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Article

Do Collagenated Xenogenic Bone Substitutes Enhance Gingival Healing and Angiogenesis Through a Barrier Membrane? An In Vitro Study

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Abstract

Research in implant dentistry has predominantly focused on bone regeneration, osseous volume maintenance, and successful osseointegration. However, soft tissue healing, which influences implant functional sealing, long-term stability, and esthetic integration, remains underexplored. This study investigated the effects of three xenogenic bone substitutes on gingival healing in vitro. Three experimental groups were established using extracts from bone substitutes diffusing through the OsteoBiol® Evolution collagen membrane: two collagenated substitutes, OsteoBiol® Gen-Os® (Gen-Os) and OsteoBiol® GTO® (GTO), and one inorganic substitute, Bio-Oss® (Bio-Oss). The substitutes were prepared in test tubes, and the extracts diffusing through the collagen membrane were used to evaluate human gingival cell (hGC) proliferation (MTT assay), migration (scratch assay), and growth factor release (ELISA). Angiogenic potential was assessed by endothelial cell proliferation, recruitment (Boyden chambers), and organization (Matrigel® assays). The indirect interaction between stimulated gingival cells and human bone marrow mesenchymal stem cells (hMSC) was investigated by analyzing hMSC recruitment and osteogenic BMP-2 secretion. Collagenated GTO and Gen-Os significantly enhanced hGC proliferation and migration in the scratch assay, with 1.8-fold and 1.6-fold increases, respectively, compared to control. All three substitutes enhanced neoangiogenesis in vitro. VEGF and FGF-2 secretion was significantly higher with GTO, showing 5-fold and 5.7-fold increases, respectively, resulting in a 3.7-fold increase in tube formation compared to control. Collagenated materials promoted hMSC recruitment, whereas BMP-2 secretion was not affected by any material. The observed effects were higher with the collagenated Gen-Os and GTO, with 2.5-fold and 2.8-fold increases, respectively, than with the non-collagenated Bio-Oss, which showed a 1.5-fold increase. These findings demonstrate that collagenated bone substitutes enhance gingival healing and angiogenic potential through barrier membranes and confirm that stimulated gingival cells indirectly promote hMSC recruitment, indicating that bone substitute effects extend beyond bone regeneration to include soft tissue healing and inter-tissue communication.

Keywords: collagenated bone substitute; thermosensitive biomaterial; gingival regeneration; angiogenesis; stem cell recruitment

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1. Introduction

Guided bone regeneration (GBR) is a cornerstone of contemporary implant dentistry, aimed at achieving reliable osseointegration and restoring sufficient alveolar bone volume to ensure long-term functional and prosthetic stability. In GBR, a bone substitute is placed to fill the osseous defect and reconstruct lost bone volume, followed by application of a barrier membrane to prevent soft tissue ingrowth and allow undisturbed bone formation within the protected space [1].

Bone substitutes are also widely used to preserve the alveolar crest following tooth extraction or trauma, as maintaining or reconstructing alveolar bone architecture is critical for future functional rehabilitation with dental implants [2,3]. Xenogenic bone substitutes are the most extensively studied due to their biocompatibility, slow resorption kinetics, and long-standing clinical effectiveness in promoting bone formation and preserving ridge dimensions [4]. Mechanistic studies have demonstrated that bone substitute application triggers local release of bioactive and chemotactic molecules, such as complement C5a [5], which enhances recruitment and osteogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs). Collagenated substitutes additionally release collagen, establishing a chemotactic gradient that recruits hMSCs to the graft site for new bone formation [6].

Bone substitutes may also modulate angiogenesis by enhancing growth factor release, endothelial cell proliferation, recruitment, and organization to form new vascular networks, an essential requirement for bone regeneration in critical-size defects [7,8]. This effect is more pronounced with collagenated materials, as collagen supports endothelial cell adhesion through $\alpha 2\beta 1$ integrins binding to RGD sequences [9], promoting vascular network formation within the material in vitro [5,10] and in newly formed bone in vivo [11]. Collagen further provides nucleation sites for mineralization of newly formed bone [12]. Clinically, xenogenic bone substitutes covered with barrier membranes effectively support bone augmentation and alveolar ridge preservation.

Most grafting materials, whether collagenated or non-collagenated, are supplied as granules. Once placed in a socket or defect, granules may collapse or displace, reducing expected bone volume. To prevent this, clinicians often stabilize the graft with a gingival graft or protective membrane, preventing soft tissue invasion during healing [13–15]. Historically, bone-regenerative and space-maintaining capacity has been the primary criterion for bone substitute selection, with regenerative procedures focusing predominantly on hard tissue reconstruction. Insufficient bone height, width, or density may impair primary implant stability and compromise long-term osseointegration, essential prerequisites for functional success.

Despite this extensive focus on bone regeneration, comparatively less attention has been devoted to soft tissue, particularly the gingival mucosa. Emerging evidence demonstrates that peri-implant soft tissues, especially the gingiva, play a critical role in long-term success, health, and esthetic outcomes of implant-supported rehabilitations. Understanding gingival healing, cellular behavior, and soft tissue regenerative mechanisms is therefore essential for comprehensive regenerative strategies. Recent findings also suggest that biomaterial selection can influence soft tissue healing and flap stability [16,17]. Given the pivotal role of gingiva, it is important to evaluate how different bone substitutes, used with barrier membranes, interact with gingival cells. To date, no study has directly compared the biological effects of collagenated versus non-collagenated xenogenic bone substitutes on human gingival cells.

In this study, an in vitro model simulating the clinical scenario of post-extraction ridge preservation was used to investigate the effects of three xenogenic bone substitutes on human gingival cells through a collagen membrane (OsteoBiol® Evolution). The tested

materials included two collagenated xenografts: Gen-Os and GTO, and one anorganic xenograft, Bio-Oss.

We evaluated the direct effects of the bone substitutes on gingival cells, the indirect effects mediated by gingival cell-secreted factors, and downstream consequences on endothelial cells and hMSCs. The study was guided by the following hypotheses: (1) bone substitutes influence gingival healing and neoangiogenesis through a barrier membrane; (2) stimulated gingival cells produce signals that modulate stem cell recruitment and support vascularization; and (3) these effects are expected to be more pronounced with the collagenated substitutes.

2. Materials and Methods

2.1. Reagents and Materials

Cell culture media, reagents, and consumables were purchased from Dominique Dutscher (Brumath, France). The evaluated materials, including their manufacturers, origins, sources, and relevant properties, are summarized in Table 1.

Table 1. Materials used (manufacturers' data).

Material	Manufacturer	Origin	Composition	Formulation	Particle Size (mm)	Collagen Content
Bio-Oss®	Geistlich Pharma, Wolhusen, Switzerland	Bovine	Cancellous bone	Granules	0.25–1	None
Gen-Os®	Tecnoss®, Torino, Italy	Porcine	Cortico-cancellous bone	Granules	0.25–1	22%
GTO®	Tecnoss®, Torino, Italy	Porcine	Collagenated bone mix granules pre-hydrated blended with a thermosensitive copolymer containing collagen types I and III	Pre-hydrated heterologous biomaterial prepared in a ready-to-use syringe	0.6–1	Collagen is embedded in the granules and included in the TSV Gel
Evolution membrane	Tecnoss®, Torino, Italy	Porcine	Heterologous collagen membrane from peritoneum tissue	Fibers	NA	Dense collagen fibers

2.2. Cell Culture

Human gingival cells (hGCs) were isolated from healthy third molars extracted for orthodontic purposes from four donors, in accordance with French legislation and with approval from the Aix-Marseille University Ethics Committee (Ref. No. 2022-05-12-003). Immediately after extraction, gingival tissues were stored in minimum essential medium (MEM) supplemented with antibiotics (300 IU/mL penicillin, 300 µg/mL streptomycin, and 0.75 µg/mL amphotericin B) and transferred to a laminar flow hood for subsequent processing under sterile conditions.

Tissue samples were rinsed with sterile phosphate-buffered saline (PBS; 0.01 M, pH 7.4), finely minced into submillimeter fragments, and cultured as explants in 100 mm culture dishes containing MEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 200 IU/mL penicillin, 200 µg/mL streptomycin, and 0.5 µg/mL amphotericin B. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

After approximately 2 weeks, confluent cells were harvested by trypsinization and subcultured in MEM supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B. Cells were maintained under the same incubation conditions and used up to passage 5. Cells derived from each donor were cultured and analyzed independently and were not pooled.

Human mesenchymal stem cells derived from bone marrow (hMSCs) were obtained from PromoCell (Heidelberg, Germany) and cultured in Mesenchymal Growth Medium

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2 (MGM-2; PromoCell) supplemented with SupplementMix at 37 °C in a humidified atmosphere of 5% CO_2.

Human umbilical vein endothelial cells (HUVECs) were obtained from PromoCell (Heidelberg, Germany) and cultured in Endothelial Cell Growth Medium 2 (ECGM-2; PromoCell) supplemented with SupplementMix under the same incubation conditions.

2.3. Preparation of Bone Filling Material Extracts

To prepare bone-filling material extracts, samples of OsteoBiol® Gen-Os® (Gen-Os), OsteoBiol® GTO® (GTO) and Bio-Oss® (Bio-Oss) were placed in 1.5 mL test tubes at a concentration of 20 mg/mL and incubated at 37 °C for 48 h, as previously described [5]. The tube caps were replaced with an OsteoBiol® Evolution membrane, and each material was incubated separately in serum-free MEM culture medium (Figure 1). This in vitro setup was designed to simulate the presence of a barrier membrane and blood-derived fluids in the alveolar socket in vivo.

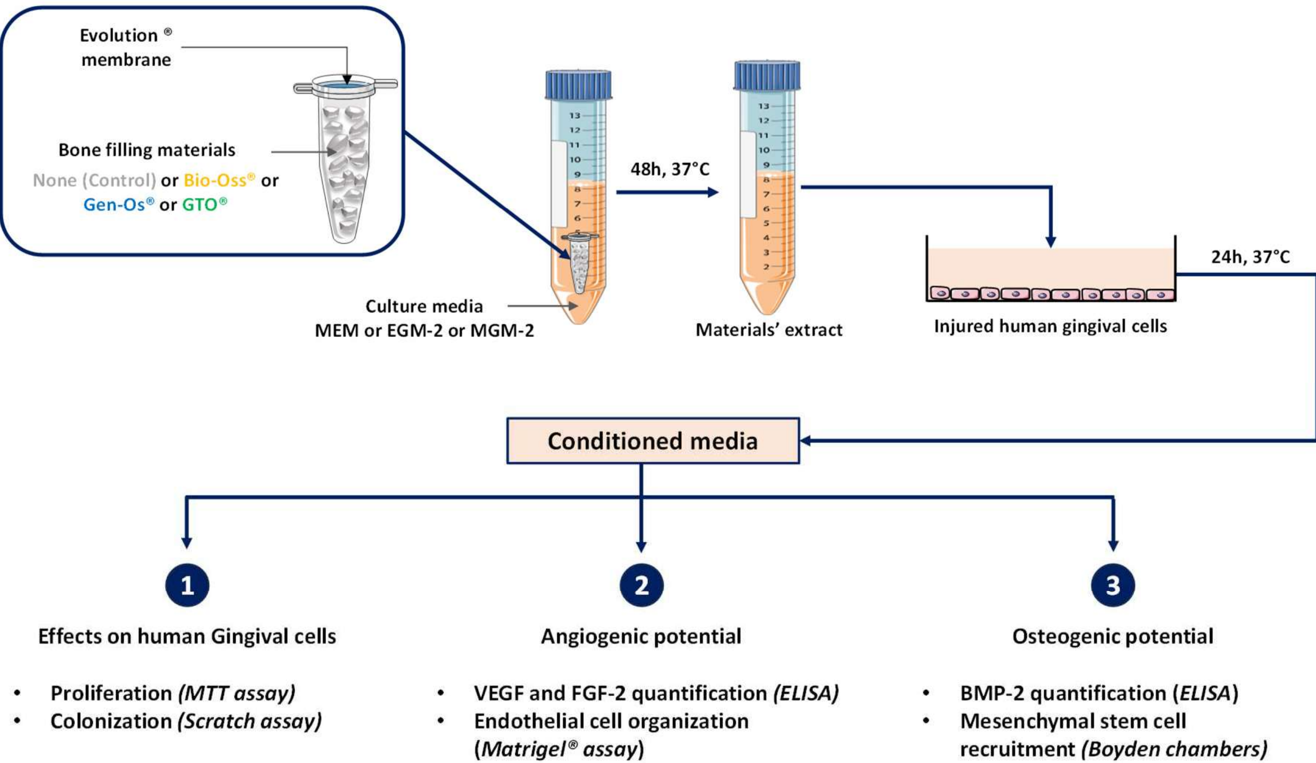

Figure 1. Schematic representation of the experimental setup. Bone-filling material extracts were prepared by incubating the materials in test tubes at 37 °C for 48 h in the appropriate cell culture media after replacing the tube caps with Evolution membranes. Mechanically injured hGCs were subsequently exposed to the resulting extracts for 24 h. The supernatants collected from injured hGCs (conditioned media) were then used to assess gingival cell proliferation and colonization, as well as angiogenic and osteogenic potential.

All membranes exhibited identical thickness, permeability, and surface area (0.4 mm^2) and were used without prior hydration. Depending on the experiment, extracts were prepared in serum-free MEM for human gingival cell assays, serum-free MGM-2 for human mesenchymal stem cells, or serum-free ECGM-2 for endothelial cell assays. After incubation, samples were centrifuged, and the supernatants were collected. Control extracts were prepared using the same protocol in the absence of bone-filling materials. The resulting extracts were subsequently used in the following experimental procedures.

2.4. Conditioned Media Preparation

Subconfluent human gingival cell cultures grown in 6-well plates were washed and mechanically injured using a sterile scalpel by creating 10 horizontal and 10 vertical linear incisions. Cells were then incubated with 2 mL of bone-filling material extract (20 mg/mL) or control extract. After 24 h of incubation, the supernatants were collected and designated as conditioned media for subsequent experiments (Figure 1).

2.5. Human Gingival Cell Proliferation

Human gingival cells were seeded at low density (1000 cells/cm^2) in 96-well culture plates and allowed to adhere for 24 h. The culture medium was then replaced with conditioned media. Cell proliferation was assessed after 1, 3, and 7 days using the MTT assay. Briefly, culture media were removed and replaced with 100 µL per well of 0.5% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution prepared in serum-free MEM. After incubation for 2 h at 37 °C, the supernatant was discarded and the resulting formazan crystals were solubilized with 100 µL per well of dimethyl sulfoxide (DMSO). Absorbance was measured at 550 nm using a microplate spectrophotometer (Σ960 Metertech, Taipei, Taiwan). Results were expressed as optical density (OD) at 550 nm.

2.6. Scratch Wound Healing Assay

The effect of the materials on hGC colonization was evaluated using a scratch wound healing assay. Confluent hGC monolayers (30,000 cells/cm^2) were established in 6-well plates, and a single standardized central scratch was generated in each well using a sterile pipette tip by the same operator. Cells were subsequently incubated with conditioned media. After 24 h of incubation at 37 °C, nuclei of cells that migrated into the wounded area were counted using fluorescence microscopy (AxioObserver A1, Zeiss, Oberkochen, Germany) in five randomly selected fields per condition. Cell counts were independently performed by two operators using ImageJ software (v1.54g), and mean values were calculated. Results were expressed as the number of migrating cells per field.

2.7. Osteogenic and Angiogenic Growth Factor Quantification

The concentrations of FGF-2, BMP-2, and VEGF in the conditioned media were measured using enzyme-linked immunosorbent assays (ELISAs) with Duoset human kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Results were expressed as picograms per milliliter (pg/mL).

2.8. Human Mesenchymal Stem Cell Migration

Migration assays were performed using 12-well plates equipped with Boyden chamber inserts (8 µm pore size). Confluent hGCs were cultured in the lower chambers, washed with MEM, mechanically injured using a scalpel, and incubated with either control or bone-filling material extract (1 mL). The upper chambers were seeded with 100 µL of hMSCs (10^4 cells/well). After 24 h of incubation, cells that had migrated to the lower side of the membrane were fixed in cold 70% ethanol for 15 min and stained with DAPI for 20 min. Migrating cells were counted in five randomly selected fields using fluorescence microscopy (Axiovert 200, Zeiss).

2.9. Endothelial Cell Organization

To assess angiogenic potential, HUVECs (4 × 10^4 cells/well) were seeded onto Matrigel® (ThermoFisher, Waltham, MA, USA) in Lab-Tek chambers and cultured with the previously described conditioned media. After 24 h of incubation at 37 °C in a humidified atmosphere of 5% CO_2, endothelial cell organization was examined using phase-contrast microscopy (AxioObserver A1, Zeiss). Closed tube-like structures (loops) were manually

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counted in five randomly selected fields per condition using ImageJ software. Results were expressed as the mean number of tube-like structures per field.

2.10. Statistical Analysis

Data processing and statistical analyses were performed using the Prism 10.2.1 software (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro–Wilk test, and the homogeneity of variances was evaluated using the Brown–Forsythe test. For comparisons among more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used when data met the assumptions of normality and homogeneity of variances. Two-way ANOVA was performed to assess the effects of conditioned media and time on gingival cell viability, followed by Tukey's post hoc. All results were presented as means ± standard deviations ($n = 4$). Differences were considered statistically significant at $p < 0.05$. Different lowercase letters above the boxes indicate statistically significant differences among groups according to Tukey's post hoc test ($p < 0.05$).

3. Results

3.1. Collagenated Bone Filling Materials Increased hGC Proliferation and Colonization

Human gingival cell proliferation was significantly enhanced by the collagenated materials Gen-Os and GTO at 1, 3, and 7 days compared with the control and Bio-Oss (Figure 2A). At day 3, Gen-Os induced a significantly greater proliferative response than GTO ($p < 0.0001$).

The scratch wound-healing assay is illustrated by representative images used for cell quantification (Figure 2(Ba–d)). Both collagenated materials, Gen-Os and GTO, significantly enhanced hGC colonization of the wound area compared with the control ($p = 0.0044$ and $p = 0.0132$, respectively) and Bio-Oss ($p = 0.0030$ and $p = 0.0090$, respectively) (Figure 2(Be)).

3.2. Collagenated GTO Increases Angiogenic Growth Factor Secretion by hGCs and Tube-like Structure Formation

GTO significantly increased the secretion of VEGF and FGF-2 compared with all other materials and the control ($p < 0.001$ for both) (Figure 3A).

After 24 h of endothelial cell culture on Matrigel® in the presence of conditioned media, anastomosis and formation of closed tube-like structures were observed, indicating in vitro neoangiogenic activity (Figure 3(Ba–d)). Quantitative analysis demonstrated a significant increase in the number of newly formed tube-like structures with Gen-Os and GTO compared with the control ($p = 0.0257$ and $p < 0.001$, respectively); this effect was significantly greater with GTO ($p < 0.001$) (Figure 3(Be)).

3.3. Osteogenic Growth Factor BMP-2 Secretion and hMSC Migration

No significant effect of the bone grafting materials on BMP-2 secretion by human gingival cells was observed (Figure 4A). In the human mesenchymal stem cell migration assay, illustrated with representative images (Figure 4(Ba–d)), both Gen-Os and GTO significantly enhanced hMSC migration toward injured hGCs compared with the control ($p < 0.0001$ for both) and Bio-Oss ($p < 0.0001$ for both) (Figure 4(Be)).

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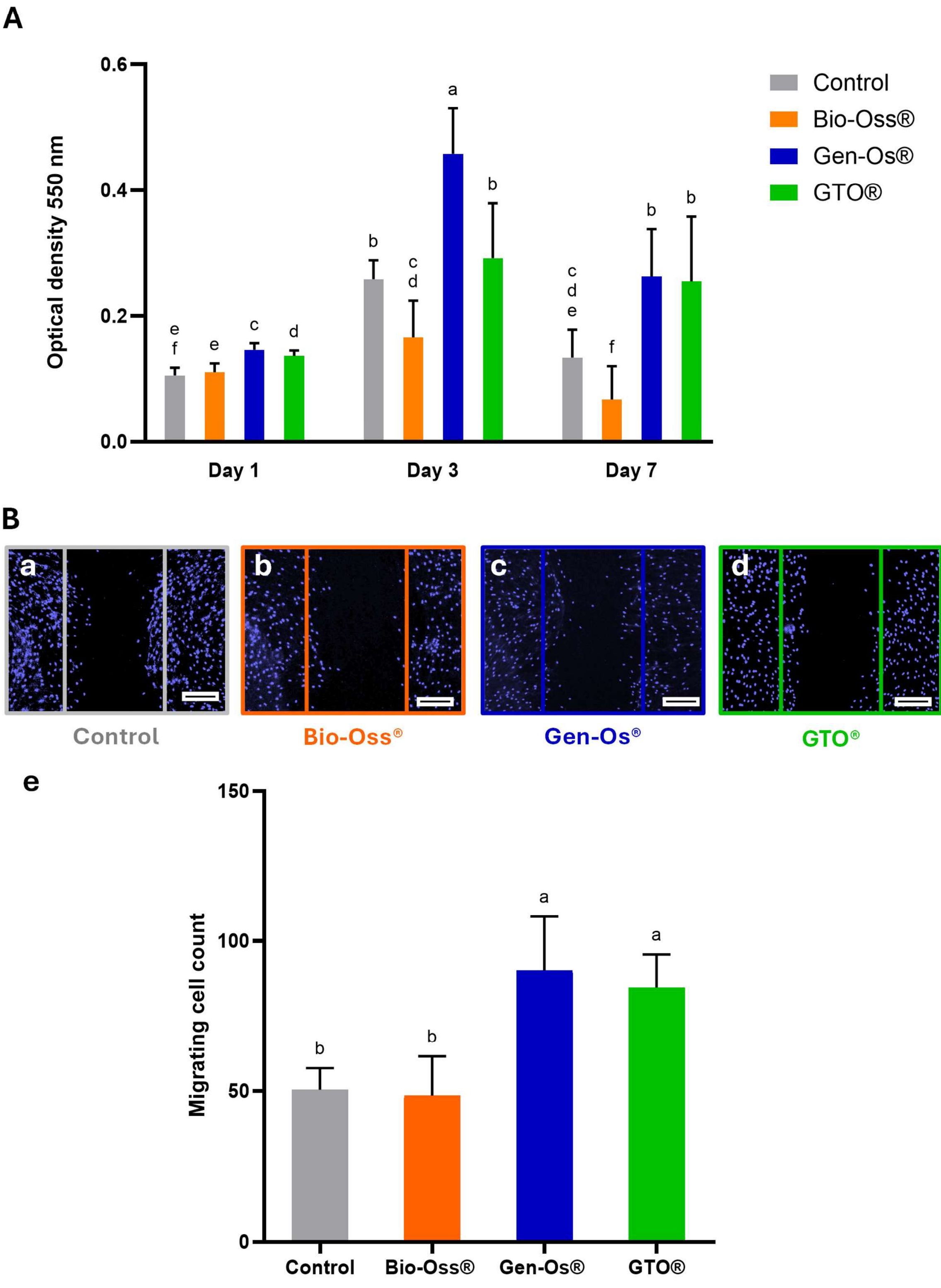

Figure 2. (**A**) Effects of the materials on hGC proliferation. The proliferation of hGCs significantly increased with Gen-Os and GTO at 1, 3 and 7 days compared to the control and Bio-Oss. (**B**) Scratch wound healing assays of hGC cultured for 24 h with conditioned media. (**a**–**d**) Representative pictures: (**a**) control, (**b**) Bio-Oss, (**c**) Gen-Os, and (**d**) GTO. The two vertical dashed lines delineate the initial cell-free zone at the injury site, and cells migrating to colonize this area were counted. Scale bars = 500 μm. (**e**) Number of migrating cells to the injury area significantly increased with Gen-Os and GTO compared with the control and Bio-Oss. Results are from 4 independent biological replicates. Different lowercase letters above the boxes indicate statistically significant differences among groups according to Tukey's post hoc test ($p < 0.05$).

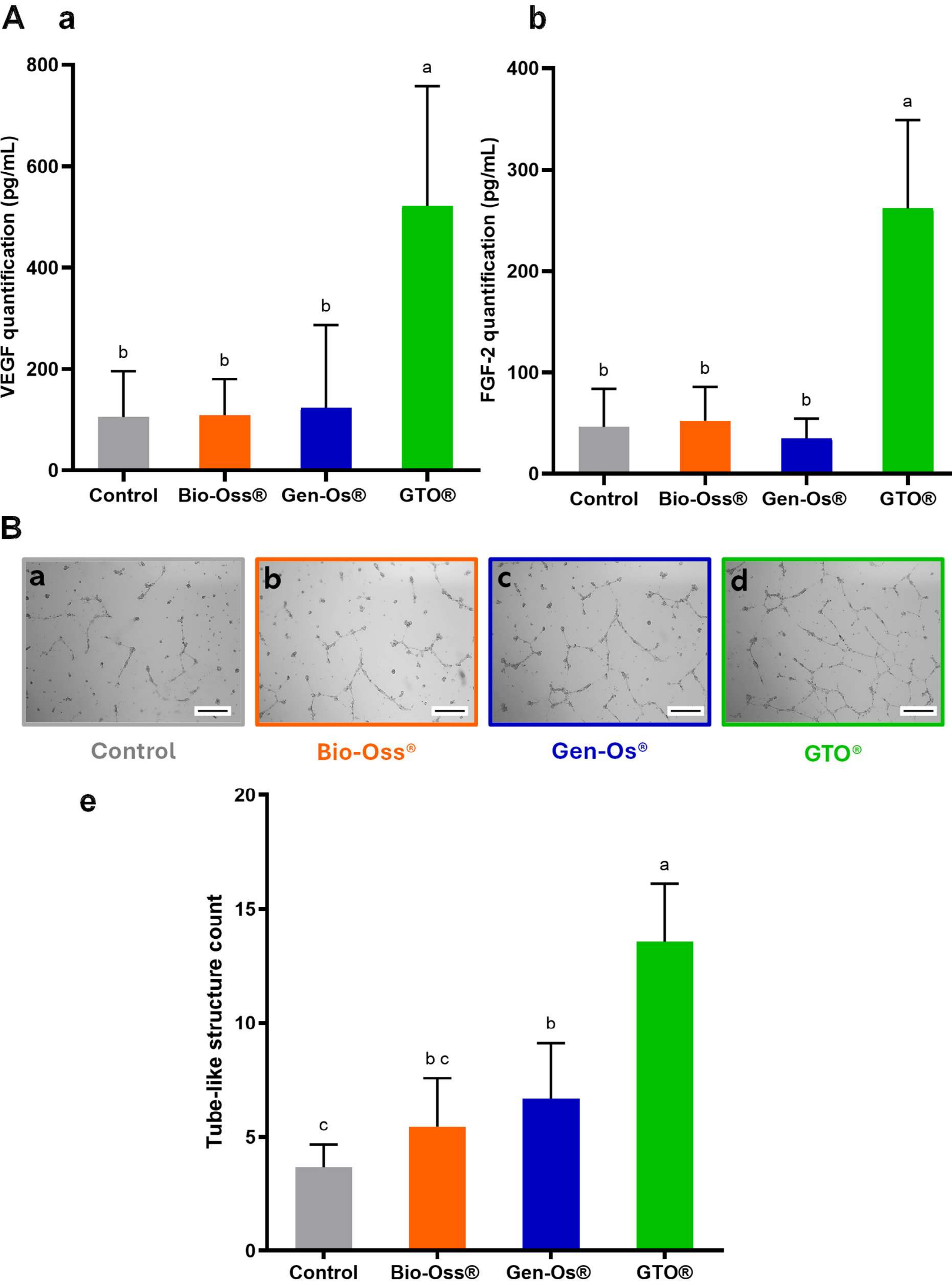

Figure 3. (**A**) Angiogenic growth factor secretion by human gingival cells. GTO significantly increased the secretion of FGF-2 (**a**) and VEGF (**b**) compared with the control. Results are expressed in pg/mL. (**B**) Effects of bone grafting materials on in vitro neoangiogenesis. (**a**–**d**) Representative images of neoangiogenesis in the (**a**) control, (**b**) Bio-Oss, (**c**) Gen-Os, and (**d**) GTO groups (scale bar = 50 μm). (**e**) Quantification of loop structures, expressed as the mean number of tube-like structures per field. Loop formation was significantly increased by Gen-Os and GTO compared with the control, with a significantly greater effect observed for GTO. Data represent four independent biological replicates. Different lowercase letters indicate statistically significant differences among groups (Tukey's post hoc test, $p < 0.05$).

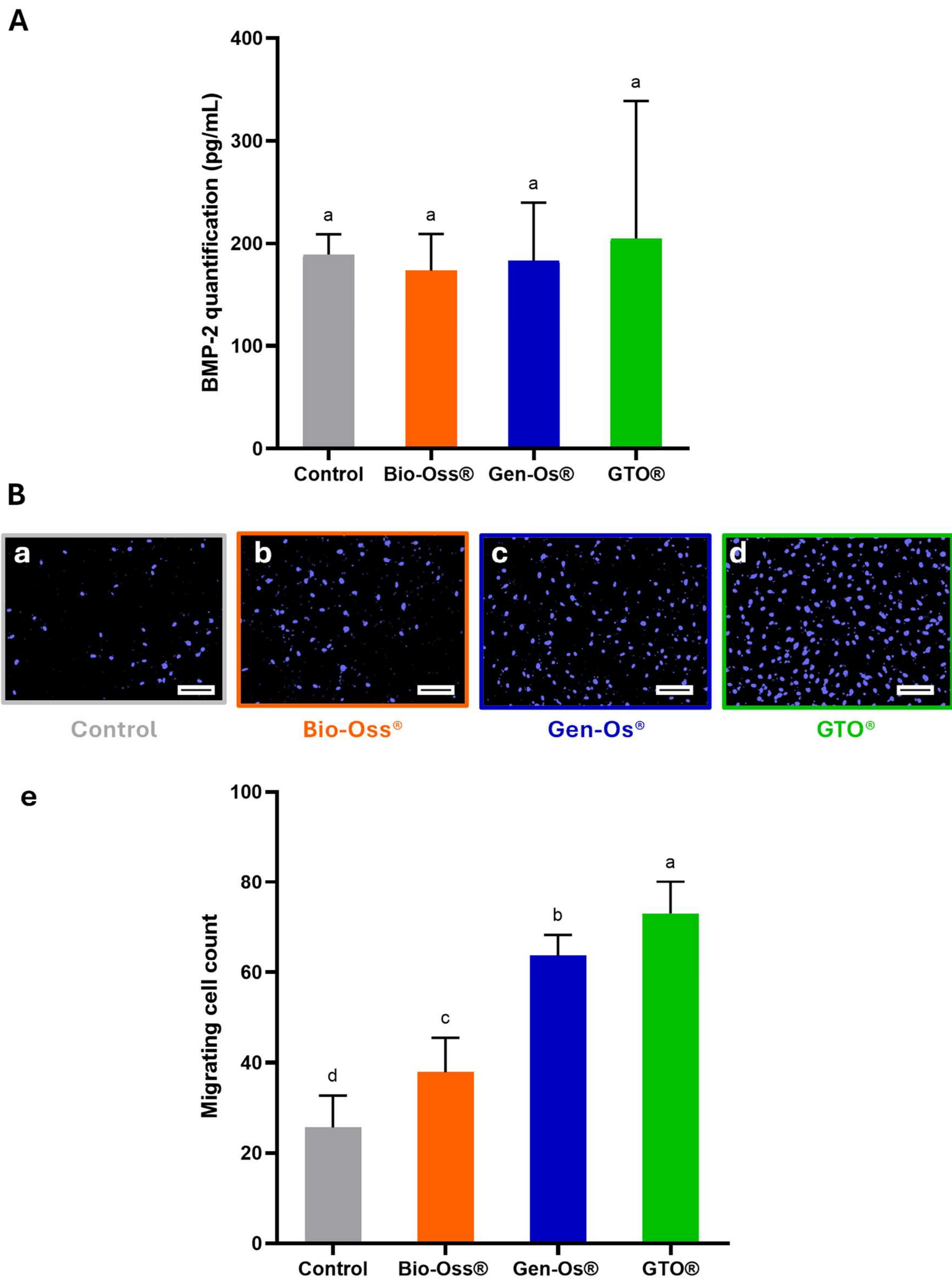

Figure 4. (**A**) BMP-2 secretion by human gingival cells. ELISA analysis showed that none of the tested materials affected BMP-2 secretion. (**B**) Effects of the materials on human mesenchymal stem cell migration assessed using Boyden chambers. (**a**–**d**) Representative images used for quantification of migrating cells: (**a**) control, (**b**) Bio-Oss, (**c**) Gen-Os, and (**d**) GTO (scale bar = 50 µm). (**e**) Gen-Os and GTO significantly increased hMSC migration compared with the control and Bio-Oss. Data represent four independent biological replicates. Different lowercase letters indicate statistically significant differences among groups (Tukey's post hoc test, $p < 0.05$).

4. Discussion

The added value of this study is the demonstration that bone grafting materials influence gingival cell angiogenic and healing potential through a protective barrier membrane. This was shown by evaluating the effects of bone substitutes on hGC proliferation, colonization of the injured area, secretion of angiogenic growth factors, endothelial cell organization into tube-like structures, and hMSC migration (Figure 5).

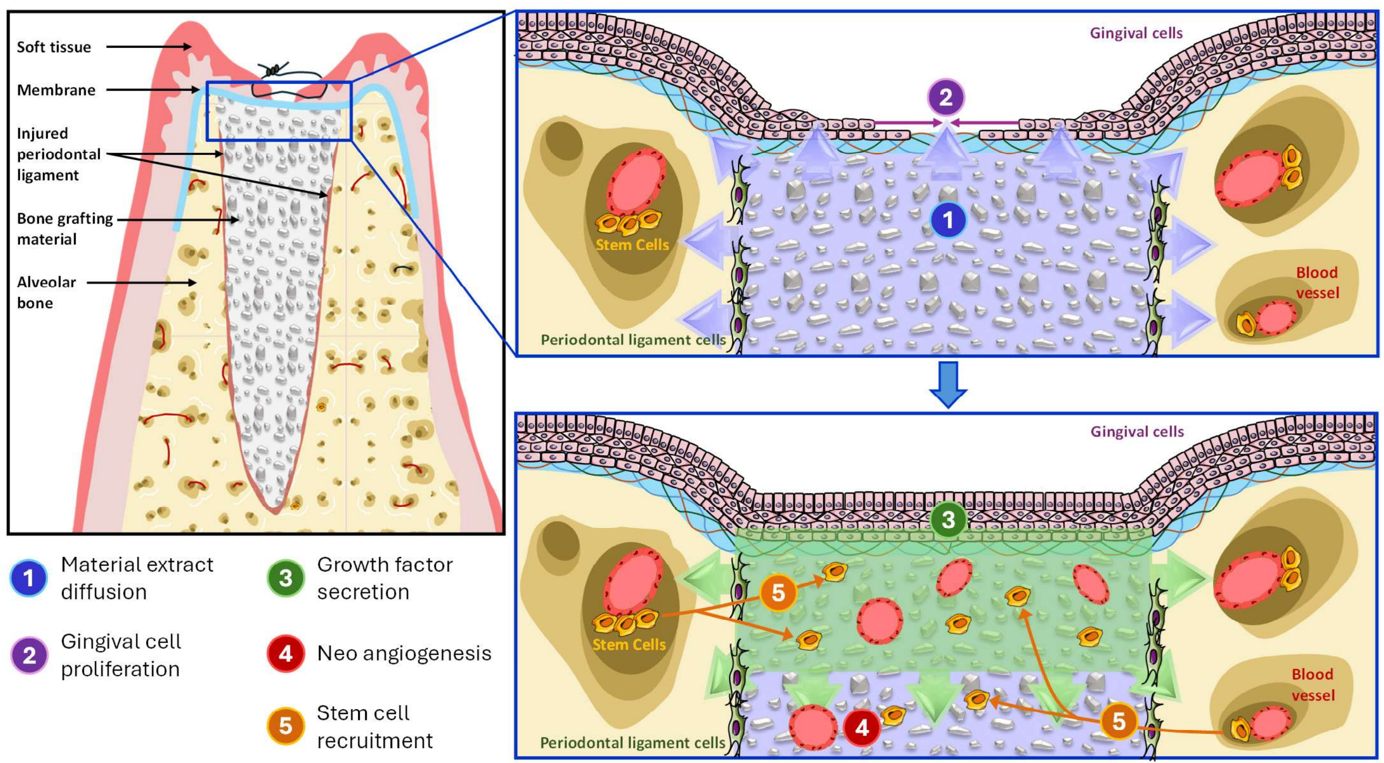

Figure 5. Schematic representation of the interaction between bone grafting materials and gingival cells. Tooth extraction results in alveolar bone resorption. The ridge preservation technique involves placement of a bone grafting material into the extraction socket to limit the bone resorption process. Following application, material extracts interact with injured gingival cells through the collagen membrane (1), inducing cell proliferation (2). In response, gingival cells secrete growth factors (3), which promote neoangiogenesis (4) and mesenchymal stem cell recruitment (5).

These findings highlight an emerging interest that, in addition to their established role in promoting bone regeneration, bone substitutes also support soft tissue healing, a critical factor for oral rehabilitation and esthetics. Soft tissue healing, often underappreciated, is essential in implantology, particularly for the healing and integration of the gingival mucosa surrounding the implant site. Effective gingival healing contributes not only to functional sealing and long-term implant stability but also to the esthetic integration of prosthetic restorations, especially in the anterior zone [18].

A second key finding of this study is that stimulated gingival cells influence the early stages of bone regeneration through the barrier membrane, as the supernatant from stimulated gingival cells differentially affected human mesenchymal stem cell recruitment. This effect was biomaterial-dependent, with significantly higher recruitment observed for collagenated bone substitutes. Because this response is mediated by the gingival cells, it can be inferred that hMSCs migrate toward the gingival cells and subsequently colonize the underlying bone substitute. Thus, hMSC recruitment occurs indirectly via signals from stimulated gingival cells rather than through direct contact with the biomaterials.

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Analysis of BMP-2 secretion showed that collagenated materials did not enhance BMP-2 production by gingival cells, whereas previous studies reported a significant increase in BMP-2 from periodontal ligament cells. This finding is consistent with expectations, as BMP-2 is primarily expressed and released by mineralizing cells, and its secretion is not typically upregulated in gingival cells.

While collagenated bone substitutes have been shown to enhance the angiogenic and osteogenic potential of human periodontal ligament cells [5], the present study demonstrates that these materials also interact with gingival cells through the barrier membrane. This interaction not only influences gingival healing but also differentially modulates mesenchymal stem cell recruitment. To our knowledge, the role of stimulated gingival cells in the early stages of bone regeneration has not been previously investigated, highlighting a potential synergistic effect of both periodontal ligament and gingival cells on bone marrow stem cell recruitment.

Our data also indicates that the observed effects vary depending only on the bone substitute as the same barrier membrane was used throughout this study. Notably, our results show that the gingival healing and angiogenic potential was higher with collagenated substitutes as compared to the inorganic bone substitute. Interestingly, similar results were obtained on periodontal ligament cells with the same materials which showed that the hPDLCs osteogenic and angiogenic potentials were higher with the collagenated materials than with the inorganic one [5]. Also, the angiogenic potential observed in this study was highest with GTO. This substitute is a collagenated bone mix granules prehydrated blended with a thermosensitive copolymer containing collagen types I and III (OsteoBiol® TSV Gel). It sets at the body temperature and keeps the space required for bone regeneration. Upon placement of the material, collagen can be released from both the gel and upon hydrolysis of the granules. Indeed, collagen concentration released from GTO was significantly higher than that from Gen-Os which contains collagen only in the granules [5]. This collagen generates a gradient for stem cell recruitment by haptotaxis and provides support for neo angiogenesis. Also, collagen is a major component of the basement membrane and allows adhesion of endothelial cells through interaction of Collagen RGD sequence and endothelial cell $\alpha 2\beta 1$ integrins binding sites [12]. This result confirms that of our previous investigation showing that collagenated materials enhance hPDLCs angiogenic and osteogenic potentials [5]. The osteogenic potential of collagenated substitutes is in line with another study on Human Dental Pulp Stem Cells which showed that Collagen-based xenograft materials enhanced viability and promoted osteogenic differentiation [19]. It provides confirmation of another study on primary cultures of osteoblasts which investigated the effects of collagenated materials on cell proliferation, adhesion, morphology, collagen and calcium deposition, alkaline phosphatase activity, gene expression of osteogenic markers and integrins and reported that the collagenated materials supported the osteoblast activity [20].

Despite growing interest in the dual regeneration of hard and soft gingival tissues in oral surgery and implantology, research specifically addressing the effect of bone-filling materials on gingival tissue regeneration in vitro remains remarkably sparse.

Most research on soft tissue outcomes in implantology has focused on soft tissue grafts or matrices designed to enhance gingival healing, rather than on the bone substitute materials placed beneath the soft tissues. However, emerging clinical evidence suggests that bone substitutes may also influence soft tissue healing dynamics. Several preclinical and clinical studies have demonstrated that combining xenogeneic bone substitutes with collagen membranes improves soft tissue outcomes.

In a canine model of guided bone regeneration, sites treated with deproteinized bovine bone mineral and covered with either collagen or synthetic membranes exhibited

superior soft tissue contour compared with membrane-only controls; the combined use of graft and membrane consistently enhanced soft tissue volume and contour during early healing phases [21]. Similarly, randomized clinical trials have shown that alveolar ridge preservation using collagenated deproteinized bovine bone mineral (DBBM-C) in combination with barrier membranes results in superior maintenance of ridge volume compared with spontaneous healing, correlating with improved protection and support of the overlying soft tissue architecture [22]. Our findings provide a plausible cellular mechanism supporting previous clinical observations, highlighting a potential shift from viewing bone substitutes as merely space-maintaining to recognizing their role in active cellular modulation.

Although these findings provide insight into the cellular mechanisms underlying interactions between bone substitutes and gingival cells, the experimental design relies on two-dimensional (2D) monolayer cultures, which represent a simplified model and limit direct clinical extrapolation. The in vitro setup, consisting of a test tube covered with a membrane to simulate the barrier membrane and the presence of blood fluids within the alveolar socket, remains distant from the in vivo clinical scenario. Several critical factors were not addressed, including the three-dimensional organization of multiple cell types within the extracellular matrix, the absence of blood circulation and inflammatory responses, and the lack of mechanical forces.

Nevertheless, this model offers mechanistic insight into how bone substitutes modulate gingival cell behavior and how this interaction may influence soft tissue healing and bone regeneration. The use of a 2D culture system likely underestimates the magnitude and complexity of signaling events that occur within a three-dimensional tissue environment at the injury site, where additional signals may be locally generated during inflammation. For example, C5a released from periodontal ligament cells and from plasma following complement activation has been shown to enhance bone marrow mesenchymal stem cell recruitment [5], and such mechanisms may further contribute to bone regeneration in vivo.

In addition to its effects on soft tissue regeneration, the present study provides valuable insight into bone regeneration, which appears to depend on multiple interconnected factors. First, the composition of the bone filling material itself is critical, as collagenated xenograft materials provide superior support for cell adhesion, neoangiogenesis, and mineralization. Second, the interaction of the filling material with various cell populations at the application site including bone marrow mesenchymal stem cells, periodontal ligament cells, endothelial cells, and gingival cells. Depending on the material and the cellular response it elicits, these cells contribute to angiogenic, chemotactic, and differentiation signals that drive regeneration.

Finally, this study provides novel evidence that collagenated xenograft bone substitutes positively influence gingival healing, an area that has received limited investigation, and advances our understanding of early bone regeneration dynamics. Our findings suggest that, upon stimulation, gingival cells release signaling molecules that, when transmitted through the bioactive interface of the barrier membrane, may enhance mesenchymal stem cell recruitment and contribute to the initial phases of bone regeneration [23–25].

5. Conclusions

This study highlights the emerging interest in dual-phase collagenated bone-filling materials, reflecting a shift from viewing bone and gingiva as separate healing domains toward recognizing the interdependence of hard and soft tissue regeneration. Incorporating materials that support gingival cell activity, vascularization, and soft tissue stability may enhance overall peri-implant health while improving long-term esthetic outcomes. Such an

approach aligns with the broader goal of developing regenerative strategies that optimize the biological, functional, and esthetic success of implant therapy.

Author Contributions: Conceptualization, I.A., C.J., J.-H.C. and R.L.; methodology, I.A., C.J., J.-H.C. and T.G.; validation, I.A., C.J., T.G. and J.-H.C.; data curation, C.J. and J.-H.C.; original draft preparation, I.A., C.J., T.G., R.L., R.I. and J.-H.C.; writing—review and editing, I.A., C.J., T.G., R.L., R.I. and J.-H.C.; supervision, C.J. and I.A.; funding acquisition, I.A.; project administration, I.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Aix-Marseille University Ethics Committee (Ref. No. 2022-05-12-003).

Informed Consent Statement: Verbal informed consent was obtained from the patients. Verbal consent was obtained rather than written because obtaining verbal consent was in accordance with the national legislation and local ethical committee agreement.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BMP-2	Bone morphogenetic protein 2
DBBM-C	Deproteinized bovine bone mineral C
DMSO	Dimethyl sulfoxide
ECGM-2	Endothelial cell growth medium 2
ELISA	Enzyme-linked immunosorbent assay
FGF-2	Fibroblast growth factor 2
GBR	Guided bone regeneration
hGC	Human gingival cell
hMSC	Human bone marrow mesenchymal stem cell
hPDLC	Human periodontal ligament cell
HUVEC	Human umbilical vein endothelial cell
MEM	Minimum essential medium
MGM-2	Mesenchymal growth medium 2
OD	Optical density
PBS	Phosphate-buffered saline
VEGF	Vascular endothelial growth factor

References

1. López-Valverde, N.; Macedo De Sousa, B.; Blanco Rueda, J.A. Changes of the Alveolar Bone Ridge Using Bone Mineral Grafts and Collagen Membranes after Tooth Extraction: A Systematic Review and Meta-Analysis. *Bioengineering* **2024**, *11*, 565. [CrossRef]
2. Araújo, M.G.; Lindhe, J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J. Clin. Periodontol.* **2005**, *32*, 212–218. [CrossRef] [PubMed]
3. Avila-Ortiz, G.; Elangovan, S.; Kramer, K.W.O.; Blanchette, D.; Dawson, D.V. Effect of alveolar ridge preservation after tooth extraction: A systematic review and meta-analysis. *J. Dent. Res.* **2014**, *93*, 950–958. [CrossRef] [PubMed]
4. Canellas, J.V.D.S.; Soares, B.N.; Ritto, F.G.; Vettore, M.V.; Vidigal Júnior, G.M.; Fischer, R.G.; Medeiros, P.J.D. What grafting materials produce greater alveolar ridge preservation after tooth extraction? A systematic review and network meta-analysis. *J. Cranio-Maxillofac. Surg.* **2021**, *49*, 1064–1071. [CrossRef]

5. Jeanneau, C.; Catherine, J.-H.; Giraud, T.; Lan, R.; About, I. The Added Value of a Collagenated Thermosensitive Bone Substitute as a Scaffold for Bone Regeneration. *Materials* **2024**, *17*, 625. [CrossRef] [PubMed]
6. Abdelgawad, M.E.; Søe, K.; Andersen, T.L.; Merrild, D.M.H.; Christiansen, P.; Kjærsgaard-Andersen, P.; Delaisse, J.-M. Does collagen trigger the recruitment of osteoblasts into vacated bone resorption lacunae during bone remodeling? *Bone* **2014**, *67*, 181–188. [CrossRef]
7. Hankenson, K.D.; Dishowitz, M.; Gray, C.; Schenker, M. Angiogenesis in Bone Regeneration. *Injury* **2011**, *42*, 556–561. [CrossRef]
8. Jang, H.-J.; Yoon, J.-K.; Jang, H.-J.; Yoon, J.-K. The Role of Vasculature and Angiogenic Strategies in Bone Regeneration. *Biomimetics* **2024**, *9*, 75. [CrossRef]
9. Sweeney, S.M.; DiLullo, G.; Slater, S.J.; Martinez, J.; Iozzo, R.V.; Lauer-Fields, J.L.; Fields, G.B.; Antonio, J.D.S. Angiogenesis in Collagen I Requires α2β1 Ligation of a GFP*GER Sequence and Possibly p38 MAPK Activation and Focal Adhesion Disassembly. *J. Biol. Chem.* **2003**, *278*, 30516–30524. [CrossRef]
10. Rombouts, C.; Jeanneau, C.; Camilleri, J.; Laurent, P.; About, I. Characterization and angiogenic potential of xenogeneic bone grafting materials: Role of periodontal ligament cells. *Dent. Mater. J.* **2016**, *35*, 900–907. [CrossRef]
11. Miyauchi, Y.; Izutani, T.; Teranishi, Y.; Iida, T.; Nakajima, Y.; Xavier, S.P.; Baba, S. Healing Patterns of Non-Collagenated Bovine and Collagenated Porcine Xenografts Used for Sinus Floor Elevation: A Histological Study in Rabbits. *J. Funct. Biomater.* **2022**, *13*, 276. [CrossRef]
12. Senger, D.R.; Claffey, K.P.; Benes, J.E.; Perruzzi, C.A.; Sergiou, A.P.; Detmar, M. Angiogenesis promoted by vascular endothelial growth factor: Regulation through α1β1 and α2β1 integrins. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13612–13617. [CrossRef]
13. Tan, W.L.; Wong, T.L.T.; Wong, M.C.M.; Lang, N.P. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. *Clin. Oral Implant. Res.* **2012**, *23*, 1–21. [CrossRef]
14. Atieh, M.A.; Alsabeeha, N.H.M.; Payne, A.G.T.; Duncan, W.; Faggion, C.M.; Esposito, M. Interventions for replacing missing teeth: Alveolar ridge preservation techniques for dental implant site development. *Cochrane Database Syst. Rev.* **2015**, *2015*, CD010176. [CrossRef]
15. El-Sioufi, I.; Oikonomou, I.; Koletsi, D.; Bobetsis, Y.A.; Madianos, P.N.; Vassilopoulos, S. Clinical evaluation of different alveolar ridge preservation techniques after tooth extraction: A randomized clinical trial. *Clin. Oral Investig.* **2023**, *27*, 4471–4480. [CrossRef]
16. Burkhardt, R.; Lang, N.P. Fundamental principles in periodontal plastic surgery and mucosal augmentation—A narrative review. *J. Clin. Periodontol.* **2014**, *41*, S98–S107. [CrossRef]
17. Canullo, L.; Pesce, P.; Antonacci, D.; Ravidà, A.; Galli, M.; Khijmatgar, S.; Tommasato, G.; Sculean, A.; Del Fabbro, M. Soft tissue dimensional changes after alveolar ridge preservation using different sealing materials: A systematic review and network meta-analysis. *Clin. Oral Investig.* **2022**, *26*, 13–39. [CrossRef] [PubMed]
18. Hadzik, J.; Błaszczyszyn, A.; Gedrange, T.; Dominiak, M. Soft-Tissue Augmentation around Dental Implants with a Connective Tissue Graft (CTG) and Xenogeneic Collagen Matrix (CMX)-5-Year Follow-Up. *J. Clin. Med.* **2023**, *12*, 924. [CrossRef] [PubMed]
19. Pelaez-Cruz, P.; López Jornet, P.; Pons-Fuster, E. Collagen Formulation in Xenogeneic Bone Substitutes Influences Cellular Responses in Periodontal Regeneration: An In Vitro Study. *Biomimetics* **2025**, *10*, 608. [CrossRef] [PubMed]
20. Pierfelice, T.V.; Cinquini, C.; Petrini, M.; D'Amico, E.; D'Arcangelo, C.; Barone, A.; Iezzi, G. Evaluation of Collagenic Porcine Bone Blended with a Collagen Gel for Bone Regeneration: An In Vitro Study. *Int. J. Mol. Sci.* **2025**, *26*, 7621. [CrossRef]
21. Di Raimondo, R.; Sanz-Esporrín, J.; Sanz-Martin, I.; Plá, R.; Luengo, F.; Vignoletti, F.; Nuñez, J.; Sanz, M. Hard and soft tissue changes after guided bone regeneration using two different barrier membranes: An experimental in vivo investigation. *Clin. Oral Investig.* **2021**, *25*, 2213–2227. [CrossRef] [PubMed]
22. Kim, H.; Han, H.-S.; Ghanaati, S.; Zadeh, H.H.; Kim, S.; Cho, Y.-D. Alveolar Ridge Preservation Using a Collagenated Xenograft: A Randomized Clinical Trial. *Int. Dent. J.* **2025**, *75*, 1155–1164. [CrossRef] [PubMed]
23. Turri, A.; Elgali, I.; Vazirisani, F.; Johansson, A.; Emanuelsson, L.; Dahlin, C.; Thomsen, P.; Omar, O. Guided bone regeneration is promoted by the molecular events in the membrane compartment. *Biomaterials* **2016**, *84*, 167–183. [CrossRef]
24. Alkildani, S.; Ren, Y.; Liu, L.; Rimashevskiy, D.; Schnettler, R.; Radenković, M.; Najman, S.; Stojanović, S.; Jung, O.; Barbeck, M. Analyses of the Cellular Interactions between the Ossification of Collagen-Based Barrier Membranes and the Underlying Bone Defects. *Int. J. Mol. Sci.* **2023**, *24*, 6833. [CrossRef]
25. Omar, O.; Elgali, I.; Dahlin, C.; Thomsen, P. Barrier membranes: More than the barrier effect? *J. Clin. Periodontol.* **2019**, *46*, 103–123. [CrossRef] [PubMed]

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