Publication status: This preprint has been published elsewhere.
DOI of the published preprint: https://doi.org/10.1590/1807-2577.20250043

Fibroblast cellular responses to calcium hydroxyapatite-based biomaterials

Roger Kirschner, Henrique Ballassini Abdalla, Juliana Trindade Clemente-Napimoga, Marcelo Henrique Napimoga

https://doi.org/10.1590/SciELOPreprints.15345

Submitted on: 2026-03-11
Posted on: 2026-03-11 (version 1)
(YYYY-MM-DD)

e20250043

Fibroblast cellular responses to calcium hydroxyapatite-based biomaterials

Respostas celulares de fibroblastos a biomateriais à base de hidroxiapatita de cálcio

Roger KIRSCHNER[a]

https://orcid.org/0000-0002-8673-945X

Henrique Ballassini ABDALLA[a]

https://orcid.org/0000-0002-7517-2830

Juliana Trindade CLEMENTE-NAPIMOGA[a]

https://orcid.org/0000-0003-1068-3039

Marcelo Henrique NAPIMOGA[a]*

https://orcid.org/0000-0003-4472-365X

[a] Instituto de Pesquisas São Leopoldo Mandic, Faculdade São Leopoldo Mandic, Campinas, SP, Brasil

How to cite:

Kirschner R, Abdalla HB, Clemente-Napimoga JT, Napimoga MH. Fibroblast cellular responses to calcium hydroxyapatite-based biomaterials. Rev Odontol UNESP. 2026;55:e20250043. https://doi.org/

Resumo

Introdução: A hidroxiapatita de cálcio (CaHa) é amplamente utilizada em procedimentos estéticos como bioestimulador de colágeno. **Objetivo**: Este estudo avaliou como diferentes concentrações de CaHA (0,5% a 2,5%) em três formulações comerciais (Stiim®, Rennova® e Radiesse®) influenciam a viabilidade celular, a proliferação de fibroblastos humanos e a expressão gênica de marcadores da matriz extracelular, incluindo colágeno tipo I e III, osteocalcina e osteopontina. **Material e método**: As CaHa foram avaliadas quanto à viabilidade celular, proliferação e expressão gênica de marcadores de matriz extracelular em fibroblastos humanos (n=3, por tempo avaliado). A viabilidade celular foi analisada pelos ensaios de MTT e exclusão por azul de tripano, a proliferação por contagem celular e a expressão gênica de colágeno tipo I e III, osteocalcina e osteopontina por RT-PCR. **Resultado**: Stiim® apresentou aumento significativo da viabilidade em baixas concentrações (1% e 2%) após 24 h e em 0,5% e 1% após 72 h, enquanto a concentração de 2,5% reduziu drasticamente a viabilidade. Radiesse® demonstrou maior estabilidade, com estímulo precoce de proliferação e indução sustentada de colágeno tipo I e III. Rennova® exibiu resposta mais tardia e variável, com redução da proliferação em concentrações intermediárias. A expressão de osteocalcina e osteopontina variou entre as formulações, com Radiesse® apresentando efeito estimulatório mais intenso. Os resultados indicam que os efeitos da CaHa sobre fibroblastos dependem da formulação e da concentração, influenciando diretamente o comportamento celular e o potencial bioestimulador. **Conclusão**: Esses achados, baseados em expressão genica, sugerem que a CaHA pode estimular a neocolagênese.

Descritores: Hidroxiapatita de cálcio; estética; cosmético dérmico; dermatologia; harmonização orofacial; colágeno; fibroblastos; biomateriais; STIIM; Diamond; Radiesse.

Abstract

Introduction: Calcium hydroxyapatite (CaHa) is widely used in aesthetic procedures as a collagen biostimulator. **Objective**: This study evaluated how different concentrations of CaHA (0.5% to 2.5%) in three commercial formulations (Stiim®, Rennova®, and Radiesse®) influence cell viability, human fibroblast proliferation, and gene expression of extracellular matrix markers, including type I and III collagen, osteocalcin, and osteopontin. **Material and method:** CaHa were evaluated on cell viability, proliferation, and gene expression of extracellular matrix markers in human fibroblasts (n=3, each time-point). Cell viability was assessed using MTT and trypan blue exclusion assays, proliferation by cell counting, and gene expression of type I and III collagen, osteocalcin, and osteopontin by RT-PCR. **Result**: Stiim® showed a significant increase in viability at lower concentrations (1% and 2%) after 24 h and at 0.5% and 1% after 72 h, while the 2.5% concentration drastically reduced viability. Radiesse® demonstrated greater stability, with early proliferation stimulation and sustained induction of both type I and III collagen. Rennova® exhibited a later and more variable response, with reduced proliferation at intermediate concentrations. Osteocalcin and osteopontin expression varied between formulations, with Radiesse® showing a stronger stimulatory effect. The results indicate that HA effects on fibroblasts are formulation- and concentration-dependent, directly influencing cellular behavior and biostimulatory potential. **Conclusion**: These gene expression findings suggest that CaHA may promote pathways associated with dermal neocollagenesis.

Descriptors: Calcium hydroxyapatite; aesthetic; dermal cosmetic; dermatology; orofacial harmonization; collagen; fibroblastos; biomaterials; STIIM; Diamond; Radiesse.

INTRODUCTION

Calcium hydroxyapatite (CaHA) is a crystalline compound that closely resembles the mineral phase of human bone, providing it with a natural affinity for biological tissues. Owing to its structural compatibility and biocompatibility, CaHA has attracted increasing attention as a multifunctional biomaterial, with applications ranging from orthopedic use to tissue repair and regenerative therapies[1,2]. Its composition, mainly constituted by calcium and phosphate ions, underpins both its stability and capacity to support cellular activity and tissue renewal.

In the field of aesthetic medicine, CaHA offers distinctive advantages by triggering the body's own mechanisms of collagen synthesis. Collagen, a central component of the extracellular matrix, is indispensable for maintaining skin resilience, elasticity, and mechanical strength. When CaHA is administered intradermally, it stimulates dermal fibroblasts, leading to enhanced production of type I and III collagen, which are the predominant isoforms in the skin[3]. Through this process, CaHA not only provides immediate structural support and volume restoration but also initiates long-term tissue remodeling. Clinical experience has consistently shown that CaHA achieves durable outcomes in facial rejuvenation, with gradual replacement of the carrier gel by newly formed collagen, resulting in thicker, firmer, and more elastic skin[4–6]. Evidence has also extended its benefits beyond the face, with reports of improved skin quality and texture in the cervical region underlining its versatility and safety[7].

Once in the dermis, CaHA microspheres function as a biostimulatory scaffold that provides local mechanical cues and promotes direct fibroblast–particle interactions associated with increased collagen production, thereby offering a plausible physical basis for downstream signaling activation. In fibroblasts, mechanotransduction is well established to engage ERK (extracellular signal-regulated kinase) as a key pathway that

is sensitive to matrix loading and tension, linking mechanical stimuli to proliferative and matrix-synthetic responses. Complementarily, the TGF-β axis is a central regulator of fibroblast activation and myofibroblast differentiation, and its activity is tightly intertwined with mechanical inputs within fibrotic and reparative contexts, collectively driving extracellular matrix deposition and remodeling. Taken together, these mechanistic frameworks—ERK-mediated mechanotransduction and TGF-β–driven activation—provide a biologically grounded rationale for how mechanically active scaffolds such as CaHA could modulate gene expression programs involved in neocollagenesis, while acknowledging that causal links specific to CaHA require direct protein-level and functional validation[8,9].

The increasing demand for minimally invasive procedures has fueled the growth of the global aesthetics market, with biostimulatory fillers such as CaHA occupying a central role. As new products and technologies emerge, comprehensive understanding of the biological mechanisms activated by these materials becomes essential. Investigating how CaHA interacts with host tissues particularly in promoting neocollagenesis and modulating cellular responses is fundamental for ensuring efficacy, safety, and predictable clinical outcomes. Such knowledge contributes not only to the development of next-generation biomaterials but also to the refinement of evidence-based approaches in aesthetic practice.

MATERIAL AND METHOD

Cell Line and Culture Conditions

Gingival fibroblasts were obtained from explants of healthy attached human gingiva from three different donors, which were harvested during periodontal surgery for

crown lengthening[10]. The current cell passage for this study is 13th. The cells were previously cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Nova Biotecnologia, São Paulo, SP, Brazil) and 1% antibiotic–antimycotic solution (Sigma, St. Louis, MO, USA). The cultures were maintained in a CO_2 incubator (Thermo Scientific, Forma II Water Jacket, USA) under a humidified atmosphere containing 95% air and 5% carbon dioxide, and the medium was refreshed every two days. Once cells reached confluence, detachment was achieved using a solution containing 1 mM ethylenediaminetetraacetic acid (EDTA; Gibco/Invitrogen, Grand Island, NY, USA) and 2.5 mg/mL trypsin (Gibco).

Calcium Hydroxyapatite Preparations

Three commercially available CaHA formulations were tested: Stiin® (CG Bio Co. Ltd., South Korea), Radiesse® (Merz North America Inc., USA), and Diamond® (Innovapharma Brasil Farmacêutica Ltda., Brazil). All three products consist of calcium hydroxyapatite microspheres dispersed in a carboxymethylcellulose (CMC) gel carrier, forming a semi-solid injectable suspension, which is the standard presentation of CaHA-based biostimulators. To maintain experimental consistency, all products were handled in their original commercial form and diluted in culture medium on the day of each assay. The study was conducted independently, without funding, material supply, or methodological involvement from the manufacturers. On the day of each assay, CaHA products were diluted in culture medium ranging from 0.5% to 2.5%. This range was defined based on preliminary experiments from our research group, which identified non-cytotoxic yet bioactive doses for human dermal fibroblasts, and is in line with

previous in vitro studies evaluating dose-dependent effects of hydroxyapatite on fibroblast viability and extracellular matrix gene expression[5]. The following production batches were used: Stiin® (S2Q23013), Diamond® (2282301), and Radiesse® (A00155260).

Cell Viability Assay (MTT)

For viability assessment, human fibroblasts were seeded into 96-well plates at a density of 2×10^5 cells per well and incubated for 24 h before treatment. Cells were then exposed to Stiin®, Radiesse®, or Diamond® at concentrations ranging from 0.5% to 2.5%. Viability was analyzed after 24, 48, and 72 h using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay. For this, 10% of MTT solution (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. After removing the supernatant, 100 µL of dimethyl sulfoxide (DMSO; Sigma) was added to dissolve the formazan crystals. Plates were shaken for 5 min, and 100 µL of the resulting solution was transferred to a new plate. Absorbance was measured at 570–650 nm using a microplate reader (Epoch; Bio-Tek, Winooski, VT, USA). Cell viability was expressed as the value of optical density (OD). All assays were performed in triplicate at each time point, and the analyses were conducted in a blinded manner.

Cell Proliferation

Cell proliferation was assessed using the Trypan Blue vital exclusion assay at 24, 48, and 72 hours after seeding. Briefly, 10 µL of the cell suspension was mixed with 10 µL of Trypan Blue, and 10 µL of the resulting mixture was loaded onto a hemocytometer

(Neubauer chamber; Fisher Scientific, Pittsburgh, PA, USA). Viable and non-viable cells were counted under an inverted phase-contrast microscope (Nikon Eclipse TS100), and total cell number was determined according to standard procedures. Results were expressed as the number of cells $\times 10^4$.

Reverse Transcription and Quantitative PCR (RT-qPCR)

Quantitative gene expression analyses were performed using the SYBR Green system (Thermo Fisher Scientific, Waltham, MA, USA) on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). At 24, 48, and 72 h, total RNA was isolated from cell cultures using the Illustra RNAspin Mini Kit (GE Healthcare, Milwaukee, WI, USA), following the manufacturer's instructions. RNA quality and concentration were determined spectrophotometrically at 260, 280, 230, and 320 nm (GE Healthcare). First-strand cDNA was synthesized from 1 µg of total RNA in a Veriti® Thermal Cycler (Applied Biosystems) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), yielding 40 ng of cDNA. Relative quantification was performed using GAPDH as the housekeeping gene, and expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method. The control group was used as the reference (calibrator) for the $2^{-\Delta\Delta Ct}$ calculation. All the genes sequencing are summarized in Table 1.

Statistical Analysis

The data were initially tested for normality. Thereafter, data were analyzed by One-way ANOVA, followed by Bonferroni post hoc test for multiple comparisons, using GraphPad 10.2. All data are presented as mean ± SD. A P-value less than 0.05 was considered significant.

RESULT

Comparison of cell viability among different commercial calcium hydroxyapatite formulations.

To investigate the influence of different concentrations of commercial calcium hydroxyapatite compounds on fibroblast cells, cell viability and proliferation were assessed using the MTT assay and the Trypan Blue dye exclusion assay, respectively.Figure 1A presents the data for the Stiim brand. At 24 hours of incubation, the 1% and 2% concentrations produced a statistically significant increase in cell viability ($p<0.05$) compared with the control. At 48 hours, no statistically significant differences were detected at any tested concentration ($p>0,05$). After 72 hours of exposure to Stiim calcium hydroxyapatite, a significant increase in viability was observed at 0.5% and 1% ($p<0.05$), whereas a marked decrease in viability occurred at 2.5% ($p<0.05$). Figure 1B shows the results for the Diamond formulation, revealing a later time- and dose-dependent response pattern. During the first 24 and 48 hours of exposure, no statistically significant differences ($p>0.05$) in cell viability were observed at any concentration. After 72 hours, only the 2.5% concentration preserved viability comparable to the control ($p>0.05$), while all other concentrations from 0.5% to 2% displayed a statistically significant reduction in viability ($p<0.05$). Finally, Figure 1C presents the data for Radiesse, indicating stable cell viability: none of the concentrations at any of the three time points differed significantly from the control ($p>0.05$).

Comparison of Cellular Proliferation Among Different Commercial calcium hydroxyapatite Formulations

The next stage of testing was conducted to evaluate the influence of different concentrations of hydroxyapatite brands on the fibroblast proliferation capacity over the analyzed time points. In Figure 2A, after 24 hours of contact with the Stiim hydroxyapatite, it was observed that, except for the 2.5% concentration, which showed no statistical difference ($p > 0.05$), the other concentrations demonstrated a statistically significant inhibitory effect on fibroblast proliferation ($p < 0.05$). The same pattern was observed after 48 hours, in which only the 2% concentration did not differ significantly from the control ($p > 0.05$), while all other concentrations showed a statistically significant reduction in cell proliferation ($p < 0.05$). In Figure 2B, after 24 hours of contact with the Diamond hydroxyapatite, the tested concentrations of 1.5%, 2%, and 2.5% resulted in a statistically significant reduction in fibroblast proliferation compared to the control group (0%) ($p < 0.05$). At 48 hours, only the 2.5% concentration exhibited a statistically significant reduction in proliferation; however, after 72 hours, concentrations ranging from 0.5% to 2% showed a significant decrease in proliferation, while only the 2.5% concentration presented a statistically significant increase in fibroblast proliferation ($p < 0.0001$). In Figure 2C, regarding Radiesse analysis, after 24 hours, there was a statistically significant increase in fibroblast numbers at concentrations of 1% ($p < 0.001$) and 2.5% ($p < 0.0001$), whereas no significant differences were observed at the other concentrations. At subsequent evaluations, after 48 hours, concentrations from 1.5% to 2.5% showed a statistically significant reduction in cell proliferation compared to the control group ($p < 0.05$), and after 72 hours, all tested concentrations exhibited a statistically significant decrease in proliferation ($p < 0.001$). These data suggest that Radiesse exerts a more pronounced inhibitory effect on fibroblast proliferation over time.

Evaluation of Type I Collagen gene expression in the Presence of Different Concentrations of Commercial calcium hydroxyapatites

One of the main characteristics of hydroxyapatite formulations commercially used for aesthetic purposes is their ability to stimulate collagen production in the skin, primarily by fibroblast cells. Therefore, we evaluated this collagen-inducing mRNA expression potential for type I collagen using polymerase chain reaction (PCR) analysis.

In Figure 3A, at a concentration of 0.5%, Radiesse hydroxyapatite showed a statistically significant increase in gene expression compared to the other brands and the control group ($p < 0.01$) across all three time points analyzed. At 24 h and 72 h, Stiim hydroxyapatite also promoted a significant increase in gene expression relative to the control and Diamond ($p < 0.05$). In Figure 3B, referring to the 1% concentration, Radiesse maintained the same pattern at 24 h, showing significantly higher gene expression than the other brands and the control ($p < 0.01$). Both Stiim and Diamond also exhibited a significant increase in type I collagen mRNA expression compared to the control ($p < 0.05$). In Figure 3C, at a concentration of 1.5%, Radiesse demonstrated a statistically significant increase in type I collagen gene expression at all three time points when compared to the other brands ($p < 0.05$). After 24 h, Diamond also showed a significant increase in gene expression compared to Stiim and the control. Interestingly, after 72 h, both Stiim and Diamond exhibited a reduction in type I collagen gene expression, with a statistically significant difference relative to the control and Radiesse ($p < 0.05$).

Evaluation of Type III Collagen gene expression in the Presence of Different Concentrations of Commercial calcium hydroxyapatites

Type III collagen also plays an important role in skin architecture; therefore, the next analysis aimed to quantify the messenger RNA expression of this gene after exposure

to commercial hydroxyapatites at 24, 48, and 72 hours. In Figure 4A, at a concentration of 0.5%, type III collagen mRNA expression after 24 hours was significantly higher in the Stiim and Radiesse groups compared to the control and Diamond ($p < 0.05$). After 48 hours, only Radiesse maintained elevated gene expression levels compared to the other groups ($p < 0.05$). At 72 hours, both the control group and Radiesse exhibited significantly higher gene expression compared to the other brands ($p < 0.05$). In Figure 4B, corresponding to the 1% concentration, no statistically significant differences were observed between groups at 24 and 48 hours ($p > 0.05$). However, at 72 hours, Diamond showed a significant reduction in gene expression compared to the other groups ($p < 0.05$). In Figure 4C, at a concentration of 1.5%, after 24 hours, Stiim showed significantly increased mRNA expression compared to the control and Diamond ($p < 0.05$). At 48 hours, Radiesse induced the highest gene expression among the groups ($p < 0.05$). At 72 hours, both Stiim and Diamond exhibited a significant reduction in mRNA expression compared to the control and Radiesse ($p < 0.05$).

Evaluation of Osteocalcin gene expression in the Presence of Different Concentrations of Commercial calcium hydroxyapatites

In Figure 5A, at a concentration of 0.5%, all three calcium hydroxyapatite brands showed a statistically significant increase in osteocalcin expression compared to the control group ($p < 0.05$) after 24 hours. At 48 hours, only Radiesse maintained elevated gene expression levels relative to the control ($p < 0.05$), while the other brands showed no significant differences ($p > 0.05$). After 72 hours, Radiesse continued to induce higher osteocalcin mRNA expression compared to the other groups ($p < 0.05$), whereas Diamond exhibited a significant reduction in gene expression, differing from the control, Stiim, and Radiesse ($p < 0.05$). In Figure 5B, at a concentration of 1%, no significant differences

were observed among the groups at 24 or 48 hours ($p > 0.05$). However, at 72 hours, the Stiim group showed a significant increase in osteocalcin gene expression compared to Radiesse ($p < 0.05$). Finally, in Figure 5C, corresponding to the 1.5% concentration, no statistically significant differences were detected among the groups at any of the evaluated time points ($p > 0.05$).

Evaluation of Osteopontin Production in the Presence of Different Concentrations of Commercial calcium hydroxyapatites

In Figures 6A and 6B, at concentrations of 0.5% and 1%, no statistically significant differences were observed in osteopontin gene expression at any of the analyzed time points ($p > 0.05$). However, at the 1.5% concentration (Figure 6C), after 24 hours, the Radiesse group exhibited a significant increase in gene expression compared to the Stiim and Diamond groups ($p < 0.05$), a pattern that persisted at 48 hours. At 72 hours, however, no statistically significant differences were detected among the groups ($p > 0.05$).

DISCUSSION

Calcium hydroxyapatite (CaHA) has been established as a reliable biostimulatory agent in aesthetic medicine for more than a decade, with consistent evidence supporting its ability to induce type I and III collagen production. When deposited in dermal or subdermal layers, CaHA microspheres act as a scaffold that facilitates fibroblast recruitment and triggers robust neocollagenesis[4,7,11].

In the present study, Radiesse demonstrated the strongest overall bioactivity, as reflected by elevated expression of collagen types I and III, together with osteocalcin and osteopontin, across a broad range of concentrations and time points. Stiim elicited intermediate responses, including an early induction of type III collagen and a marked

increase in osteocalcin at 1% after 72 h. In contrast, Diamond generally exhibited weaker responses, with reduced expression trends at later intervals.

These findings highlight brand- and concentration-dependent differences in fibroblast behavior. Previous reports have indicated that composites containing approximately 70% CaHA maintain high cell viability, with progressive increases in metabolic activity over several days[12]. This aligns with the present observation of enhanced viability at lower Stiim concentrations (1–2%) at 24 h, as well as the proliferative effects of Radiesse at 1–2.5%. Conversely, the reduction in cell responses at higher concentrations (2.5%) after 72 h may reflect cytotoxic stress or particle overload, phenomena described in studies using hydroxyapatite microcrystals.

Radiesse has previously been shown to upregulate type III collagen at 24 h, followed by type I collagen at 72 h[5,6]. The present results reproduce this sequential activation pattern, as demonstrated in Figures 3 and 4. Ex vivo and in vitro models similarly report COLIII induction at early time points, followed by COLI increases, supporting the temporal dynamics identified here5. Clinically, reviews have shown that type III and I collagen levels rise progressively from 4 to 12 months following CaHA injection[13,14], suggesting that the in vitro data generated here represent the initial stages of a prolonged neocollagenesis process. Beyond collagen induction, CaHA has also been reported to enhance elastin synthesis and reinforce the extracellular matrix, contributing to sustained improvements in skin density, elasticity, and overall quality[2,15]. The fibroblast gene expression profiles obtained in this study are in line with these mechanisms.

In addition to its effects on dermal markers, CaHA has been shown to stimulate osteogenic differentiation in experimental models. Specifically, osteocalcin and osteopontin—both characteristic of mineralized tissue—are induced in osteoblast cultures exposed to CaHA[16]. Although fibroblasts were used in the present work, the detection of osteogenic markers suggests that CaHA may promote partial phenotypic reprogramming or activate broader pathways of matrix remodeling. For instance, the increases in osteopontin observed at 0.5% and 1.5% CaHA in Stiim and Radiesse (Figures 5 and 6) are consistent with preclinical evidence linking CaHA exposure to this protein.

Osteocalcin, while primarily secreted by osteoblasts, can also be expressed by dermal fibroblasts and other mesenchymal cell types under osteogenic stimuli, such as

hydroxyapatite exposure. It plays a regulatory role in extracellular matrix turnover and metabolic signaling during tissue remodeling[17]. Osteopontin, in contrast, is a multifunctional glycoprotein involved in tissue regeneration, immune regulation, and angiogenesis. It promotes cell adhesion through integrin and CD44 binding, supports fibroblast migration, and functions as a chemoattractant for immune cells during wound healing. In addition, it contributes to collagen deposition and vascularization, reinforcing its importance in regenerative processes[18].

Several limitations must be acknowledged. First, these findings were generated in 2D monolayer fibroblast cultures, which do not fully replicate the mechanical, vascular, and immunological features of human dermis. Second, gene expression analysis alone cannot confirm protein synthesis or functional ECM remodeling; therefore, protein-level validation and longer-term studies are necessary. Third, the relevance of osteopontin and osteocalcin in aesthetic applications is not yet fully defined, and their roles should be interpreted cautiously. Further research should explore protein secretion, ECM deposition, and interactions with immune and vascular cells to better contextualize these findings.

Taken together, these gene expression findings suggest that CaHA may promote pathways associated with dermal neocollagenesis and may influence markers linked to mineralized tissue formation. Given the cell culture-based nature of this study and the absence of protein-level validation, these observations should be interpreted as preliminary and caution to in vivo extrapolation.

FIGURES

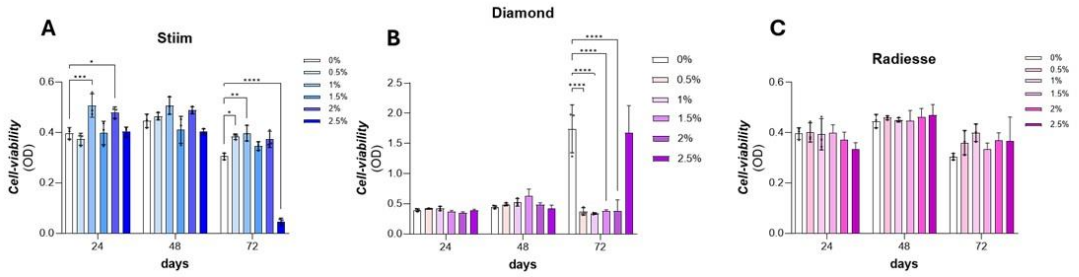

Figure 1. Cell viability of fibroblasts exposed to three Calcium hydroxyapatite (CaHA) brands. Fibroblast cultures were treated with (A) Stiim, (B) Rennova, or (C) Radiesse at concentrations of 0% (control), 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (v/v). Cell viability was assessed using the MTT assay at the specified time points. Data are presented as mean ± SD. All assays were performed in triplicate at each time point.. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols indicate statistical significance compared with the control group ($p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$).

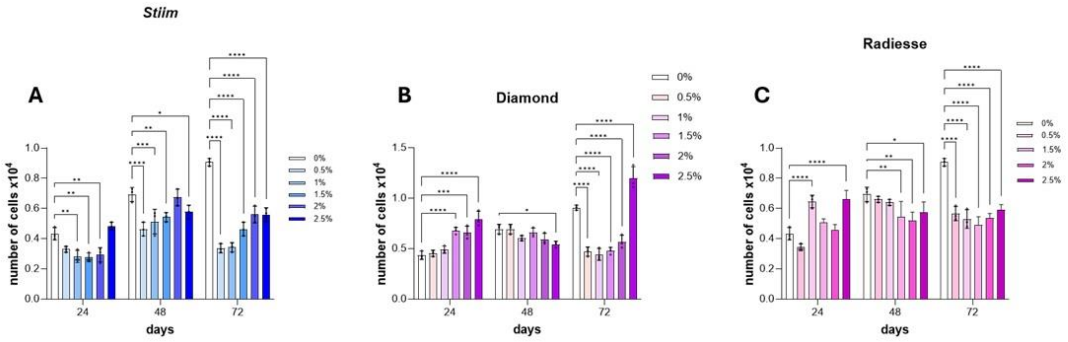

Figure 2. Cell proliferation of fibroblasts exposed to three Calcium hydroxyapatite (CaHA) brands. Fibroblast cultures were treated with (A) Stiim, (B) Rennova, and (C) Radiesse at concentrations of 0% (control), 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (v/v). Cell proliferation was assessed using the Trypan Blue exclusion at the indicated time points. Data are presented as mean ± SD. All assays were performed in triplicate at each time point. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols indicate statistical significance compared with the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

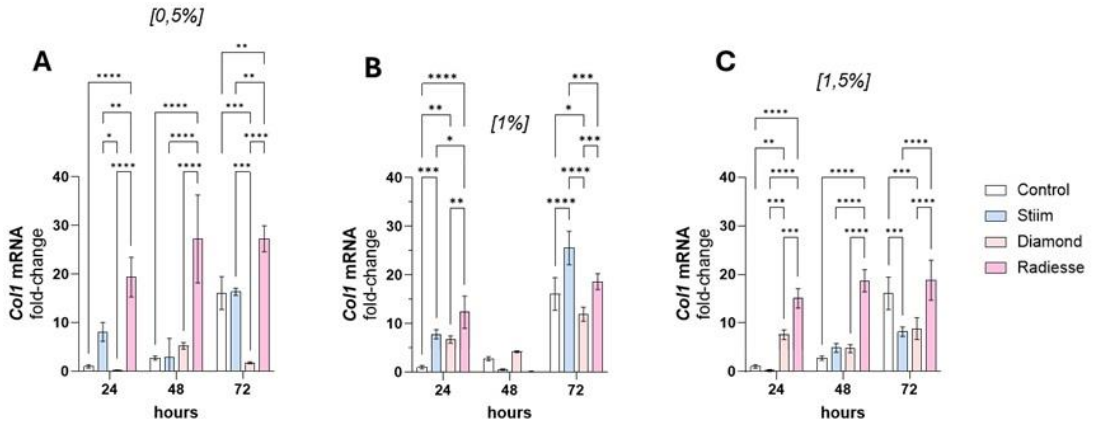

Figure 3. Type I collagen mRNA expression in fibroblasts treated with Calcium hydroxyapatite (CaHA) formulations. Fibroblast cultures were exposed to Stiim, Rennova, or Radiesse, as well as to a control group (0%), at concentrations of 0.5% (A), 1.0% (B), and 1.5% (C). Type I collagen gene expression was quantified by RT-qPCR, using GAPDH as the housekeeping gene and the $2^{-\Delta\Delta Ct}$ method, with the control condition as the calibrator. Data are presented as mean ± SD. All assays were performed in triplicate at each time point. Statistical comparisons were performed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols indicate statistical significance relative to the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

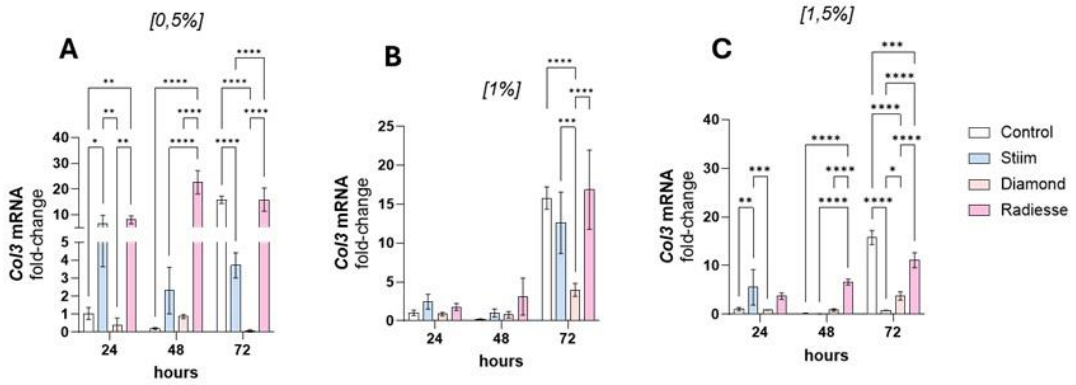

Figure 4. Type III collagen mRNA expression in fibroblast cultures treated with hydroxyapatite (HA) formulations. Fibroblasts were exposed to different brands of calcium hydroxyapatite (Stiim, Rennova, and Radiesse) at concentrations of 0.5% (A), 1.0% (B), and 1.5% (C), along with the control condition (0%). Type III collagen gene expression was quantified by RT-qPCR, using GAPDH as the housekeeping gene and the $2^{-\Delta\Delta Ct}$ method, with the control group serving as the calibrator. Data are presented as mean ± SD. All assays were performed in triplicate at each time point. Statistical analysis was conducted using one-way ANOVA followed by Bonferroni's post hoc test. Symbols indicate statistical significance relative to the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

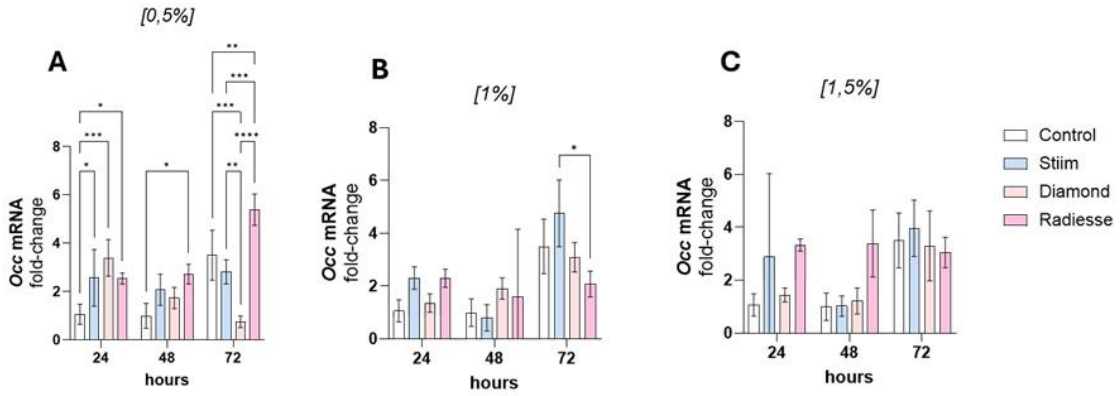

Figure 5. Osteocalcin (OCN) mRNA expression in fibroblast cultures treated with calcium hydroxyapatite (CaHA) formulations. Fibroblasts were exposed to different brands of calcium hydroxyapatite (Stiim, Rennova, and Radiesse) at concentrations of 0.5% (A), 1.0% (B), and 1.5% (C), as well as to the control condition (0%). Osteocalcin gene expression was quantified by RT-qPCR, using GAPDH as the housekeeping gene and the $2^{-\Delta\Delta Ct}$ method, with the control group serving as the calibrator. Data are reported as mean ± SD. All assays were performed in triplicate at each time point. Statistical comparisons were performed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols indicate statistical significance relative to the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

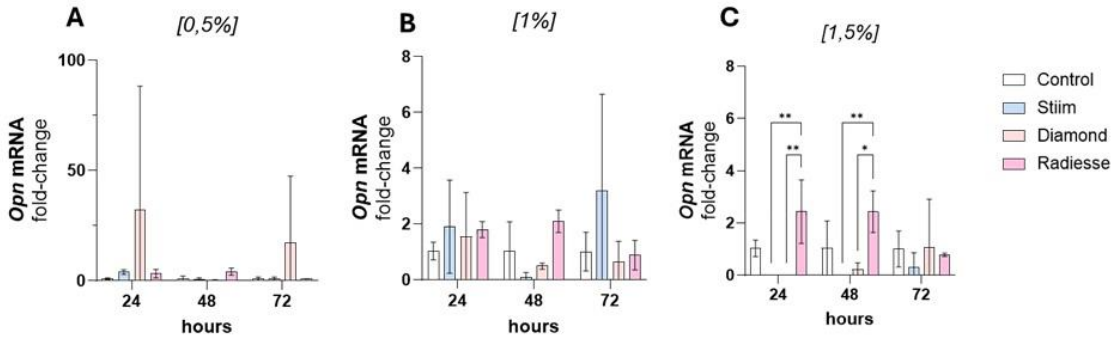

Figure 6. Osteopontin (OPN) mRNA expression in fibroblast cultures treated with calcium hydroxyapatite (CaHA) formulations. Fibroblasts were exposed to different brands of calcium hydroxyapatite (Stiim, Rennova, and Radiesse) at concentrations of 0.5% (A), 1.0% (B), and 1.5% (C), in addition to the control condition (0%). Osteopontin gene expression was quantified by RT-qPCR, using GAPDH as the housekeeping gene and the $2^{-\Delta\Delta Ct}$ method, with the control group serving as the calibrator. Data are shown as mean ± SD. All assays were performed in triplicate at each time point. Statistical comparisons were performed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols denote statistical significance relative to the control group ((* $p < 0.05$, ** $p < 0.01$).

Table 1: PCR primers for genes encoding Collagen I (COL I), Collagen III (Col III), osteopontin (OPN), osteocalcin (OC) and GAPDH.

Human COL I

Forward - CCAGAAGAACTGGTACATCAGCAA

Reverse – GGACATCAGGCGCAGGAA

Human COL III

Forward - CGCCCTCCTAATGGTCAAGG

Reverse - AGGGCCTGAAGGACCAGCTT

Human OPN

Forward – GCCGAGGTGATAGTGTGGTT

Reverse – AACGGGGATGGCCTTGTATG

Human OC

Forward – ACCTGTATCAATGGCTGGGAG

Reverse – AGCAGAGCGACACCCTAGAC

Human GAPDH

Forward – ACCCACTCCTCCACCTTTGA

Reverse - TGTTGCTGTAGCCAAATTCGTT

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, for providing access to scientific literature through its journals portal (#001).

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: RK, HBA, JTC-N, MHN

Conducted experiments: RK, HBA

Contributed new reagents or analytic tools: HBA, JTC-N, MHN

Performed data analysis: RK, HBA, JTC-N, MHN

Wrote or contributed to the writing of the manuscript: RK, HBA, JTC-N, MHN

All authors agreed with the final version of the manuscript.

REFERENCES

1. Figueredo VO, Miot HA, Soares Dias J, Nunes GJB, Barros de Souza M, Bagatin E. Efficacy and safety of 2 injection techniques for hand biostimulatory treatment with diluted calcium hydroxylapatite. Dermatol Surg. 2020 Oct;46 Suppl 1:S54-S61. https://orcid.org/10.1097/DSS.0000000000002334. PMid: 32976172.
2. Mazzuco R, Evangelista C, Gobbato DO, de Almeida LM. Clinical and histological comparative outcomes after injections of poly-L-lactic acid and calcium hydroxyapatite in arms: a split side study. J Cosmet Dermatol. 2022 Dec;21(12):6727-6733. https://orcid.org/10.1111/jocd.15356. Epub 2022 Sep 20. PMid: 36098704.
3. Dallara JM, Baspeyras M, Bui P, Cartier H, Charavel MH, Dumas L. Calcium hydroxylapatite for jawline rejuvenation: consensus recommendations. J Cosmet Dermatol. 2014 Mar;13(1):3-14. https://orcid.org/10.1111/jocd.12074. PMid: 24641600.

4. Berlin AL, Hussain M, Goldberg DJ. Calcium hydroxylapatite filler for facial rejuvenation: a histologic and immunohistochemical analysis. Dermatol Surg. 2008 Jun;34 Suppl 1:S64-7. https://orcid.org/10.1111/j.1524-4725.2008.34245.x. PMid: 18547184.
5. Nowag B, Casabona G, Kippenberger S, Zöller N, Hengl T. Calcium hydroxylapatite microspheres activate fibroblasts through direct contact to stimulate neocollagenesis. J Cosmet Dermatol. 2023 Feb;22(2):426-432. https://orcid.org/10.1111/jocd.15521. Epub 2022 Dec 27. PMid: 36575882.
6. Amiri M, Meçani R, Niehot CD, Phillips T, Kolb J, Daughtry H, et al. Skin regeneration-related mechanisms of calcium hydroxylapatite (CaHA): a systematic review. Front Med (Lausanne). 2023 Jun 2;10:1195934. https://orcid.org/10.3389/fmed.2023.1195934. PMid: 37332763
7. Marmur ES, Phelps R, Goldberg DJ. Clinical, histologic and electron microscopic findings after injection of a calcium hydroxylapatite filler. J Cosmet Laser Ther. 2004 Dec;6(4):223-6. https://orcid.org/10.1080/147641704100003048. PMid: 16020207.
8. Younesi FS, Miller AE, Barker TH, Rossi FMV, Hinz B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. Nat Rev Mol Cell Biol. 2024 Aug;25(8):617-638. doi: 10.1038/s41580-024-00716-0. Epub 2024 Apr 8. Erratum in: Nat Rev Mol Cell Biol. 2024 Aug;25(8):671. https://orcid.org/10.1038/s41580-024-00744-w. PMid: 38589640.
9. Rosenfeldt H, Grinnell F. Fibroblast quiescence and the disruption of ERK signaling in mechanically unloaded collagen matrices. J Biol Chem. 2000 Feb 4;275(5):3088-92. https://orcid.org/10.1074/jbc.275.5.3088.
10. Moretti D, Teixeira LN, Perri de Carvalho PS, Vedovatto E, Martinez EF. Biologic behavior of pressed lithium disilicate ceramic and zirconia on human gingival

fibroblasts: an in vitro study. Int J Periodontics Restorative Dent. 2022 Sep-Oct;42(5):e153-e159. https://orcid.org/10.11607/prd.5978. PMid: 36044702.

11. Coleman KM, Voigts R, DeVore DP, Termin P, Coleman WP 3rd. Neocollagenesis after injection of calcium hydroxylapatite composition in a canine model. Dermatol Surg. 2008 Jun;34 Suppl 1:S53-5. https://orcid.org/10.1111/j.1524-4725.2008.34243.x. PMid: 18547182.
12. Thuy Ba Linh N, Mondal D, Lee BT. In vitro study of CaTiO3-hydroxyapatite composites for bone tissue engineering. ASAIO J. 2014 Nov-Dec;60(6):722-9. https://orcid.org/10.1097/MAT.0000000000000126. PMid: 25238497.
13. van Loghem J. Calcium hydroxylapatite in regenerative aesthetics: mechanistic insights and mode of action. Aesthet Surg J. 2025 Mar 17;45(4):393-403. https://orcid.org/10.1093/asj/sjae196. PMid: 39365034.
14. Hong JY, Park KY. Dual Benefits of calcium hydroxyapatite filler: a prospective study on midface volume restoration and skin quality enhancement. J Cosmet Dermatol. 2025 Jun;24(6):e70265. https://orcid.org/10.1111/jocd.70265. PMid: 40439277
15. Courderot-Masuyer C, Robin S, Tauzin H, Humbert P. Evaluation of lifting and antiwrinkle effects of calcium hydroxylapatite filler. In vitro quantification of contractile forces of human wrinkle and normal aged fibroblasts treated with calcium hydroxylapatite. J Cosmet Dermatol. 2016 Sep;15(3):260-8. https://orcid.org/10.1111/jocd.12215. Epub 2016 Mar 17. PMid: 26990784.
16. Bargowo L, Kusumawardhani B, Perdana S, Wijaksana IKE, Saskianti T, Ridwan RD, et al. Expression of osteopontin and osteocalcin in Osteoblast cells exposed to a combination of polymethylmethacrylate (PMMA) and hydroxyapatite (HAp): a prospective observational study. Medicine (Baltimore). 2024 Oct 18;103(42):e40088. https://orcid.org/10.1097/MD.0000000000040088. PMid: 39432596

17. Mera P, Laue K, Ferron M, Confavreux C, Wei J, Galán-Díez M, et al. Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. Cell Metab. 2016 Jun 14;23(6):1078-1092. https://orcid.org/10.1016/j.cmet.2016.05.004. Erratum in: Cell Metab. 2017 Jan 10;25(1):218. https://orcid.org/10.1016/j.cmet.2016.12.003. PMid: 27304508

18. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science. 2000 Feb 4;287(5454):860-4. https://orcid.org/10.1126/science.287.5454.860. PMid: 10657301.

CONFLICTS OF INTERESTS

The authors report no conflict of interest

DATA AVAILABILITY

The contents underlying the research text are included in the manuscript. The contents are already available.

*CORRESPONDING AUTHOR

Dr. Marcelo Henrique Napimoga, Faculdade São Leopoldo Mandic, Instituto e Centro de Pesquisas São Leopoldo Mandic, Rua José Rocha Junqueira, 13, Swift, 13405-755 Campinas,SP, Brasil, e-mail: marcelo.napimoga@gmail.com; marcelo.napimoga@slmandic.edu.br

Received: November 11, 2025

Accepted: March 7, 2026

Critérios SciELO Brasil

Formulário sobre Conformidade com a Ciência Aberta

versão 29 de junho de 2020

Por meio deste formulário os autores informam o periódico sobre a conformidade do manuscrito com as práticas de comunicação da Ciência Aberta. Os autores são solicitados a informar: (a) se o manuscrito é um preprint e, em caso positivo, sua localização; (b) se dados, códigos de programas e outros materiais subjacentes ao texto do manuscrito estão devidamente citados e referenciados; e, (c) se aceitam opções de abertura no processo de avaliação por pares.

Preprints

Depósito do manuscrito em um servidor de preprints reconhecido pelo periódico.

O manuscrito é um preprint?	
( )	Sim - Nome do servidor de Preprints: DOI do Preprint:
(X)	Não

Disponibilidade de Dados de Pesquisa e outros Materiais

Autores são encorajados a disponibilizar todos os conteúdos (dados, códigos de programa e outros materiais) subjacentes ao texto do manuscrito anteriormente ou no momento da publicação. Exceções são permitidas em casos de questões legais e éticas. O objetivo é facilitar a avaliação do manuscrito e, se aprovado, contribuir para a preservação e reuso dos conteúdos e a reprodutibilidade das pesquisas.

Os conteúdos subjacentes ao texto do manuscrito já estão disponíveis em sua totalidade e sem restrições ou assim estarão no momento da publicação?	
(x)	Sim: (x) os conteúdos subjacentes ao texto da pesquisa estão contidos no manuscrito (x) os conteúdos já estão disponíveis ( ) os conteúdos estarão disponíveis no momento da publicação do artigo Segue títulos e respectivas URLs, números de acesso ou DOIs dos arquivos dos conteúdos subjacentes ao texto do artigo (use uma linha para cada dado):
( )	Não: ( ) dados estão disponíveis sob demanda dos pareceristas ( ) após a publicação os dados estarão disponíveis sob demanda aos autores – condição justificada no manuscrito ( ) os dados não podem ser disponibilizados publicamente. Justifique a seguir:

Aberturas na avaliação por pares

Os autores poderão optar por um ou mais meios de abertura do processo de *peer review* oferecidos pelo periódico.

Quando oferecida a opção, os autores concordam com a publicação dos pareceres da avaliação de aprovação do manuscrito?	
(x)	Sim
( )	Não
Quando oferecida a opção, os autores concordam em interagir diretamente com pareceristas responsáveis pela avaliação do manuscrito?	
(x)	Sim
( )	Não

This preprint was submitted under the following conditions:

- The authors declare that the necessary Terms of Free and Informed Consent of participants or patients in the research were obtained and are described in the manuscript, when applicable.
- The authors declare that the preparation of the manuscript followed the ethical norms of scientific communication.
- The authors declare that they are aware that they are solely responsible for the content of the preprint and that the deposit in SciELO Preprints does not mean any commitment on the part of SciELO, except its preservation and dissemination.
- The authors declare that the data, applications, and other content underlying the manuscript are referenced.
- The deposited manuscript is in PDF format.
- The authors declare that the research that originated the manuscript followed good ethical practices and that the necessary approvals from research ethics committees, when applicable, are described in the manuscript.
- The authors declare that once a manuscript is posted on the SciELO Preprints server, it can only be taken down on request to the SciELO Preprints server Editorial Secretariat, who will post a retraction notice in its place.
- The authors agree that the approved manuscript will be made available under a Creative Commons CC-BY license.
- The submitting author declares that the contributions of all authors and conflict of interest statement are included explicitly and in specific sections of the manuscript.
- The authors declare that the manuscript was not deposited and/or previously made available on another preprint server or published by a journal.
- If the manuscript is being reviewed or being prepared for publishing but not yet published by a journal, the authors declare that they have received authorization from the journal to make this deposit.
- The submitting author declares that all authors of the manuscript agree with the submission to SciELO Preprints.