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Cobalt ions sensitize TRPV2 and TRPV4 channels in synovial fibroblasts, leading to cytokine secretion

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Cobalt-chromium alloys are widely used in orthopedic implants due to their excellent toughness, wear resistance, and biocompatibility. However, cobalt ions released as consequence of corrosion or wear, trigger cytokine secretion and promote inflammation and pain in periprosthetic tissues. Transient receptor potential (TRP) channels are a family of voltage-dependent Ca^{2+} permeable channels involved in various physiological and pathological processes. Because of their permeability and modulation by divalent cations, we studied how TRP channels' activity is influenced by cobalt ions. We used primary human synovial fibroblasts and through qPCR we found relevant expression of TRPC1, TRPC4, TRPV2, TRPV4, TRPM4 and TRPM7 mRNA in synovial fibroblasts. Next, we exposed synovial fibroblasts to cobalt ions and/or selective pharmacology of TRPV2 and TRPV4 channels. We observed that TRPV2 and TRPV4 are sensitized by cobalt exposure, increasing intracellular calcium in synovial fibroblasts. Furthermore, exposure to TRPV2 and TRPV4 antagonists inhibited the basal long-term intracellular calcium increase, and reduced the secretion of IL-6, IL-8, TNF-a, and VEGF-a triggered by cobalt exposure. However, the sole activation of TRPV2 and TRPV4 did not trigger secretion or expression of these cytokines. Our findings demonstrate for the first time that metal ions released from orthopedic implants, can modulate the function of TRP channels and may contribute to the pathogenesis of fibrosis and inflammation associated with biomedical implants. Notably, we propose a molecular mechanism in which TRPV2 and TRPV4 channels are potentially involved in mediating inflammatory and fibrotic responses in peri-implant tissues. However, further studies are necessary to elucidate the regulatory role of cytosolic calcium in the development of adverse local tissue reactions.

KEYWORDS

cobalt cell damage, hip arthroplasty, metabolic stress, calcium signaling, ALTRs, hip pseudotumors

1 Introduction

For 60 years, cobalt-chromium alloys have been the most common metal alloy for orthopedic implants. Their robustness and wear resistance allow outstanding mechanical performance (Szczęsny et al., 2022), while its biocompatibility provides a long-term solution for millions of patients world-wide (Whitehouse et al., 2024). However, the emergence of adverse local tissue reactions to hip implants has led to a high number of revision surgeries and pushed the search for more biocompatible options, such as zirconium and ceramic, whose use has dramatically increased in recent years (National Joint Registry, 2017). Although their use in hip implants has been reduced, cobalt-chromium remain to be the most used alloy in orthopedic implants for knee, elbows, and ankle replacements, and are highly used in coronary and ureteral stents, as well as dental prosthetics. As such, understanding the mechanisms of cell and tissue damage observed in adverse local tissue reactions is critical to elucidate the processes that underlie inflammation and fibrosis generated by cobalt-chromium alloys, to improve treatment strategies and the development of new biomedical materials.

Despite titanium alloys present the highest biocompatibility, bone integration, and acceptable mechanical properties for use in orthopedic implants (Ad et al., 2024), they do not perform well under wear or sliding contact as is the case of hip or knee articulations (Marin and Lanzutti, 2023). Hence, cobalt–chromium alloys are the predominant choice for articulating surfaces. Their high chromium content promotes the formation of a passive oxide layer, which confers excellent corrosion resistance (Mani et al., 2024); however, this passive film can be disrupted by mechanical wear at modular junctions such as taper connections (Jacobs et al., 2014; Eltit et al., 2019). Surface damage initiates fretting–corrosion cycles, characterized by alternating phases of mechanical wear and passive film reformation, ultimately leading to progressive metal ion release. In the femoral head–neck articulation, this crevice corrosion contributes to elevated release of metal ions and particles, which can subsequently be detected in patients' serum and peri-implant tissues (Vendittoli et al., 2011; Hart et al., 2012).

Adverse local tissue reactions to cobalt chromium alloys are clinically characterized by swelling and pain that is only resolved by the removal of the metal implants (Pandit et al., 2008; Murray et al., 2011). While the etiological factor is the release of cobalt in form of particles or soluble ions (Co^{2+}) (Eltit et al., 2019; Wang et al., 2022; Urish et al., 2019; Wang et al., 2020; Samelko et al., 2016), their pathogenesis is still not fully understood. A hypoxia-mimicking effect of Co^{2+} and increased oxidative stress have been widely described in a variety of cell models (Salloum et al., 2021; Caicedo et al., 2010; Salloum et al., 2018), triggering cytokine secretion and cell death (VanOs et al., 2014; Hallab and Jacobs, 2017; Kanaji et al., 2014; Liu et al., 2022). Studies in human synovial fibroblasts have shown that Co^{2+} triggers mitophagy and a cytokine storm, which in chronic doses generate cell senescence and cell death (Grant et al., 2024; Eltit et al., 2021a). These cellular events induce physiological events like pain, swelling, chronic inflammation, fibrosis, and necrosis (Ricciardi et al., 2016; Eltit et al., 2021b; Eltit et al., 2017). Open questions as how Co^{2+} enter the cells and the molecular mechanisms that trigger the cytokine storm are currently unresolved.

Transient receptor potential (TRP) are voltage-dependent cation channels located in the cell membrane, that sense a variety of cellular and environmental signals (Vriens et al., 2004a; Zhang et al., 2023). Mammals express 28 different TRP channels that are classified into 7 subfamilies according to their aminoacidic sequence (TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV). TRP channels respond to different physical or chemical stimuli such as heat, cold, stress, tension, hormones, oxidative stress, pH, chemicals, or divalent cations (mostly $Zn^{2+}$and Mg^{2+}) (Jimenez et al., 2020; Persoons et al., 2021; Held et al., 2021). The activation of TRP channels cause the influx of extracellular cations like Mg^{2+} and Ca^{2+}, which can trigger a variety of intracellular events such as chemo-electrical transmission, gene expression, protein phosphorylation, protein secretion, among many others (Held et al., 2015; Held et al., 2016). These events result in a myriad of physiological and pathological responses (Jimenez et al., 2020; Gatica et al., 2019; Kecskes et al., 2023; Held and Tóth, 2021; Van Hoeymissen et al., 2020). In synovial tissues a variety of TRP channels have been identified, which multiple functions presumably include proprioception in the joints (Matta et al., 2023; Kochukov et al., 2006).

Here we hypothesize that TRP channels in synovial fibroblasts are sensitive to Co^{2+} and could trigger inflammatory changes observed in adverse local tissue reactions to metal implants. To perform this study, primary human synovial fibroblasts were stimulated by Co^{2+} in microfluorimetric experiments. Our results show for first time that TRP channel activity could be modulated by metals used in implant alloys. Consequently, this channel modulation results in increased secretion of inflammatory cytokines suggesting that this could be a pathway that causes inflammation and fibrosis in periprosthetic tissues. Because of pharmacological blockers of TRP channels, our study shows possible therapeutic alternatives for preventing fibrosis and inflammation triggered by metal implants.

2 Materials and methods

2.1 Patients and cells

All procedures were performed under the ethics approval from the University of British Columbia (H14-03050). Synovial tissue samples from patients undergoing primary hip replacement surgery due to osteoarthritis were obtained (demographics of patients in supplementary table SI). The specimens were preserved in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies; Burlington, ON) with 1% penicillin/streptomycin on ice for no more than 1 h. The specimens were mechanically disrupted into ~1 mm pieces, digested with 0.1% collagenase I (Life Technologies; Burlington, ON) in DMEM with 2% penicillin/streptomycin (Gibco) for 90 min at 37 °C under agitation. The digested tissue was filtered using 40 μm grids (Falcon, Corning Inc.; NY), and the obtained suspension was seeded in 10 cm petri dishes. Adherent cells were washed after 12 h and cultured with DMEM 5% glucose, 10% FBS (Life Technologies; Burlington, ON), and 2% penicillin/streptomycin. The purity of fibroblasts in culture was confirmed by immunofluorescent stain of the alpha smooth muscle actin (αSMA). At passage 3, the cells were seeded to perform the experiments described below.

2.2 qPCR for TRP channels in synovial fibroblasts

RNA expression of TRP channels in human primary synovial cells was investigated by qPCR. RNeasy Mini kit (Qiagen, Netherlands) was used for RNA extraction following the manufacturers' instructions. RNA concentration and quality were determined via the Nanodrop method (Isogen Life Science, Belgium) and Experion RNA Analysis kit (Bio-Rad, Belgium). cDNA was generated from 1 µg of RNA using the First-Strand cDNA Synthesis Kit (GE Healthcare, Belgium). RT-qPCR was performed on triplicate cDNA samples using specific TaqMan gene expression assays (supplementary Table SII) (Life Technologies, Belgium) in the StepOne PCR system (Applied Biosystems, Belgium).

2.3 Microfluorimetric imaging

We activated and inhibited TRPV2 and TRPV4 using the agonists and antagonists listed in Supplementary Table SIII. Briefly, as agonist for TRPV2 we used Tetrahydrocannabinol (THC) (De Petrocellis et al., 2011) or Cannabidiol (CBD) (De et al., 2012), and for TRPV4 we used the compound GSK1016790A (De Clercq et al., 2015); as antagonists for TRPV2 we used Loratadine (Van den Eynde et al., 2022), and for TRPV4 we used the compound GSK2193874 (Arredondo Zamarripa et al., 2017). Exposure times to cobalt chloride ($CoCl_2$), agonists, and/or antagonists for each condition are provided in the Results section.

To evaluate the activation of TRP channels, we measured total intracellular calcium ions (Ca^{2+}) by using FURA-2 a.m. reaction and high-speed camera protocol as previously described (De Clercq et al., 2017). Briefly, the fibroblasts were incubated with 2 µM Fura-2 acetoxymethyl ester for 30 min at 37 °C. After alternating illumination at 340 and 380 nm using a Lambda XL illuminator (Sutter instruments, Novato, United States), the fluorescent signal was recorded using an Orca Flash 4.0 camera (Hamamatsu Photonics Belgium, Mont-Saint-Guibert, Belgium) on a Nikon Eclipse Ti fluorescence microscope (Nikon Benelux, Brussels, Belgium). We calculated the absolute calcium ions concentrations from the (F340/F380) ratio after correction for the individual background, using the Grynkiewicz equation (Grynkiewicz et al., 1985) The KD of Fura-2 and the isocoefficient α were assumed as described previously (Vriens et al., 2011).

Ionomycin (2 µM, Sigma) was applied at the end of every experiment as positive control. All the experiments were performed in technical triplicates and if not indicated differently, in cells obtained from minimally 3 different patients (biological triplicates). The standard imaging solution (Bath solution) contained in mM: 150 NaCl, 10 HEPES, 2 $CaCl_2$, 1 $MgCl_2$ and 10 Glucose (pH 7.4 with NaOH). For recordings in 0 mM calcium, the $CaCl_2$ was omitted from the solution.

2.4 Cytokine secretion

Culture media from fibroblasts was collected following 24 h of exposure to Co^{2+} and/or agonist and antagonists of TRPV2 and TRPV4 channels. Cytokine secretion was quantified as previously described (Eltit et al., 2021b). Briefly, after centrifugation, 100 µL aliquots of synovial fluid supernatant were separated and stored at −20 C until analyzed. The concentration of 48 cytokines, chemokines and growth factors associated with inflammation, necrosis, or fibrosis, were quantified, using the Bio-Plex Suspension Array System (48 plex-Eve Technologies, Calgary, AB). The obtained values of cytokine concentration were not normally distributed; thus, we perform Log(10) transformation of the values and confirmed the normal by Shapiro-Wilk test. Then a one-way ANOVA with a Tukey post-hoc and Benjamini-Hochberg correction was used to determine the factors differentially detected between groups.

3 Results

3.1 Cobalt increases the intracellular calcium concentration in synovial fibroblasts

To test our hypothesis that cobalt-induced inflammation is related to the activity of calcium permeable ion channels in synovial fibroblasts, we investigated the direct effect of Co^{2+} stimulation on the cytosolic calcium levels of synovial fibroblasts. Primary hip synovial fibroblasts of 3 independent patients were exposed to 0.1 mM $CoCl_2$ during microfluorimetric calcium measurements (Figure 1). Stimulation by 0.1 mM $CoCl_2$ produced no effect on the cytosolic calcium levels in a calcium free extracellular medium. In contrast, in the presence of 2 mM calcium in the extracellular medium, application of 0.1 mM $CoCl_2$ induced a robust and transient increase in intracellular calcium concentration (Figure 1A). These results demonstrate that Co^{2+} triggers calcium intake, and that the source of that calcium is extracellular.

3.2 TRP channels are expressed in synovial fibroblasts

The previous results demonstrate the presence of a calcium flux into synovial fibroblasts from the extracellular environment. Therefore, we investigated the RNA expression levels of TRP channels in human synovial fibroblasts by qRT-PCR. RNA-expression levels of TRPC1, TRPC4, TRPV2, TRPV4, TRPM4, and TRPM7 were detected in synovial fibroblast in standard culture conditions (Figure 2). Interestingly, the expression of TRPC4, TRPV2, TRPV4 and TRPM4 channels significantly decreased in synovial cells exposed to different doses of Co^{2+} (0.1 and 0.5 mM $CoCl_2$) while TRPA1 shows an increase in expression at 0.1 mM of Co^{2+}.

3.3 Cobalt sensitizes TRPV2 and TRPV4 in synovial fibroblasts

Following the change in RNA expression of TRP channels induced by Co^{2+} exposure and the existence of selective

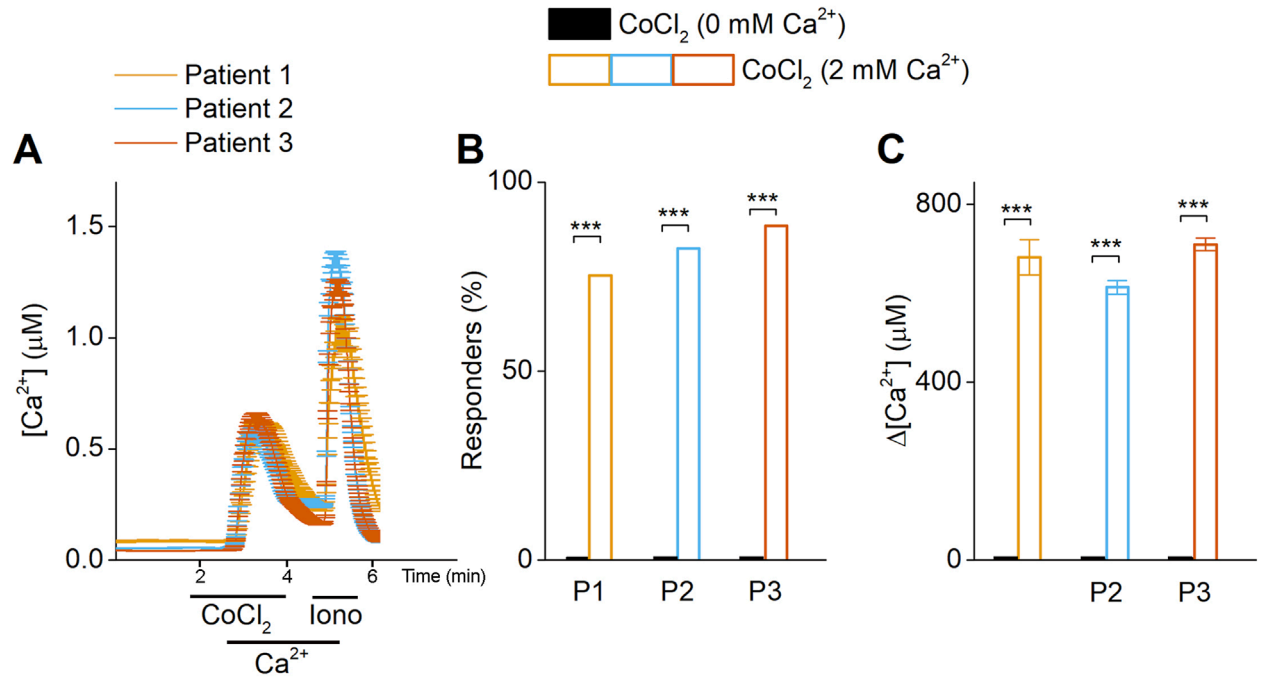

FIGURE 1
Co^{2+} triggers calcium flux from the extracellular compartment. **(A)** Time course of Ca^{2+} imaging measurements in synovial fibroblasts. At basal conditions and after the exposure of cobalt ($CoCl_2$, 0.1 mM) in calcium-free media, intracellular calcium is not increased. The introduction of calcium (Ca^{2+}) triggers the intracellular calcium increase, demonstrating that calcium influx is due to extracellular influx. **(B)** Percentage of responder cells, obtained from 3 different patients. **(C)** Intracellular calcium peak after cobalt exposure.

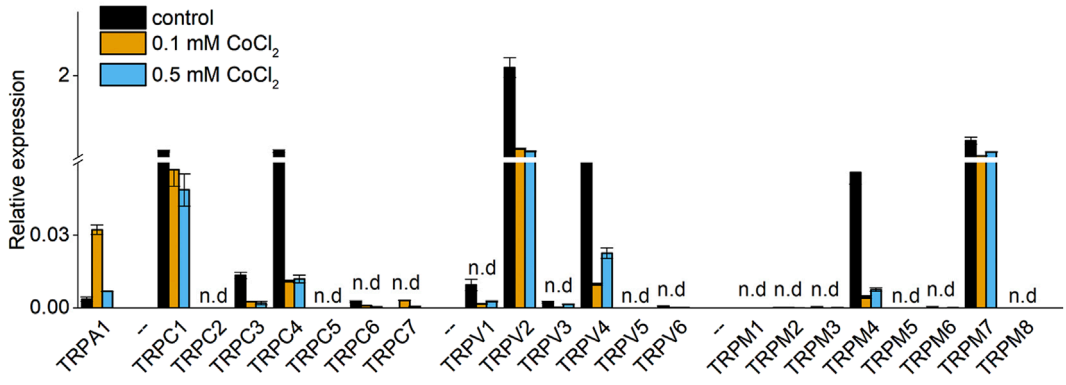

FIGURE 2
Relative expression of TRP channels in synovial fibroblasts in absence (Control), 0.1 mM or 0.5 mM $CoCl_2$. Bars represent mean + - Std. deviation observed in 3 different patients.

pharmacology, we investigated the functional expression of TRPV2 and TRPV4 in synovial fibroblasts.

Stimulation with the TRPV2 selective agonist THC (50 μM) (De Petrocellis et al., 2011) induced an influx in fluorescence in synovial fibroblasts. Interestingly, 24 h preincubation with $CoCl_2$ at 0.1 and 0.5 mM induced an increase in basal calcium concentration compared to the non-exposed controls (Figures 3A,B). Although, preincubation by $CoCl_2$ does not significantly increase the number of responding cells (Figure 3C), it produces a significant increase in the fluorescence amplitude after THC-stimulation compared to non-treated cells (Figures 3A,D). These findings demonstrate that Co^{2+} modulates TRPV2 channel activity, leading to increased intracellular calcium concentrations both at baseline and following specific TRPV2 stimulation.

Similar results were obtained after stimulation by the TRPV4 agonist (GSK1016790A) (Thorneloe et al., 2008) (Figure 4). Preincubation with 0.1 and 0.5 mM $CoCl_2$ significantly increased the amplitude of calcium influx (Figures 4A,D). The number of GSK1016790-responding cells was increased when exposed to 0.1 mM of $CoCl_2$, compared to control (Figure 4C). These results

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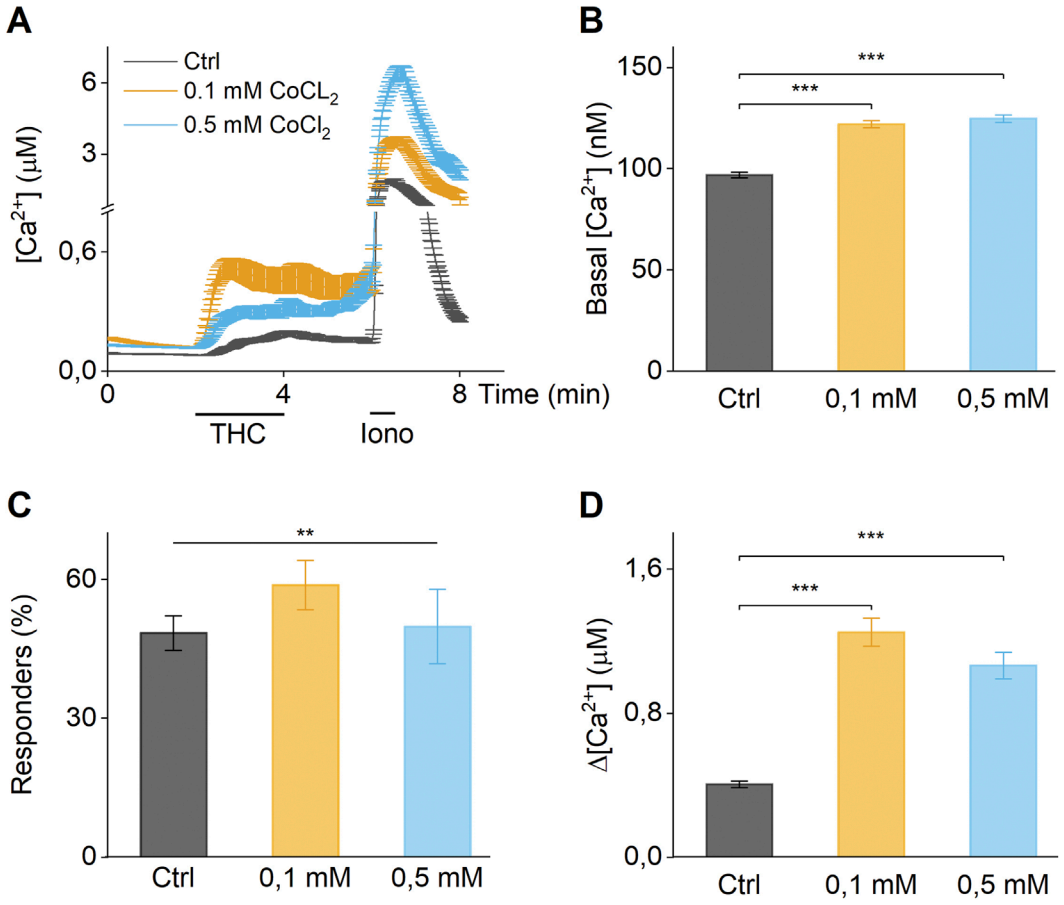

FIGURE 3
Co^{2+} increase activity of TRPV2. **(A)** Time course of calcium imaging measurements in synovial fibroblasts in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. The application of the selective TRPV2 agonist THC shows increased response in cells exposed to cobalt. **(B)** Basal intracellular calcium concentration of cells in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. **(C)** Percentage of responder cells to THC stimuli in absence (Ctrl), 0.1 mM or 0.5 mM cobalt chloride. **(D)** Differential calcium concentration of peak after THC stimulation v/s basal concentration in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. **(A)** Data of cells from a single patient, **(B–D)** Biological triplicates.

demonstrates that TRPV4 channel activity is modulated by the presence of extracellular Co^{2+}, resulting in elevated basal calcium content and increased calcium amplitudes upon stimulation by a selective agonist.

3.4 Pharmacological block of TRPV2 can alleviate effects of long-term $CoCl_2$ exposure

Next, we wanted to test whether pharmacological inhibition of Co^{2+} sensitive TRP channels TRPV2 and TRPV4 could alleviate Co^{2+}-induced increases in intracellular calcium. Therefore, we focused on TRPV2, which exhibited a more pronounced channel sensitization as shown in our experiments above. TRPV2 was reported to be blocked by the antihistaminic drug loratadine (Van den Eynde et al., 2022). In fact, 10 µM loratadine significantly reduced the amount of responsive synovial fibroblast cells (Figures 5A,B) as well as the amplitude in intracellular calcium increases (Figures 5A,C) upon THC application. Notably, the same concentration of loratadine reduced the Co^{2+}-induced increases in basal calcium concentrations of synovial fibroblasts to a level close to the basal calcium concentrations of non-treated fibroblast cells, while direct long-term loratadine incubation did not affect basal calcium levels (Figures 5D,E). Interestingly, this effect could not be explained by a block of the transient increases of intracellular calcium induced by the application of Co^{2+} (Figures 5F,G). This lack of blocking capability of the cobalt-induced transient calcium influx was also observed after TRPV4 blocking (Supplementary Figure S1). These results show that TRPV2 has a potential role in the increased intracellular calcium concentration induced by Co^{2+}. However, other mechanisms (like ion channels/receptors) will also be involved in the transient calcium increase induced by cobalt exposure.

3.5 Inhibition of TRPV2 and TRPV4 activity reduces secretion of inflammatory cytokines after cobalt exposure in synovial fibroblasts

To investigate the potential involvement of TRPV2 and TRPV4 in the secretion of cytokines induced by cobalt incubation, cytokine

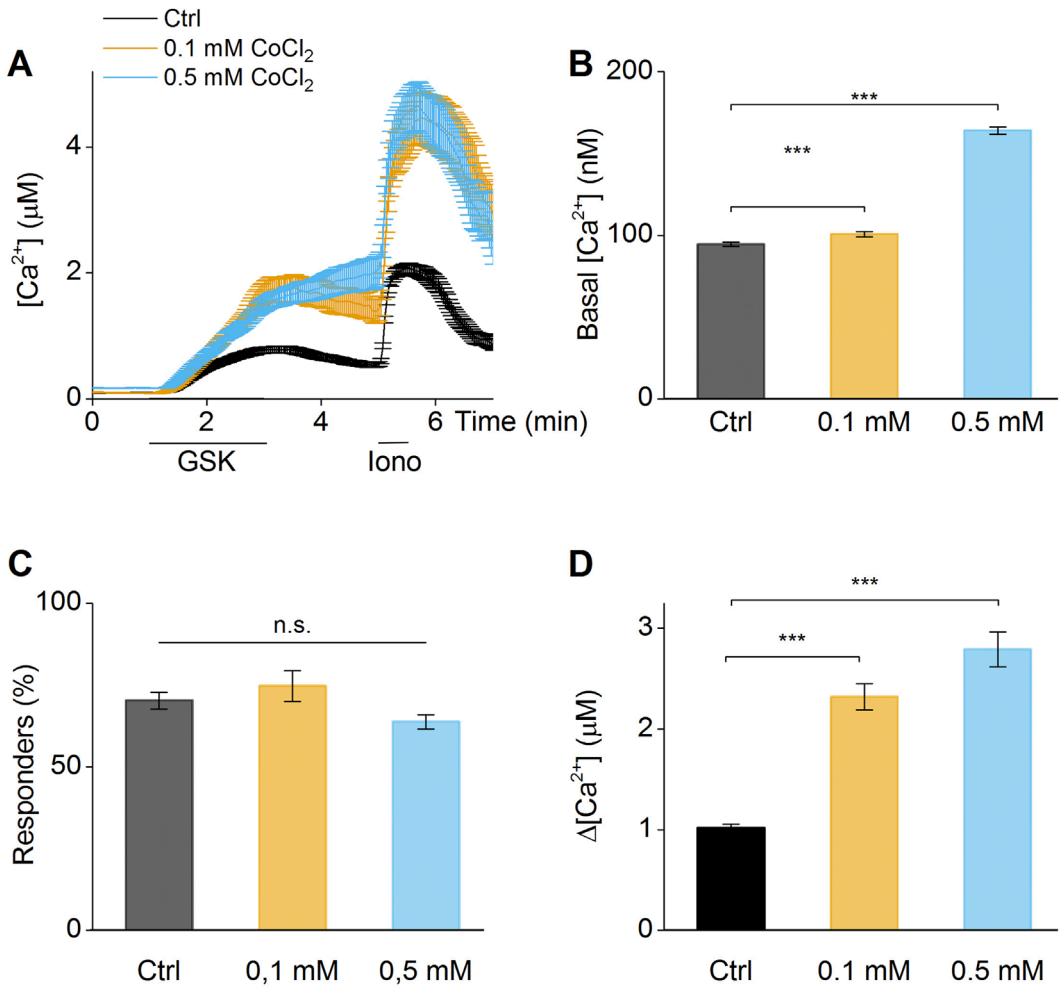

FIGURE 4
Co^{2+}increase activity of TRPV4. **(A)** Time course of calcium imaging measurements in synovial fibroblasts in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. The application of the selective TRPV4 agonist (GSK1016790A) shows increased response in cells exposed to cobalt. **(B)** Basal intracellular calcium concentration of cells in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. **(C)** Percentage of responder cells to GSK1016790A stimuli in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. **(D)** Differential calcium concentration of peak after GSK1016790A stimulation v/s basal concentration in absence (Ctrl), 0.1 mM or 0.5 mM Co^{2+}. **(A)** Data of cells from a single patient, **(B–D)** Biological triplicates.

release experiments were performed in the presence of selective pharmacology of TRPV2 (loratadine) (Van den Eynde et al., 2022) and TRPV4 (GSK2193874) (Arredondo Zamarripa et al., 2017) (Figure 6). In the presence of the TRPV2 antagonist loratadine, the IL-6 secretion was reduced by 50%, the secretion of IL-8 and VEGF by 30% while the secretion of TNF-α was not affected (Figure 6). In addition, incubation with the TRPV4 antagonist, GSK2193874, resulted in the significant reduction of IL-6 secretion (~25%) while no significant reduction was observed of other cytokines (Figure 6). The combined effect of TRPV2 and TRPV4 antagonists significantly reduce the secretion of IL-6, IL-8, TNF-α and VEGF-α, in rates between 30%–50% (Figure 5). In conclusion, these results demonstrated that the cobalt-induced modulation of both TRPV2 and TRPV4 channels resulted in an altered secretion of cytokines that potentially could trigger inflammation in periprosthetic human tissues.

3.6 TRPV2 and TRPV4 activation do not trigger cytokine expression or secretion

We next evaluated whether activation of TRPV2 and TRPV4 channels alone could trigger cytokine secretion. We stimulated synovial fibroblasts with the specific agonist for TRPV2 (CBD) (De et al., 2012) and TRPV4 (GSK1016790A) (De Clercq et al., 2015), either alone or in combination with $CoCl_2$. We did not observe significant changes in cytokine secretion following stimulation with either agonist, in both cobalt-exposed and unexposed cells (Supplementary Figure S2). We also found no changes in cytokine gene expression under the same conditions (Supplementary Figure S3). These results are not due to reduced cell viability, as we confirmed sustained viability after long-term exposure to various concentrations of $CoCl_2$ (Supplementary Figure S4). Together, these findings suggest that TRPV2 and TRPV4 activity contributes to cobalt-induced

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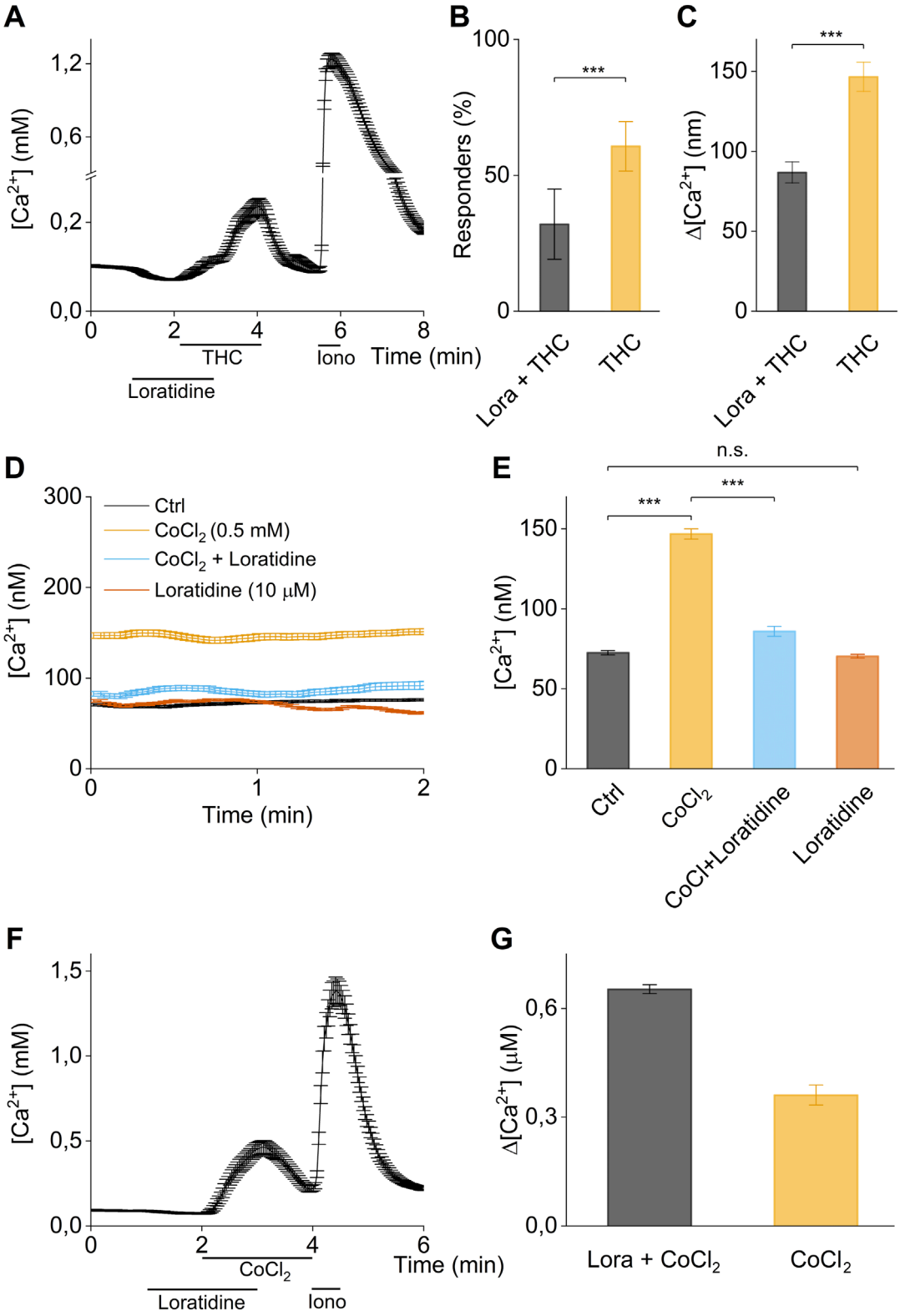

FIGURE 5
TRPV2 inhibition decreases basal calcium intracellular concentration but is not capable of reducing the cobalt-induced transient calcium increase. **(A)** Time course of calcium imaging measurements in synovial fibroblasts from a single patient. The application of the selective TRPV2 antagonist (Loratadine) shows decreased response in cells exposed to the selective agonist THC. **(B)** Responder cells to THC in presence or absence of Loratadine. **(C)** Amplitude of response to THC in presence or absence of Loratadine. **(D)** Basal calcium concentration after long term exposure to cobalt chloride in presence or absence of loratadine. **(E)** Differences in basal calcium concentration after long term exposure. **(F)** Time course of calcium imaging measurements in synovial fibroblasts from a single patient. The application of the selective TRPV2 antagonist (Loratadine) cannot block the transient calcium increase triggered by cobalt chloride. **(G)** Differential calcium concentration of peak after Loratadine + cobalt chloride and after removal of loratadine. Charts correspond to biological triplicates.

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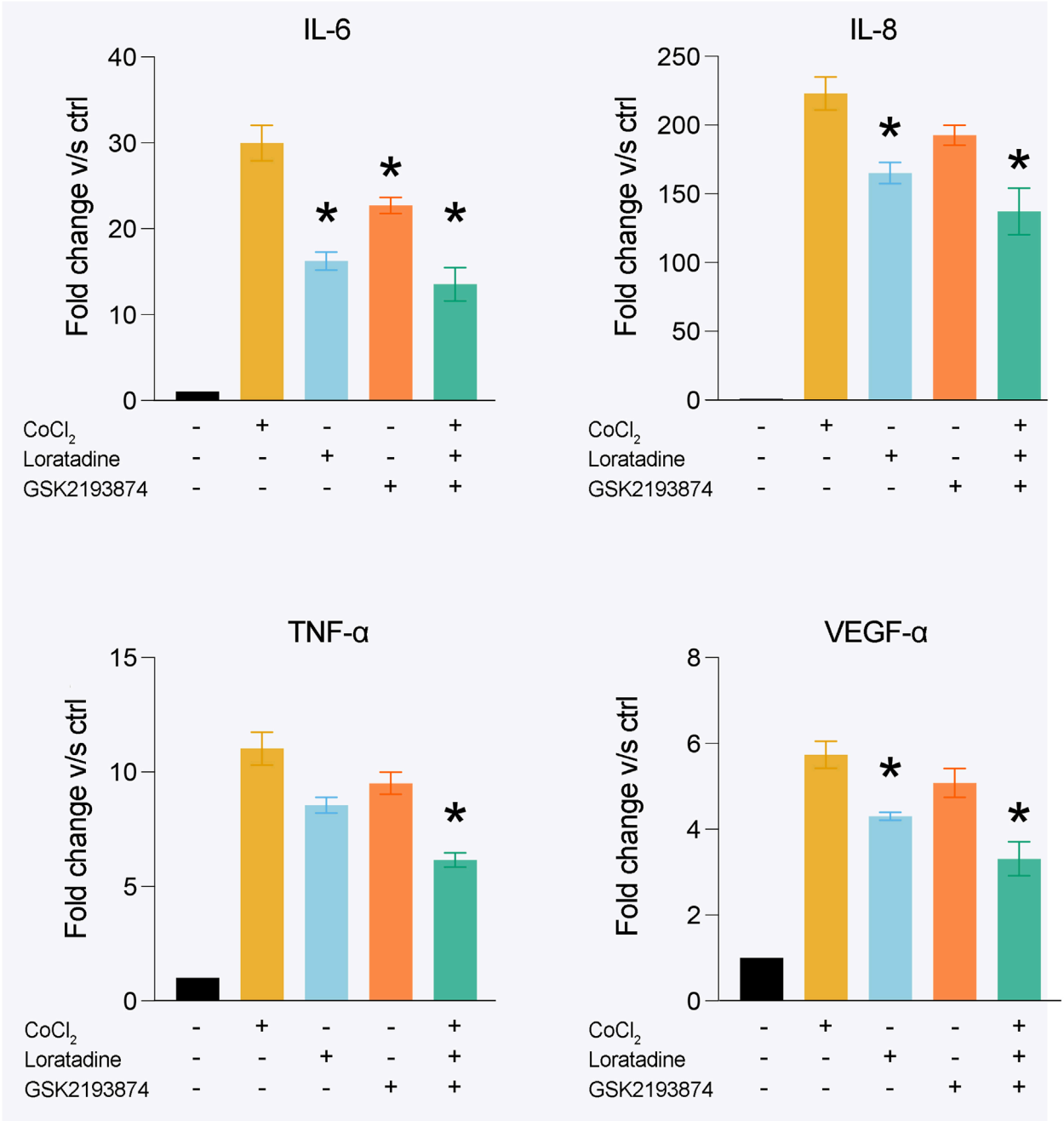

FIGURE 6
Inhibition of TRPV2 and TRPV4 activity decrease cytokine secretion after cobalt exposure. After 24 h of exposure to 0.1 mM of cobalt, the secretion of IL-6, IL-8, TNF- α and VEGF- α is increased. By using the specific TRPV2 antagonist Loratadine, there is decrease in the secretion of IL-6, IL-8 and VEGF-α, while the exposure to the specific TRPV4 antagonist GSK2193874 produce a decreased secretion of IL-6. Combining both antagonists showed an additive effect, reducing the secretion of IL-6, IL-8, TNF-α and VEGF-α. The plots represent the mean +SEM of fibroblasts obtained from 6 patients.

cytokine secretion, but this effect does not result from their canonical calcium channel activity alone.

4 Discussion

Cobalt alloys remain one of the most common materials used in medical implants. Its' mechanical robustness, and low corrosion rates allows long-term solutions with minimal deleterious effects. The arise of adverse local tissue reactions to cobalt-based implants, due to the release of Co^{2+} as consequence of wear and corrosion, generated concerns about possible mechanisms of cell damage induced by Co^{2+} that may affect peri-implant tissues. In this study, we demonstrate for the first time how Co^{2+} ions modulate the activity of calcium permeable ion channels in the plasma membrane of human cells. We demonstrate that Co^{2+} enhances the activity

of TRPV2 and TRPV4 channels in human synovial fibroblasts, resulting in increased intracellular calcium influx, which results in increased secretion of inflammatory cytokines.

Cobalt is the most abundant element in alloys for biomedical implants, that also contain 28% of chromium, 8% molybdenum and 1%–2% of trace elements. Its biocompatibility is due mostly to a dense and stable passive layer of ~4 nm thickness composed of chromium oxide. This superficial passive layer, prevent corrosion of the alloys (Wang et al., 2019). For those reasons cobalt-chromium alloys are considered bioinert. However, the mechanical disruption of this passive layer exposes other elements, such as cobalt and molybdenum that are subject to corrosion (Wang et al., 2022). Cobalt, chromium and molybdenum are then solubilized, until a new passive layer is formed by oxidation of chromium atoms (Wang et al., 2020). The ions released by the corrosion process follow different paths. While Cr^{3+} forms stable oxides and phosphates which precipitate in the extracellular environment (Hart et al., 2010; Di Laura et al., 2017) Co^{2+} remains in solution, contacting cells and affecting their metabolism and function (Hallab and Jacobs, 2009; Hallab et al., 2005). In this study, we demonstrate how Co^{2+} affect the channel activity of TRPV2 and TRPV4 channels resulting in an increased intracellular calcium content, affecting the secretion of cytokines.

TRPV2 and TRPV4 belong to the vanilloid subfamily of TRP channels and are involved in sensing osmolarity and mechanical stimuli (Doñate-Macián et al., 2019; Sato et al., 2013; Perálvarez-Marín et al., 2013). Since calcium signaling is critical for the response and adaptation of tendons and ligaments to mechanical forces (Wall et al., 2016), their presence in hip synovial fibroblasts might be related to these functions. TRPV2 is activated by mechanical stretching, and by chemicals such as cannabinoids (Vriens et al., 2009). TRPV4 is activated by osmotic changes (increasing its activity under hypotonic conditions), moderate temperatures (24–38 °C), shear stress, flavonoids, and organic chemical crystals (Vriens et al., 2004b; Lan et al., 2021; Garcia-Elias et al., 2014). To the best our knowledge, this is the first study demonstrating that Co^{2+} modulates TRPV2 and TRPV4 channel activity and has an agonist function over these channels (and acts as an agonist for these channels).

We observed that Co^{2+} induces a transient calcium increase in synovial fibroblasts. This transient effect could be due to osmotic change, electrochemical changes, or other non-specific effects (Garcia-Elias et al., 2014). The fact that specific antagonists of TRPV2 and TRPV4 could not inhibit the transient response to Co^{2+}, reinforces the idea of non-specific effect of Co^{2+} in the transient calcium increase. Furthermore, this transient effect could be related to other TRP channels or intracellular store depletion. However, the fact that long-term exposure to Co^{2+} triggers an increased response to specific TRPV2 and TRPV4 agonists (observed as increased calcium intake) reveals that Co^{2+} specifically sensitizes the channel activity of TRPV2 and TRPV4. Furthermore, the long-term effect of the TRPV2 antagonist loratadine, in reducing the basal level of calcium in cobalt-exposed cells, suggests that this sensitization of TRPV2 and TRPV4 has a cellular effect that could be observed over time. Twenty-four-hour inhibition of TRPV2 and TRPV4 reduced cobalt-induced cytokine secretion, supporting a long-term effect of these channels, and potentially explaining the prolonged effects of low-concentration Co^{2+} observed clinically.

Activation of TRPV2 and TRPV4 induced a robust a calcium influx that could trigger a myriad of intracellular signaling events. Previous studies demonstrated that Co^{2+} exposure activates the transcription factors Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Hypoxia-inducible factor 1 alpha (HIF-1α) (Eltit et al., 2021a; Samelko et al., 2013; Nyga et al., 2015). NF-kB is the transcription factor for the expression of cytokines such as TNF-α, IL-1β, IL-6, and IL-8; while HIF-1α is directly related with the expression of VEGF-α. In the present study, we describe that TRPV2 and TRPV4 are sensitized by Co^{2+}, enhancing the calcium signaling after. The inhibition of TRPV2 and TRPV4 reduces the secretion of IL-6, IL-8 and VEGF-α. However, the fact that cytokine secretion does not occur after TRPV2 or TRPV4 activation suggests that the activity of both channels is not directly related to NF-κB and HIF-1α activation, and that a mechanism independent of the canonical activity of TRPV2 and TRPV4 is involved.

The secretion of cytokines by synovial fibroblasts after Co^{2+} exposure has demonstrated the activation of endothelial cells and the migration of monocytic cells (Eltit et al., 2021a), which can be associated with the inflammation, necrosis, and fibrosis surrounding failed orthopedic implants (Murray et al., 2011; Ricciardi et al., 2016; Kurmis et al., 2019; Grammatopoulos et al., 2013). The long-term effects of cobalt ions on synovial fibroblasts were elegantly demonstrated by Xu et al. (2020) (Xu et al., 2020). In their study, synovial fibroblasts extracted from patients with failed metal-on-metal implants exhibited increased collagen secretion, resistance to apoptosis, and enhanced pro-inflammatory cytokine release. Remarkably, these alterations persisted after cell extraction and culture in cobalt-free medium, suggesting that long-term cobalt exposure may induce stable, imprinted changes in synovial fibroblasts. This imprinted inflammation phenotype has been previously demonstrated in models of dermatitis in human dermal fibroblasts (Sharma et al., 2018). Moreover, due to the availability of inhibitors such as loratadine (TRPV2) and GSK2193874 (TRPV4), adverse reactions to metal implants could potentially be treated pharmacologically. In addition, material-based strategies are also critical for mitigating Co^{2+} release. Alloy optimization can enhance passive film stability and reduce dissolution (Metikoš-Huković et al., 2006), while surface engineering techniques, such as ceramic or diamond-like carbon coatings, have been attempted to minimize wear and corrosion at modular junctions (Roy et al., 2024; Fernandez-Fairen, 2022). Advances in manufacturing, like additive processing and nanostructured alloys, may further improve resistance to fretting-corrosion (Zhang et al., 2024). These material-based solutions may reduce cobalt exposure which activates the mentioned biological responses.

As main limitation of our work, we restricted our analysis to TRPV2 and TRPV4, while other TRP channels are expressed in synovial fibroblasts. Important expression is achieved by TRPC1, TRPC4 TRPM4and TRPM7 and they can also be sensitized by cobalt chloride. Among those, TRPM4 and TRPC4 dramatically drop their expression in presence of cobalt, and TRPM7 and TRPC1 are among the most expressed in this cell type. Importantly, there exists other calcium channels like Piezo1, which responds to mechanical stimuli (Hennes et al., 2019), and has been described in synovial cells (He et al., 2024). Furthermore, Piezo1 has been shown to signal through NFκB (Yu et al., 2025), triggering inflammation and pain in osteoarthritis (Li et al., 2024). Weather mechanosensitive

channels like Piezo1 are sensitized by Co stimulation, is matter of investigation.

Another limitation is that we conducted our experiments in concentrations of Co^{2+} that are higher than what is has been described in peri-implant fluids or tissues (Langton et al., 2019; Lehtovirta et al., 2018), and the time of exposure of the cells is limited to days compared to the years of exposure of peri-implant tissues. As well, our results correspond to *in-vitro* observations, and patients or animal models are needed to understand the effect of the cobalt-mediated activation of TRPV2 and TRPV4 in the complex peri-implant tissue environment. To test the role of TRPV2 and TRPV4 in mediating cell permeability to implant-derived cations, further work is needed, including patch clamp experiments, or specific TRP channel knock out cell lines among other techniques. Finally, while we performed experiments with pharmacology of TRPV2 and TRPV4, showed high expression in synovial fibroblasts and could potentially also contribute to inflammation and pain in periprosthetic tissues. However, other TRP channels, plasma membrane channels, receptors and intracellular receptors could also be involved in the altered cytosolic calcium levels associated to cobalt incubation. Finally, TRPV2 and TRPV4, which we studied using pharmacological experiments, are highly expressed in synovial fibroblasts and could potentially contribute to inflammation and pain in periprosthetic tissues. However, other TRP channels, plasma membrane channels, receptors, and intracellular receptors could also be involved in the altered cytosolic calcium levels associated with cobalt incubation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of British Columbia (H14-03050). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KH: Conceptualization, Data curation, Writing – review and editing, Methodology, Investigation, Writing – original draft, Resources, Software, Formal Analysis. SK: Formal Analysis, Data curation, Writing – original draft, Writing – review and editing, Investigation. JN: Writing – original draft, Formal Analysis, Investigation, Writing – review and editing, Data curation. RM: Formal Analysis, Resources, Writing – original draft, Methodology, Conceptualization, Writing – review and editing. FS: Methodology, Writing – original draft, Writing – review and editing, Conceptualization. MC: Project administration, Writing – original draft, Conceptualization, Writing – review and editing, Funding acquisition, Supervision, Resources. JV: Conceptualization, Supervision, Writing – original draft, Resources, Writing – review and editing, Funding acquisition, Project administration, Formal Analysis, Methodology. FE: Methodology, Supervision, Writing – original draft, Investigation, Formal Analysis, Software, Data curation, Resources, Funding acquisition, Validation, Conceptualization, Project administration, Writing – review and editing, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmats.2025.1653364/full#supplementary-material

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