), što upućuje na osteointegraciju; crvene strelice označavaju krvne žile koje podržavaju revaskularizaciju, a zelene strelice ističu slojeve osteoblasta na sučelju NB/CB-a; postupan prijelaz iz vezivnoga tkiva (CT) u NB odražava progresivno sazrijevanje tkiva

inter-individual variation was detected between the Cerabone® Plus and Cerabone® Plus + Statin groups.

Across all samples, connective tissue (CT) represented the predominant tissue component, followed by newly formed bone (NB) and residual bone substitute material (BSM). Quantitatively, CT accounted for approximately 59 ± 12 % of the total tissue in the control group and 64 ± 9 % in the Cerabone® Plus + Statin group. The difference between groups was not statistically significant (p = 0.31; Cohen's d = –0.36), but a trend toward higher CT fraction (~5 %) in the Cerabone® Plus + Statin group was observed.

The relative proportions of NB/TV and BSM/TV were also similar between treatments. NB/TV represented 31.0 ± 15.5 % in the Cerabone® Plus group and 26.2 ± 11.6 % in the Cerabone® Plus + Simvastatin group, with no significant difference between groups (p = 0.45; Cohen's d = 0.27). BSM/TV constituted approximately 10 % of the total tissue volume in both conditions (9.9 ± 8.5 % vs. 9.5 ± 9.7 %), indicating comparable graft resorption behavior at the 3-month interval (Table 2, Figure 1).

Collectively, these data indicate that while statin addition did not significantly modify the quantitative tissue composition, it preserved the normal healing pattern observed with Cerabone® Plus alone. The modest (~5 %) increase in CT/TV observed in the Cerabone® Plus + Statin sites may reflect a transient early-stage soft-tissue predominance rather than reduced osteogenic activity, which is consistent with known early angiogenic/fibroblastic effects of statins.

Discussion

This clinical split-mouth pilot study evaluated the effect of adding locally applied simvastatin to a bovine-derived xenograft (Cerabone® Plus) on alveolar bone regeneration following socket preservation. The quantitative histomorphometric analysis at three months revealed no statistically significant differences in NB/TV, BSM/TV, or CT/TV between Cerabone® Plus and Cerabone® Plus + Statin sites. Nevertheless, a consistent intra-individual trend toward a slightly

čenja (slika 1.). Nisu otkrivene statistički značajne razlike između skupina Cerabone® Plus i Cerabone® Plus + Statin.

U svim uzorcima vezivno tkivo (CT) činilo je prevladavajuću komponentu, a slijedila ga je novonastala kost (NB) i ostatni koštani nadomjestak (BSM). Kvantitativno, CT je činio približno 59 ± 12 % ukupnoga volumena tkiva u kontrolnoj skupini (64 ± 9 % u Cerabone® Plus + Statin skupini). Razlika između skupina nije bila statistički značajna (p = 0,31; Cohenov d = –0,36), ali je zabilježen trend prema većem udjelu CT-a (~5 %) u skupini Cerabone® Plus + Statin.

Relativni udjeli NB/TV-a i BSM/TV-a također su bili slični između tretmana. NB/TV iznosio je 31,0 ± 15,5 % u skupini Cerabone® Plus i 26,2 ± 11,6 % u skupini Cerabone® Plus + Statin, bez značajne razlike između skupina (p = 0,45; Cohenov d = 0,27). BSM/TV činio je približno 10 % ukupnoga volumena tkiva u oba stanja (9,9 ± 8,5 % prema 9,5 ± 9,7 %), što upućuje na usporedivu retenciju ostatnoga biomaterijala poslije 3-mjesečnoga intervala (tablica 2., slika 2.).

Ukupno gledano, ti podatci pokazuju da dodatak statina nije značajno promijenio kvantitativni sastav tkiva u očuvanim modelima alveolarnoga grebena liječenima Cerabone® Plusom samim ili u kombinaciji (5 – 6 %) s povećanjem CT/TV-a zabilježenim u skupini Cerabone® Plus + Statin, što može odražavati prolaznu fazu rane dominacije mekog tkiva, a ne smanjenu osteogenu aktivnost, u skladu s poznatim ranim angiogenim/fibroblastičnim učincima statina.

Rasprava

Ova klinička pilot-studija s razdvojenim ustima (*split-mouth*) procjenjivala je učinak dodavanja lokalno primijenjenoga simvastatina bovinom ksenograftu (Cerabone® Plus) na regeneraciju alveolarne kosti nakon očuvanja alveole. Kvantitativna histomorfometrijska analiza poslije tri mjeseca nije pokazala statistički značajne razlike u NB/TV-u, BSM/TV-u ili CT/TV-u između mjesta liječenih Cerabone® Plusom i Cerabone® Plusom + Statin. Ipak, uočena je dosljedhigher CT/TV fraction (~5 %) was observed in the Cerabone® Plus + Statin group. Although not statistically significant, this subtle shift is biologically plausible and may reflect early modulation of fibrovascular tissue rather than a clear osteoanabolic effect.

The present findings align with preclinical and translational data indicating that statins exert complex, dose- and carrier-dependent effects on bone formation. Mundy et al. (26) first identified statins as stimulators of the BMP-2 promoter, linking HMG-CoA reductase inhibition to osteoanabolic signalling. Subsequent animal and biomaterial studies have shown that local statin delivery can increase bone formation and neovascularisation, but that the magnitude and duration of response depend on the statin type, concentration, and delivery system. Recent reviews have highlighted that statin-loaded scaffolds, hydrogels, and microspheres can enhance osteogenesis and angiogenesis when sustained local levels are achieved, yet translation to predictable clinical benefit remains challenging (23,24,27,28).

In humans, data remain inconsistent. While systemic statin use has been associated with beneficial skeletal effects, controlled local-delivery studies show variable outcomes. Randomized clinical trials of locally delivered simvastatin in periodontal intrabony defects have reported mixed results: some demonstrated adjunctive gains in clinical attachment or radiographic fill, whereas others showed no significant benefit, particularly in anatomically constrained lesions. More recent clinical trials and systematic reviews confirm that local statin efficacy depends strongly on dose, carrier, defect morphology, and release kinetics (29,30). Meta-analytic syntheses likewise indicate that local statins can improve certain periodontal parameters, yet effect sizes vary considerably across studies (31,32). A recent systematic review focusing on socket healing after tooth extraction suggested potentially favorable outcomes with local simvastatin, though substantial methodological heterogeneity persists (33).

In the current study, bone formation occurred predictably in both groups, with NB/TV constituting approximately 30 % of the total tissue and BSM/TV around 10 %. These proportions are broadly consistent with reports in the literature regarding slowly resorbing xenogeneic bovine grafts at early healing stages, where limited graft resorption and moderate new bone formation are expected (25, 34). The similarity between Cerabone® Plus and Cerabone® Plus + Statin sites indicates that the simvastatin dose and release profile achieved with simple mixing did not substantially modulate osteogenic activity within the three-month interval. The low resorption rate and compact pore architecture typical of this class of xenografts may have further restricted drug diffusion to osteogenic cells within the defect (35,36). The modest increase in CT/TV in simvastatin-treated sites, although not statistically significant, warrants mechanistic consideration. Statins are known to enhance angiogenesis and fibroblast proliferation through up-regulation of VEGF, eNOS, and hypoxia-inducible factor-1α pathways, and these pleiotropic effects may initially favour a fibrovascular tissue phenotype (37). In the context of bone healing, such vascular activation typically precedes mineralisation; therefore, a higher

na unutarpojedinačna tendencija prema nešto većem udjelu CT/TV-a (~5 %) u skupini Cerabone® Plus + Statin. Iako nije statistički značajna, ova suptilna promjena biološki je opravdana i može odražavati ranu modulaciju fibrovaskularnoga tkiva, a ne jasan osteoanabolički učinak.

Dobiveni rezultati podudaraju se s pretkliničkim i translacijskim podatcima koji pokazuju da statini imaju složene učinke na formiranje kosti, ovisne o dozi i načinu primjene. Mundy i suradnici (26) prvi su identificirali statine kao stimulatore promotora BMP-a 2, povezujući inhibiciju HMG-CoA reduktaze s osteoanaboličkim signaliziranjem. U naknadnim studijama na životinjama i biomaterijalima pokazano je da lokalna primjena statina može povećati stvaranje kosti i neovaskularizaciju, ali da veličina i trajanje odgovora ovise o vrsti statina, koncentraciji i sustavu dostave. U nedavnim pregledima ističe se da nosači s ugrađenim statinima (scaffoldi), hidrogeli i mikrosfere mogu potaknuti osteogenezu i angiogenezu kada se postignu stabilne lokalne koncentracije, no prijenos u predvidivu kliničku korist i dalje je izazovan (23, 24, 27, 28).

Kod ljudi su podatci i dalje nedosljedni. Iako je sistemska primjena statina povezana s povoljnim učincima na skelet, kontrolirane studije lokalne primjene pokazuju varijabilne rezultate. Randomizirana klinička ispitivanja lokalnoga simvastatina u parodontnim intrakoštanim defektima daju mješovite rezultate: u nekima su zabilježena poboljšanja kliničkoga pripoja ili radiološkog ispuna, a u drugima nije pokazana značajna korist, osobito u anatomski ograničenim lezijama. Novija klinička ispitivanja i sustavni pregledi potvrđuju da učinkovitost lokalnih statina snažno ovisi o dozi, nosaču, morfologiji defekta i kinetici otpuštanja (29, 30). Metaanalize također pokazuju da lokalni statini mogu poboljšati određene parodontne parametre, ali se veličina učinka znatno razlikuje među studijama (31, 32). Nedavni sustavni pregled o cijeljenju alveole nakon ekstrakcije zuba sugerira potencijalno povoljne ishode uz lokalni simvastatin, no metodološka heterogenost i dalje je velika (33).

U ovoj studiji formiranje kosti bilo je predvidivo u objema skupinama, pri čemu je NB/TV činio približno 30 % ukupnog tkiva, a BSM/TV oko 10 %. Te vrijednosti u skladu su s literaturom o sporije resorbirajućim ksenogenim bovinim graftovima u ranim fazama cijeljenja, gdje se očekuju ograničena resorpcija grafta i umjereno stvaranje nove kosti (25, 34). Sličnost između skupina Cerabone® Plus i Cerabone® Plus + Statin upućuje na to da doza i profil otpuštanja simvastatina postignuti jednostavnim miješanjem nisu značajno utjecali na osteogenu aktivnost unutar tromjesečnog razdoblja. Niska stopa resorpcije i kompaktna porozna struktura tipična za ovu vrstu ksenografta mogu dodatno ograničiti difuziju lijeka do osteogenih stanica unutar defekta (35, 36).

Blago povećanje CT/TV-a u skupini sa statinom, iako nije statistički značajno, zahtijeva mehanicističko objašnjenje. Poznato je da statini potiču angiogenezu i proliferaciju fibroblasta putem povećane ekspresije VEGF-a, eNOS-a i HIF-1α, te ti pleiotropni učinci na početku mogu favorizirati fibrovaskularni tip tkiva (37). U kontekstu cijeljenja kosti, takva vaskularna aktivacija obično prethodi mineralizaciji; zato viši CT/TV poslije tri mjeseca može biti prijelazna

CT/TV fraction at three months may represent an intermediate phase of enhanced granulation or fibrovascular tissue rather than a definitive impairment of bone formation. Similar early-phase increases in soft tissue have been described in statin-loaded scaffolds that subsequently support improved bone regeneration once mineralisation progresses (10,38-40).

From a pharmacological standpoint, these results reinforce that statin effects in bone are highly context-dependent and critically influenced by the delivery system. Osteoanabolic activity mediated by BMP-2 up-regulation generally requires sustained local statin concentrations in the low micromolar range (27,41), whereas angiogenic or anti-inflammatory responses can occur at lower doses. Simple admixture of simvastatin with a dense, slowly resorbing xenograft such as Cerabone® Plus is unlikely to provide controlled release, and the drug may be rapidly cleared from the defect or sequestered within the graft surface. Recent experimental work has shown that statin effects are markedly attenuated when delivered without an appropriate drug-delivery system and can be prolonged when incorporated into hydrogels, polymeric membranes, or microsphere-based carriers (42-45).

Taken together, the current data suggest that simply mixing simvastatin with a xenogeneic bone graft is unlikely to elicit measurable osteogenic enhancement at three months. Future translational efforts should prioritise delivery platforms that provide sustained, spatially controlled release, such as polymeric microspheres, smart hydrogels, electrospun membranes, or aerogel scaffolds, while maintaining the structural advantages of xenogeneic grafts (46). Integration of histomorphometry with molecular markers of angiogenesis and osteogenesis (e.g., VEGF, BMP-2, osteocalcin) and advanced imaging (micro-CT or cone-beam CT) would allow a more complete characterisation of the temporal tissue response and help distinguish transient soft-tissue modulation from durable bone formation.

This study has several limitations that must be acknowledged. First, as a pilot trial with nine patients, the sample size is small and underpowered to detect small-to-moderate differences between treatments; the resulting wide confidence intervals mean that all statistical comparisons should be interpreted cautiously and considered exploratory. Second, although the split-mouth design reduces inter-individual variability, it does not eliminate potential confounders such as site-specific anatomy or local vascular conditions. Third, no molecular or immunohistochemical markers of osteogenesis or angiogenesis (e.g., BMP-2, VEGF, osteocalcin) were evaluated, which limits mechanistic interpretation of the observed CT/TV shift. Fourth, the local pharmacokinetics of simvastatin release from Cerabone® Plus were not quantified, hence the actual drug concentration and residence time at the healing site remain unknown. Finally, the three-month endpoint represents an early healing stage; sequential or long-term assessments could better capture the temporal evolution of statin-mediated effects. Additionally, the use of a single centrally harvested 2-mm trephine biopsy may not fully represent the spatial heterogeneity of tissue composition within the entire regenerated socket. Regional coronal-apical

faza pojačanoga granulacijskoga ili fibrovaskularnoga tkiva, a ne smanjeno stvaranje kosti. Slični rani porasti mekog tkiva opisani su i kod nosača sa statinima koji poslije podupiru bolju regeneraciju kosti nakon napredovanja mineralizacije (10, 38 – 40).

S farmakološkog gledišta, ovi rezultati potvrđuju da su učinci statina na kost iznimno ovisni o kontekstu i sustavu dostave. Osteoanabolički učinak posredovan BMP-om 2 obično zahtijeva održane lokalne koncentracije statina u niskom mikromolarnom rasponu (27, 41), a angiogeni ili protuupalni učinci mogu se pojaviti i pri nižim dozama. Jednostavno miješanje simvastatina s gustim, sporo resorbirajućim ksenograftom poput Cerabone® Plusa vjerojatno ne omogućuje kontrolirano otpuštanje, pa se lijek može brzo eliminirati ili zadržati na površini grafta. Nedavna eksperimentalna istraživanja pokazuju da su učinci statina znatno slabiji bez odgovarajućega sustava dostave, a produljeni kada se uključe u hidrogelske, polimerne ili mikrosferne nosače (42 – 45).

Sveukupno, ovi podatci sugeriraju da jednostavno miješanje simvastatina sa ksenogenim koštanim graftom vjerojatno ne rezultira mjerljivim povećanjem osteogeneze poslije tri mjeseca. Buduća istraživanja trebala bi biti usmjerena na sustave dostave koji omogućuju kontrolirano i dugotrajno otpuštanje, poput polimernih mikrosfera, pametnih hidrogelova, elektroispredenih membrana ili aerogelnih nosača, uz očuvanje strukturnih prednosti ksenografta (46). Kombinacija histomorfometrije s molekularnim markerima angiogeneze i osteogeneze (npr., VEGF, BMP-2, osteokalcin) te naprednim slikovnim metodama (mikro-CT ili CBCT) omogućila bi potpuniju analizu vremenskog odgovora tkiva.

Ova studija ima nekoliko ograničenja. Prvo, to je pilot-studija s devet pacijenata, pa je uzorak malen i nedovoljan za detekciju manjih razlika i zato rezultate treba tumačiti oprezno. Drugo, iako *split-mouth* dizajn smanjuje varijabilnost među ispitanicima, ne uklanja sve moguće ometajuće čimbenike. Treće, nisu analizirani molekularni ili imunohistokemijski markeri što ograničava interpretaciju. Četvrto, lokalna farmakokinetika oslobađanja simvastatina iz Cerabone® Plus nije kvantificirana, stoga stvarna koncentracija lijeka i vrijeme zadržavanja na mjestu zacjeljivanja ostaju nepoznati. Konačno, tromjesečna završna točka predstavlja ranu fazu zacjeljivanja; sekvencijalne ili dugoročne procjene mogle bi bolje uhvatiti vremensku evoluciju učinaka posredovanih statinima. Osim toga, korištenje jedne centralno uzete 2-milimetarske trepanske biopsije možda ne u potpunosti predstavlja prostornu heterogenost sastava tkiva unutar cijele regenerirane alveole. Regionalne koronalno-apikalne ili periferne varijacije u distribuciji mineraliziranog i nemineraliziranog tkiva stoga bi mogle ostati neotkrivene, što potencijalno utječe na kvantitativne histomorfometrijske ishode.

Unatoč tim ograničenjima, ova pilot studija pruža preliminarne kliničke dokaze da je dodavanje 1 mg lokalno primijenjenog simvastatina u Cerabone® Plus biološki sigurno i ne utječe negativno na ishode očuvanja alveole nakon tri mjeseca. Unutar ograničenja malog uzorka i ranog ishoda, kombinacija se čini histološki neutralno, s mogućim trendom prema većem CT/ TV-u što može odražavati ranu fibrovaskularnu modulaciju, a ne konačnu promjenu NB/TV-a. Po-or peripheral variations in mineralised and non-mineralised tissue distribution could therefore remain undetected, potentially influencing quantitative histomorphometric outcomes.

Despite these limitations, the present pilot study provides preliminary clinical evidence that adding 1 mg of locally applied simvastatin to Cerabone® Plus is biologically safe and does not adversely affect socket preservation outcomes at three months. Within the constraints of the small sample size and early endpoint, the combination appears histologically neutral, with a possible trend toward greater CT/TV that may reflect early fibrovascular modulation rather than a definitive change in NB/TV. Larger, rigorously designed clinical trials incorporating optimised delivery systems, pharmacokinetic assessment, advanced imaging, and molecular endpoints are warranted to clarify whether local statins can be harnessed as reliable osteoanabolic adjuncts in alveolar ridge preservation and other bone regenerative procedures.

Conclusion

Within the limitations of this pilot split-mouth clinical study, the local application of 1 mg simvastatin combined with Cerabone® Plus did not produce measurable improvements in histomorphometric bone regeneration at the 3-month healing interval. Both treatment groups exhibited comparable NB/TV and BSM/TV values, indicating that simvastatin at the tested dose and delivery method did not enhance mineralised tissue formation or graft resorption during early healing. A modest increase in CT/TV observed in simvastatin-treated sites may reflect transient early fibrovascular or angiogenic activity rather than a definitive alteration in osteogenesis at this stage. Overall, the findings suggest that simple admixture of simvastatin with a slowly resorbing xenograft is biologically safe but histologically neutral at three months. Future research should investigate controlled-release delivery platforms, optimise dosing strategies, and integrate molecular or imaging-based parameters to clarify the temporal and mechanistic effects of locally applied statins in clinical bone regeneration. Larger, well-powered clinical trials are required before firm conclusions regarding efficacy can be established.

Conflict of Interest: The authors report no conflict of interest.

Author's contribution: **T.K.** – study design, surgical procedures, data acquisition; **L.Š.** – histological analysis, data interpretation; **D.G.** – study design, manuscript writing, data interpretation; **I.S.** – surgical procedures, clinical supervision; **S.L.** – data interpretation, critical revision of the manuscript; **I.P.** – methodological support, critical revision of the manuscript; **R.L.** – pharmacological expertise, data interpretation. All authors approved the final version of the manuscript

trebna su veća, rigorozno osmišljena klinička ispitivanja koja uključuju optimizirane sustave isporuke, farmakokinetičku procjenu, napredno snimanje i molekularne ishode kako bi se razjasnilo mogu li se lokalni statini koristiti kao pouzdani osteoanabolički dodaci u očuvanju alveolarnog grebena i drugim postupcima regeneracije kostiju.

Zaključak

Unutar ograničenja ove kliničke pilot-studije sa *split-mouth* dizajnom, lokalna primjena 1 mg simvastatina u kombinaciji s Cerabone® Plusom nije pokazala mjerljiva poboljšanja u histomorfometrijskoj regeneraciji kosti poslije tromjesečnog razdoblja cijeljenja. Obje skupine liječenja pokazale su usporedive vrijednosti NB/TV-a i BSM/TV-a, što upućuje na to da simvastatin u ispitanoj dozi i načinu primjene nije povećao stvaranje mineraliziranog tkiva, ni resorpciju grafta u ranoj fazi cijeljenja.

Umjereno povećanje CT/TV-a, uočeno u skupini tretiranoj simvastatinom, može odražavati prolaznu ranu fibrovaskularnu ili angiogenu aktivnost, a ne definitivnu promjenu u osteogenezi u ovoj fazi. Sveukupno, rezultati sugeriraju da je jednostavno miješanje simvastatina sa ksenogenim graftom sporije resorpcije biološki sigurno, ali histološki neutralno poslije tri mjeseca.

Buduća istraživanja trebala bi se usmjeriti na sustave kontroliranog otpuštanja, optimizaciju doziranja te uključivanje molekularnih i slikovnih parametara kako bi se razjasnili vremenski i mehanicistički učinci lokalno primijenjenih statina u regeneraciji kosti. Potrebna su veća i metodološki snažnija klinička ispitivanja prije donošenja konačnih zaključaka o učinkovitosti.

Sukob interesa: Autori nisu bili u sukobu interesa.

Doprinos autora: **T. K.** – dizajn studije, kirurški zahvati, prikupljanje podataka; **L. Š.** – histološka analiza, interpretacija podataka; **D. G.** – dizajn studije, pisanje teksta, interpretacija podataka; **I. S.** – kirurški zahvati, klinički nadzor; **S. L.** – interpretacija podataka, kritička revizija teksta; **I. P.** – metodološka podrška, kritička revizija teksta; **R. L.** – farmakološka ekspertiza, interpretacija podataka. Svi autori odobrili su konačnu verziju teksta.

Sažetak

Cilj: Statini pokazuju pleiotropne učinke na metabolizam kosti, uključujući osteogenu i angiogenu aktivnost. Svrha ove pilot-studije bila je procijeniti poboljšava li lokalna primjena simvastatina u kombinaciji sa ksenogenim koštanim nadomjestkom govedega podrijetla histomorfometrijske ishode očuvanja alveole u usporedbi s primjenom samoga koštanog nadomjestka poslije tromjesečnoga razdoblja cijeljenja. **Materijali i metode:** U ovoj prospektivnoj *split-mouth* kliničkoj studiji sudjelovalo je devet pacijenata kojima je bila potrebna bilateralna ekstrakcija zuba. Jedna alveola tretirana je samo cerabone® plusom (kontrola), a kontralateralna alveola primila je cerabone® plus u kombinaciji s lokalno primijenjenim simvastatinom (1 mg/0,2 mL fiziološke otopine). Biopsije koštanoga cilindričnoga uzorka uzete su tri mjeseca poslije augmentacije i analizirane histomorfometrijski. Kvantificirani su udjeli novostvorene kosti (NB/TV), rezidualnog materijala koštanoga nadomjestka (BSM/TV) i vezivnoga tkiva (CT/TV). Za statističku analizu korišteni su parni t-test i Wilcoxonov test predznaka rangova. **Rezultati:** Histomorfometrijska analiza pokazala je sličan sastav tkiva u obje skupine. Vezivno tkivo bilo je je dominantna komponenta u kontrolnim i statinom tretiranim mjestima (59,1 ± 12,0 % i 64,3 ± 9,4 %; p = 0,31). Novostvorena kost iznosila je 31,0 ± 15,5 % u kontrolnoj skupini i 26,2 ± 11,6 % u statinskoj skupini (p = 0,45). Rezidualni materijal koštanoga nadomjestka pokazao je usporedive vrijednosti između skupina (9,9 ± 8,5 % prema 9,5 ± 9,7 %; p = 0,91). Nisu uočene statistički značajne razlike. **Zaključci:** Dodatak lokalno primijenjenoga simvastatina ksenogenom koštanom nadomjestku govedega podrijetla nije rezultirao značajnim histomorfometrijskim razlikama u očuvanju alveole poslije tri mjeseca. Pri ispitivanoj dozi i načinu primjene simvastatin nije poboljšao ranu formaciju mineraliziranog tkiva u usporedbi s primjenom samoga koštanog nadomjestka.

Zaprimljen: 20. siječnja 2026.
Prihvaćen: 12. ožujka 2026.

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Autorske ključne riječi: statin, očuvanje alveole, regeneracija kosti, ksenograft, histomorfometrija

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