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Chitosan-triclosan particles modulate inflammatory signaling in gingival fibroblasts

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Background and Objectives: An important goal of periodontal therapy is the modulation of the inflammatory response. To this end, several pharmacological agents have been evaluated. Triclosan corresponds to an antibacterial and anti-inflammatory agent currently used in periodontal therapy. Chitosan is a natural polymer that may act as a drug delivery agent and exerts antibacterial and anti-inflammatory activities. Therefore, an association between both molecules might be useful to prevent inflammation and tissue destruction in periodontal tissues.

Material and Methods: In the present study, we have generated chitosan-triclosan particles and evaluated their morphology, charge, biocompatibility and gene expression analysis in human gingival fibroblasts.

Results: The chitosan-triclosan particles size and Z potential were 129 ± 47 nm and 51 ± 17 mV respectively. Human gingival fibroblast viability was not affected by chitosan-triclosan. A total of 1533 genes were upregulated by interleukin (IL)-1β. On the other hand, 943 were downregulated in fibroblasts stimulated with IL-1β plus chitosan-triclosan particles. Fifty-one genes were identified as molecular targets upregulated by IL-1 β and downregulated by the chitosan-triclosan particles. The gene ontology analysis revealed that these genes were enriched in categories related to biological processes, molecular function and cellular components. Furthermore, using real-time reverse transcription-polymerase chain reaction *beta-actin*, *fibronectin*, *interleukin-6* and IL-1b genes were confirmed as targets upregulated by IL-1β and downregulated by chitosan-triclosan particles.

Conclusion: Our results show that chitosan-triclosan particles are able to modulate the inflammatory response in gingival fibroblasts. This effect might be useful in the prevention and/or treatment of inflammation in periodontal diseases.

KEYWORDS
chitosan, fibroblasts, periodontal diseases, triclosan

1 | INTRODUCTION

Periodontal diseases correspond to highly prevalent inflammatory lesions that cause significant tissue destruction in the gingiva and tooth supporting structures.[1] Inflammatory cytokines including interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α) have been identified as main drivers of the inflammatory response in periodontitis.[2] The host response initiated by bacteria plays a prominent role in tissue destruction in periodontitis.[1,3] Therefore, modulation of infection and inflammation are critically important issues for the prevention and treatment of periodontal diseases.

Triclosan is a widely used lipid-soluble antibacterial agent that has been incorporated into dentifrices and mouth rinses to prevent and treat gingival inflammation.[4-7] Moreover, several studies have provided

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evidence of an additional anti-inflammatory action for this agent.[7-12] Nevertheless, a critically important restriction in the use of triclosan has been its limited substantivity or lack of retention in oral tissues.[13] Polymers and other agents have been designed to improve this weakness in triclosan.[14,15] More recently, triclosan has been combined with chitosan, a polysaccharide derived from chitin, due to its drug delivery, antimicrobial and anti-inflammatory properties.[16-18] One important aspect of using chitosan as a drug delivery agent is that it exerts both antimicrobial and anti-inflammatory activities.[17] This aspect of this drug delivery agent may be important to reduce the concentration of the active drug, in this case triclosan, in a putative formulation.

Gingival fibroblasts represent the most prevalent cell population in the gingiva playing a critical role in the modulation of tissue remodeling and inflammatory reactions.[19] IL-1β has been implicated in tissue destruction in periodontitis and is detected at increased levels in the inflamed periodontium.[3] In a recent study we have evaluated the anti-inflammatory effect of chitosan in human gingival fibroblasts challenged with IL-1β, describing that chitosan was able to downregulate IL-1β-stimulated prostaglandin E_2 production in gingival fibroblasts through the JNK pathway.[18]

Given the significant role of inflammation in periodontal disease pathogenesis, it is critically important to understand how pharmacological agents control cell signaling and in turn modulate gene expression in cells challenged by inflammatory cytokines such as IL-1β. In the present study, using a gene microarray, we have evaluated the main genes regulated by the inflammatory cytokine IL-1β in primary cultures of human gingival fibroblasts and the role of chitosan-triclosan particles as anti-inflammatory agents capable of downregulating this response.

2 | MATERIAL AND METHODS

2.1 | Cell culture

Explants were obtained from the healthy gingiva surrounding 3rd molars from 3 male adults (18-25 years) at the Institutional Clinical Facility, School of Dentistry, Pontificia Universidad Católica de Chile. Three cell cultures were used for preliminary analysis of cell viability of fibroblasts exposed to the chitosan-triclosan formulation and for experiments performed to determine the optimal proinflammatory dosage of IL-1β. Microarray analysis and real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed in 1 cell culture. Periodontal examination demonstrated sites with probing depth less than 4 mm, no loss of attachment and no bleeding on probing. The Ethical Committee, Pontificia Universidad Católica de Chile approved the protocol for tissue attainment. All donors approved and signed an informed consent. Patients did not report pre-existing medical or drug histories in the last 6 months and no smokers were included. Cells were cultured in α-minimal essential medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc., Logan, UT, USA), and Normocin (InvivoGen, San Diego, CA, USA) at 37°C in 5% CO_2 atmosphere. All experiments were performed using cells expanded between passages 3 and 8.

2.2 | Preparation of chitosan-triclosan particles

Chitosan was produced from *Dosidicus gigas* squid pens. Briefly, squid pens were treated with 8% NaOH at 60°C for 2 hours. The solution was filtered and intensively washed until obtaining a neutral pH. After this, the product was deacetylated using 40% NaOH. The solid/solution ratio was 1:10 and the reaction time was 4 hours at 60°C. The product was characterized according its viscosity average molar mass = 977 kDa by capillary viscometry and degree of acetylation = 10.5% by nuclear magnetic proton resonance using conditions described elsewhere.[20] Chitosan-tripolyphosphate (TPP) particles loaded with triclosan (2,4,4-trichloro-2-hydroxy diphenyl ether) (Calbiochem, San Diego, CA, USA) were prepared according to a previous study.[21] Briefly, chitosan (3.0 mg/mL) and triclosan (0.06 mg/L) were dissolved in an acetic aqueous solution (3 mg/mL). Simultaneously, an aqueous TPP solution (1.0 mg/mL) was prepared. After this, 2.5 mL chitosan-triclosan solution was added dropwise under constant stirring to 1 mL TPP solution generating an opalescent suspension. The particles obtained were separated by centrifuging at 17 000 *g* for 30 minutes, freeze-dried, crushed with a mortar and sieved through a 400 mesh, and stored at 4-8°C.

2.3 | Morphology of chitosan-triclosan particles

Chitosan-triclosan particles were analyzed through transmission electron microscopy (Zeiss EM 109, Göttingen, Germany). Chitosan-triclosan particles were suspended in deionized water and a drop of the suspension was placed on a copper grid. After 3 minutes the drop was removed with a filter paper and then 5% aqueous uranyl acetate was added over the grid. After 5 minutes, the drop was removed from the grid with a filter paper and directly observed on the transmission electron microscopy.

2.4 | Zeta potential

Zeta potential was measured using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Diluted samples in water were placed in disposable polystyrene cuvettes and the scatter intensity was measured at 25°C.

2.5 | Experiment design and stimulation of cell cultures

Three different experimental conditions were evaluated in this study: (i) non-stimulated cells (control) compared to chitosan-triclosan-stimulated cells (this experiment allowed us to evaluate the biological safety of the formulation); (ii) non-stimulated cells compared to IL-1β-treated fibroblasts (this experiment estimated the gene expression changes induced by the inflammatory response); and (iii) chitosan-triclosan-treated cells compared to chitosan-triclosan plus IL-1β stimulated cells (this last experiment allowed us to estimate the anti-inflammatory activity of the chitosan-triclosan formulation). Fibroblasts (7×10^6) were seeded on 150 mm cell culture dishes. The day after

this, FBS was removed and 24 hours later, cell cultures were treated for 4 hours with 200 μg/mL chitosan-triclosan and/or 300 pg/mL IL-1β. Dosages of both chitosan-triclosan and IL-1β were determined in preliminary experiments in which we established the optimum concentrations of these agents to induce a consistent inflammatory response.

2.6 | Cell viability assays (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and lactate dehydrogenase release assessment

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI, USA) was used to assess cell viability. Cells were seeded into 96-well plates (Orange Scientific, Braine-L'Alleud, Belgium) and allowed to attach overnight in the presence of FBS (Hyclone Laboratories). Cells were then exposed to different 200 μg/mL chitosan-triclosan and or 300 pg/mL IL-1β. After 24 hours, the MTS labeling reagent was added to each well, incubated for 4 hours and subsequently read at 490 nm using a microplate reader (ThermoPlate TP-Reader NM; Thermo Fisher Scientific, Rockford, IL, USA). In parallel, lactate dehydrogenase (LDH) release was read in the cell culture medium. To this end, 50 μL of cell culture medium was transferred to a 96-well plate and mixed with 50 μL of the LDH substrate mix (Promega Cytotox 96 non-radioactive cytotoxicity assay). The plate was incubated for 30 minutes at room temperature. Stop solution was then added and the absorbance at 490 nm was registered.

2.7 | RNA isolation and microarray hybridization

Total RNA extraction from cells was carried out using Tri-reagent (Ambion, Austin, TX, USA), following the manufacturer's recommendations. Genomic DNA in the RNA samples was digested with RNase-free DNase I (Ambion) The concentration, 28S/18S ribosomal RNA (rRNA) ratio, and RNA integrity number of the purified RNA samples were measured using the 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) with the RNA 6000 Pico Chip kit (Agilent). After confirming the purity of RNA samples, One microgram of total RNA was used to synthesize amplified RNA (aRNA) using the MessageAmp II mRNA amplification kit (Ambion). Five micrograms of aRNA from non-stimulated and IL-1β-treated gingival fibroblasts was coupled with Cy3 and 5 μg of aRNA from IL-1β-treated cells and triclosan-chitosan-treated cells was coupled with Cy5 dyes, according to the manufacturer's instructions. Probe quantity and dye incorporation were assessed with a scanning spectrophotometer. Three different sets of hybridization experiments were made: (i) aRNA from non-stimulated cells vs aRNA from IL-1β-treated cells; (ii) aRNA from non-stimulated cells vs aRNA from chitosan-triclosan-treated cells; and (iii) aRNA from IL-1β-treated cells vs aRNA from chitosan-triclosan-plus IL-1β-treated cells. For each experiment, the 2 dye probes were mixed and concentrated to a volume of 50 μL in a solution containing 20% formamide, 5× SSC and 0.1% sodium dodecyl sulfate, and hybridization was performed essentially according to the microarray manufacturer's instructions. Slides were incubated for 16 hours at 42°C as described.[22] A ScanArray GX laser reader (Perkin Elmer Life Sciences, Boston, MA, USA) was used for detection of the fluorescent derivatives. The overall expression of genes was performed using a Human MI Ready Array, from Microarrays Inc. (Huntsville, AL, USA). Each array contained 48 958 70-mer oligonucleotides. Spot identification and quantification were performed with Scanarray Express software (Perkin Elmer Life Sciences). Array data were analyzed using the R statistical language and environment (http://www.r-project.org), specifically with the microarray analysis tools available from the Bioconductor Project (http://www.bioconductor.org). Spots that showed qcom < or >0.5 were considered low-quality spots and were removed from the analysis.[23] Data were background subtracted and normalized using the LIMMA Bioconductor package.[24] Data obtained from biological replicates were averaged and then linear models were applied. Differentially expressed genes were determined using significance analysis of microarrays,[25] with a false discovery rate <10%. We used GOrilla,[26] a tool to identify enriched gene ontology (GO) terms, to obtain biological attributes characterizing the "decreased inflammatory response mediated from triclosan chitosan particles." All data are MIAME compliant and the genes described herein have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO number GPL22904.[25]

2.8 | Real-time reverse transcription-polymerase chain reaction

Total RNA (2 μg) was used as a template for RT reactions to synthesize single-stranded cDNA using MMLV-RT reverse transcriptase (Promega) and an oligo(dT) primer according to standard procedures. Gene-specific primer sets (Table S1) were designed by Primer3Plus to amplify DNA products of 70 and 150 bp. Real Time Reverse Transcription Quantitative Polymerase Chain Reaction (qPCR). reactions were performed as previously described.[22] Transcript levels of genes were normalized to the expression values of the topoisomerase gene, which was validated in our experimental conditions by NormFinder.[27] qPCR was performed in samples from at least 2 cultured cells, and differences in gene expression levels between samples were analyzed by Mann-Whitney *U*-test. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Particle size, morphology and cell viability

Using transmission electron microscopy, we observed that most of the particles had a rounded morphology (Fig. S1A). The size of nanoparticles and *Z* potential, obtained from 4 different batches, were 129 ± 47 nm and 51 ± 17 mV respectively (Figure S1B). Cell viability was evaluated by exposing serum-starved human gingival fibroblasts to IL-1β or chitosan-triclosan particles. After 24 hours, cell viability was evaluated through the MTS assay and by detecting LDH in the cell culture media. As shown in Figure S1C and D, chitosan-triclosan particles were well tolerated by gingival fibroblast

cell cultures, not demonstrating significant changes in mitochondrial metabolic status (MTS assay) nor in the release of LDH into the cell culture media.

3.2 | Effect of chitosan-triclosan particles on the transcriptional response of human gingival fibroblasts

As a first step, qPCR assays were performed to analyze the mRNA abundance of *il-6* and *cox-2*, 2 genes expected to be upregulated by inflammation in gingival fibroblasts challenged by IL-1β.[28] Figure 1 shows the qPCR results of the mRNA for these genes. Significant increases in the expression of both *il-6* and *cox-2* were observed after exposure to IL-1β. The chitosan-triclosan formulation did not alter the constitutive mRNA level of these genes. Moreover, both *il-6* and *cox-2* mRNA levels were significantly downregulated when cells were exposed to the chitosan-triclosan formulation in association with the IL-1β stimulus. The analysis of this initial experiment suggested that, in our experimental condition, chitosan-triclosan induced an anti-inflammatory transcriptional response in gingival fibroblasts challenged by IL-1β, a possibility previously proposed for these agents.[7,12,18]

To obtain a more complete transcriptional landscape of the effect of chitosan-triclosan particles on the inflammatory response initiated by IL-1β, the gene expression profile of each specific treatment was determined by microarray analysis. The data indicate that 1533 genes were differentially expressed between non-stimulated cells and IL-1β-treated fibroblasts (data not shown), corresponding to 5.6% of the genes present in the microarray. Among these genes, 777 were upregulated and 756 were downregulated in IL-1β-treated fibroblasts compared to control cells. Regarding the chitosan-triclosan treatment, 1761 genes were differentially expressed between IL-1β and IL-1β plus chitosan-triclosan-treated fibroblasts, corresponding to 6.5% of the genes present in the microarray (data not shown). Among these genes, 818 were upregulated and 943 were downregulated in IL-1β- + chitosan-triclosan-treated fibroblasts.

After identifying genes that were differentially expressed in each experimental condition, we searched those genes that were both upregulated in response to IL-1β and downregulated in chitosan-triclosan- + IL-1β-treated fibroblasts. Fifty-one genes exhibited this pattern and are indicated in the overlapping area in the Venn diagram of Figure 2A. The GO analysis indicated that this set of genes was enriched ($P < .05$) in categories related to biological process (ie, Wnt receptor signaling pathway cell adhesion, macromolecule biosynthetic process), molecular function (ie, transporter and oxidoreductase activity) and cellular component (ie, extracellular matrix, cytoskeleton) (Figure 2B). As these transcriptional changes suggested a potential anti-inflammatory role of the chitosan-triclosan formulation on IL-1β-stimulated human gingival fibroblasts, 18 genes were selected to perform validation experiments by qPCR analysis. Of these 18 genes, IL-6, fibronectin (FN1), beta actin (ACTB) and IL-1β were seen to be upregulated by IL-1β and downregulated by chitosan-triclosan particles (Table 1). Figure 3 depicts a model that describes a proposed anti-inflammatory effect of chitosan-triclosan particles in human gingival fibroblasts.

4 | DISCUSSION

The present study has analyzed the effect of particles consisting of chitosan-triclosan on the inflammatory response instigated by IL-1β

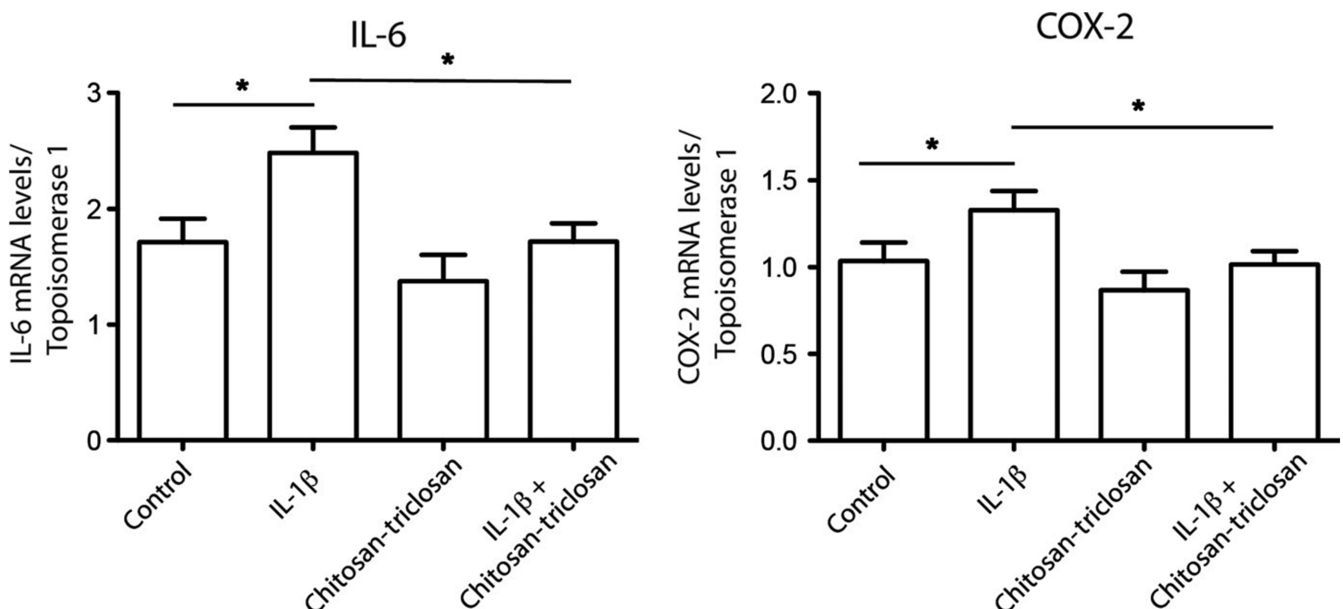

FIGURE 1 Transcript levels of IL-6 and COX-2 genes in human gingival fibroblasts exposed to IL-1β and/or a chitosan-triclosan formulation. Gingival fibroblasts were exposed, or not, to chitosan-triclosan particles and then were stimulated with IL-1β. Quantitative polymerase chain reaction analysis was performed in samples from 3 independent experiments. Graphs represent the average and standard error for mRNA levels normalized against the topoisomerase 1 gene in the corresponding sample. Asterisks indicate statistically significant differences between each indicated condition ($P < .05$). COX, cyclooxygenase; IL, interleukin

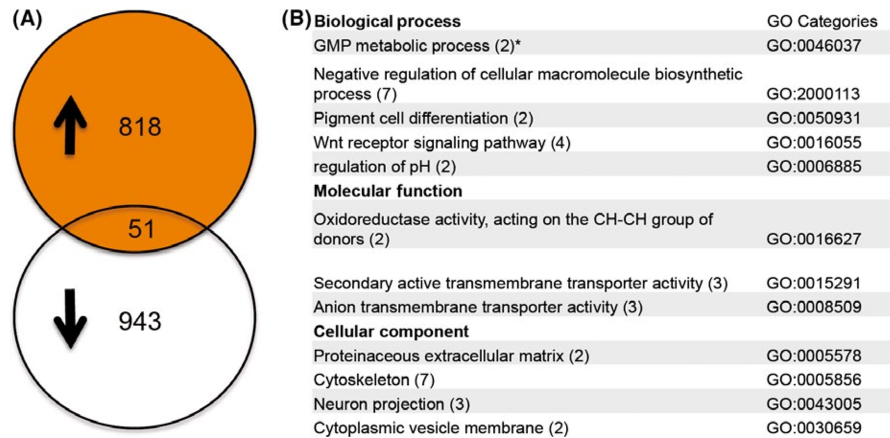

FIGURE 2 Sets of genes differentially expressed in human gingival fibroblasts exposed to IL-1β or IL-1β plus a chitosan-triclosan solution. (A) Venn diagram of common and unique genes that were upregulated (↑) or downregulated (↓) after each treatment. (B) Significantly enriched GO categories of 51 common genes that were significantly ($P < .05$) upregulated after IL-1β exposure and downregulated after chitosan-triclosan treatment. *Numbers in parentheses are numbers of genes in the GO category. GO, gene ontology; IL, interleukin

in primary cultures of human gingival fibroblasts. Particle sizes were in the nanometer range and were well tolerated by gingival fibroblasts as demonstrated by the MTS assay and the detection of LDH into the cell culture media. As assessed through real-time RT-PCR and a microarray approach, we identified that IL-1β modulates the transcriptions of several genes that were also modulated by the chitosan-triclosan

TABLE 1 Genes regulated by chitosan-triclosan particles

Gene code	Gene name	IL-1β/no stimulus	IL-1β + chitosan-triclosan/IL-1β
IL-6	Interleukin-6	10.12 ± 2.380*	−5.072 ± 0.728*
FN1	Fibronectin 1	1.817 ± 0.025*	−1.982 ± 0.038*
ACTB	Actin, beta	2.765 ± 0.064*	−4.120 ± 0.094*
IL-1β	Interleukin-1β	5.785 ± 1.095*	−1.570 ± 0.273*
IL-15RA	Interleukin 15 receptor, alpha	2.067 ± 0.438*	−1.170 ± 0.479
C1qtnf1	C1q and tumor necrosis factor related protein 1	1.453 ± 0.069*	−0.801 ± 0.122
ITGAE	Integrin, alpha E	1.174 ± 0.182*	−1.263 ± 0.140
IRAK3	IL-1 receptor-associated kinase 3	1.995 ± 0.060*	−1.042 ± 0.762
C/EPB	CCAAT/Enhancer binding protein	−1.880 ± 0.070*	0.970 ± 0.131
TNFRSF25	Tumor necrosis factor receptor superfamily member 25	−2.548 ± 0.069*	0.404 ± 0.234
ACTA2	Alpha smooth muscle actin	1.088 ± 0.118	−3.164 ± 0.116*
TLR6	Toll-like receptor 6	−0.051 ± 0.164	1.126 ± 0.094*
RELT	RELT tumor necrosis factor receptor	0.147 ± 0.302	0.675 ± 0.182
TNFRSF10C	Tumor necrosis factor receptor superfamily member 10c	−0.041 ± 0.013	0.084 ± 0.042
ILR2	Interleukin 1 receptor, type II	0.011 ± 0.140	−0.037 ± 0.140
TNFRSF14	Tumor necrosis factor receptor superfamily member 14	−0.201 ± 0.025	−0.911 ± 0.201
MYL4	Myosin, light chain 4	−0.104 ± 0.922	−0.476 ± 0.338
NFKBIL1	Nfklight polypeptide gene enhancer	−0.041 ± 0.013	0.084 ± 0.042

A group of genes was selected from the microarray data to confirm their dual modulation by IL-1β and the chitosan-triclosan particles. Table indicates gene code and name or each gene evaluated and the fold-change plus standard error for the mRNA levels for each gene under the stimulus of IL-1β and chitosan-triclosan particles as determined by quantitative reverse transcription-polymerase chain reaction.
Asterisks indicate statistically significant differences between each indicated condition ($P < .05$).

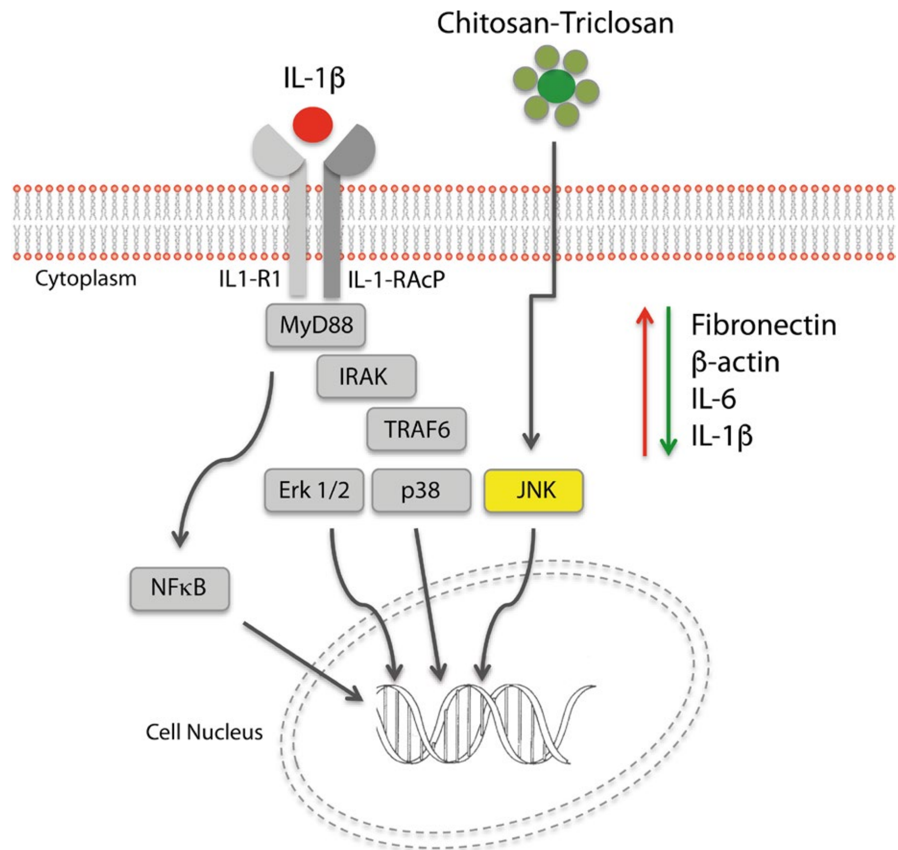

FIGURE 3 Theoretical model proposed for the modulation of chitosan-triclosan particles on IL-1β-induced inflammatory response. Our study shows that β-actin, IL-6, fibronectin and IL-1β are upregulated by IL-1β and downregulated by the chitosan-triclosan particles. Precise mechanism of action of chitosan-triclosan particles is still poorly defined. However, chitosan downregulates JNK activity in gingival fibroblasts. JNK pathway may be one of the intracellular targets modulated by chitosan-triclosan particles in gingival fibroblasts exposed to inflammatory stimuli such as IL-1β. IL, interleukin

particles. We validated a dual response, upregulation by IL-1β and downregulation by chitosan-triclosan, for the IL-1β, IL-6, β-actin and fibronectin genes. Given the significant role played by these genes in connective tissue inflammation and homeostasis, it is important to consider the potential effect of chitosan-triclosan particles in the modulation of inflammation in gingival connective tissue cells.

Periodontal disease results from the interplay between infecting bacteria and the host inflammatory response that finally causes tissue destruction.[1] Therefore, prevention of tissue damage in infected tissues should consider both the control of bacteria and the modulation of the inflammatory response. The present study has analyzed the potential effect of triclosan and chitosan particles on the inflammatory response. Both agents were able to control the growth of bacteria and modulate the inflammatory response.[12,15,18] In a recent study we described that chitosan was capable of reducing prostaglandin E_2 production at 50 μg/mL in human gingival fibroblasts.[18] In addition, the same chitosan formulation was able to inhibit the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* at 5 mg/mL.[18] Unfortunately, no studies have been performed to compare these activities in the case of triclosan using the same experimental setting. However, it is possible that a formulation incorporating both molecules may exert a potent activity of interest for the prevention or treatment of periodontal infection and inflammation. Moreover, drug delivery formulations that combine triclosan and chitosan have been designed to increase triclosan substantivity.[16] Therefore, the association between these 2 molecules may be of benefit for the prevention and/or treatment of periodontal diseases.

Through an initial analysis from the gene microarray data, we identified 51 genes that were upregulated by IL-1β and downregulated by the chitosan-triclosan particles plus IL-1β treatment. We believe these genes represent interesting targets in the modulation of the inflammatory response. From this group of genes we selected 18 to validate their dual modulation by the inflammatory stimulus and its downregulation by chitosan-triclosan particles using real-time RT-PCR. We found that IL-1β, IL-6, β-actin and fibronectin genes followed this pattern of response. It is interesting to consider that this effect of IL-1β on IL-1β transcription may represent an important mechanism that may amplify the inflammatory response in gingival fibroblasts.[29] Therefore, the ability of the chitosan-triclosan particles to downregulate this effect represents an interesting response that may collaborate in the modulation of inflammation in connective tissue cells. A synergistic role has been proposed for IL-1β and IL-6 in gingival connective tissue cells that promotes the enhancement of inflammation in gingival tissues.[30] Consequently, the effect of chitosan-triclosan particles on IL-6 transcription in IL-1β-stimulated cells suggests that these particles might modulate inflammation at an additional step. An interesting finding corresponds to the modulation of the expression of β-actin and fibronectin. It has been well demonstrated that IL-1β activates several pathways related to the cytoskeleton in human gingival fibroblasts stimulating the formation and maturation of focal adhesions.[28] IL-1β stimulates the inflammatory response by upregulating calcium release and Erk activation in a focal adhesion-dependent mechanism.[31] Moreover, it has been proposed that focal adhesions represent interesting targets for the pharmacological modulation of

inflammatory reactions in connective tissue cells.[32] Beta-actin plays a central role in the mechanical stability, contraction and cell migration in several cell types and in signaling events originating from focal adhesions in adherent cells.[33] Although β-actin has been extensively used as a housekeeping gene to validate experiments, several studies have shown that its gene expression is not stable and may be subject to transcriptional regulation under a broad array of conditions.[34-36] In addition, the modulation of the fibronectin gene is also important considering its role in the maturation of focal adhesions.[37] Fibronectin plays a prominent role in the development of fibrosis by stimulating the deposition of type I collagen fibers.[38] Our study revealed that IL-1β stimulated the transcription of the fibronectin gene and this was downregulated by the chitosan-triclosan particles. Consequently, the aforementioned particles might modulate a fibrotic response in gingival connective tissue cells.

The mechanism of action of chitosan-triclosan on the inflammatory response is far from being understood. However, at least we may consider that pathways modulated individually by chitosan and triclosan may be involved. In a previous study we identified that chitosan was able to downregulate the phosphorylation of JNK in IL-1β-stimulated gingival fibroblasts.[18] In addition, triclosan has several anti-inflammatory targets as demonstrated by different studies. In monocytes stimulated with LPS, triclosan was able to downregulate an important number of genes as identified by a gene microarray.[12] In gingival fibroblasts, triclosan was able to interrupt JNK signaling in TNF-α-stimulated cells.[7] At this moment, we are unable to identify whether a complex particle in which chitosan and triclosan are combined might change the mode of action of each individual component. Interestingly, triclosan is released from chitosan-triclosan particles as evidenced by previous studies.[16] In addition, chitosan is able to bind to mucosal surfaces, eventually providing a continuous release of triclosan in the oral environment.[16] Further studies are needed to evaluate this putative effect at the in vivo level.

IL-1β plays an important role in periodontal inflammation. Of particular interest has been the finding that gingival fibroblasts express high levels of IL-1 type 1 receptors.[39] Importantly, IL-1β reaches high levels during the progressive phase of periodontal diseases.[40] Therefore, IL-1β may trigger critical inflammatory signals in gingival fibroblasts involved in periodontal disease pathogenesis.[3] Fibroblasts probably play an important role in disease pathogenesis through their contribution to inflammation and connective tissue remodeling.[41,42] Although periodontal inflammation is a far more complex process in which several mediators are involved, this study was developed to analyze whether chitosan-triclosan particles were able to modulate inflammatory reactions in gingival connective tissue cells. Within the limitations of the present experimental design, our findings reveal that chitosan-triclosan particles are able to control the upregulation of several genes driven by IL-1β in gingival connective tissue cells.

The results of the present study contribute to identify the mechanism of action of chitosan-triclosan particles that may help to regulate the levels of inflammation in periodontal tissues. These findings are relevant for the design and future testing of chitosan-triclosan-based formulations for the prevention and treatment of periodontal diseases.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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