Investigation of foreign materials in gingival lesions: a clinicopathologic, energy-dispersive microanalysis of the lesions and in vitro confirmation of pro-inflammatory effects of the foreign materials

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Objectives. This study aimed to evaluate the clinical and histopathologic features of gingival lesions containing foreign material (GLFMs). In parallel, the composition of the foreign material and its effects in primary human gingival fibroblasts (HGFs) were investigated.

Study Design. Eighty-six GLFMs were retrieved from an oral pathology biopsy service. Clinical and microscopic data were analyzed, and the composition of the particles was identified by using energy-dispersive X-ray spectroscopy (EDX). Furthermore, HGFs were stimulated with silica (SiO_2) microparticles to investigate the production of collagen type 1 (COL-1), matrix metalloproteinase 2 (MMP2), and inflammatory cytokines.

Results. GLFMs were most commonly found in women (60.5%) and most frequently described as white plaques. Histopathologic examination identified verrucous hyperplasia in 59% and epithelial dysplasia in 28% of the cases. EDX microanalysis revealed that Si (94%) was the most frequently detected foreign element. SiO_2 microparticles induced higher COL-1 expression; higher levels of proinflammatory cytokines, such as interleukin-6 (IL-6), IL-8, and transforming growth factor-β, and increased MMP-2 activity in HGFs.

Conclusions. There was a strong association between the presence of foreign material in the gingiva and white verrucous clinical lesions. In addition, the most common element in the foreign material was Si, and our in vitro findings demonstrate the importance of silica-mediated effects on gingival fibroblasts. (Oral Surg Oral Med Oral Pathol Oral Radiol 2019;128:250–267)

Patients may present in dental clinics with focal lesions in the gingiva that may be suggestive of inflammation but do not respond to improved oral hygiene and periodontal treatment. Although systemic diseases and mucocutaneous conditions should be investigated in such situations, foreign body gingivitis (FBG) should also be included in the differential diagnosis.[1–4] FBG has been described as a localized inflammatory reaction associated with the presence of foreign material in the gingival tissues.[3,4] This condition was first reported by Daley and Wysocki in 1990,[4] when they identified 8 patients with red or red-and-white gingival lesions that usually were painful and were refractory to conventional periodontal therapy. Microscopically, these lesions showed granulomatous inflammation containing particles of foreign material. It is hypothesized that FBG arises when damage to the sulcular epithelium during hygiene or restorative procedures or during use of household cosmetic products allows for the introduction of foreign particles into the deep gingival tissues. Moreover, common oral diseases, such as gingivitis and periodontitis, result in ulceration of the sulcular epithelium, and this predisposes the patient even further to the ingress of foreign particles into the gingival tissues. Other more rare oral diseases, such as lichen planus and vesiculobullous diseases, may also disrupt the integrity of the gingival epithelium and allow particles to be more easily introduced into the gingival connective tissue.

Statement of Clinical Relevance

We found a strong association between the presence of foreign material in gingival biopsy specimens and epithelial dysplasia. The most common element found in the foreign material was Si, and we demonstrated that silica (SiO_2) may disturb the homeostasis of gingival tissues.

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The foreign material in FBG cases is believed to arise from a dental origin, given the frequency with which dental materials are brushed, rubbed, cut, or burnished adjacent to the gingival tissues,[3] but could also be introduced through use of common cosmetic or other household products. Furthermore, studies using energy dispersive X-ray spectroscopy (EDX) analysis of foreign particles have shown that many different elements that are not normal biologic constituents of human tissues may be identified in FBG lesions. Silicon (Si) has been one of the elements most frequently found (61% of the cases in 2 studies), along with aluminum (Al).[3,5] Si is the second most abundant element in the earth's crust and is a major component of several materials used in dentistry, including tooth paste, polishing paste, pumice, and carborundum disks. In nature, Si is found in practically all rocks and in sand and is typically combined with oxygen as silica (SiO_2) or with oxygen and other chemical elements (e.g., Al, Mg, or Ca) as silicates. It is well-known that the inflammatory response induced by chronic exposure to SiO_2 causes the progressive and incurable interstitial lung disease known as *silicosis*. Silicosis is caused by inhalation of crystalline SiO_2, mostly in occupational settings and is the most prevalent occupational disease in the world. Most importantly, available evidence from combined pooled/meta-analyses and case control studies supports the conclusion that crystalline SiO_2 is a human carcinogen.[6–11] Accordingly, since 1997, the International Agency for Research on Cancer (IARC) has classified crystalline SiO_2 as a group I human carcinogen.[12] The association between SiO_2 exposure and an increased risk of lung cancer has been found in all major histologic types of lung cancer, particularly small cell and squamous cell carcinomas.[13,14] SiO_2-dependent carcinogenesis has been well documented in animal models and the mechanisms involved include the induction of chronic inflammation mediated by the continuous activation of macrophages in their effort to clear the SiO_2 particles accompanied by the production of factors that cause epithelial cell genotoxicity, injury, and proliferation. Moreover, it is believed that silicosis results in an immunosuppressive microenvironment; therefore, once tumor arises in the silicotic lung, tumor survival and growth are enhanced because the immune system might not be able to efficiently fight against the tumor cells.[7,15]

This research project arose from the frequent observation of foreign particles, similar to those described in previous FBG studies,[5,16] in the gingival biopsy specimens received for histopathologic examination at the Pacific Oral Pathology Laboratory (POPL) at the University of Pacific Arthur A. Dugoni School of Dentistry. In many instances, however, these particles were detected in lesions that were described by the referring clinicians as "leukoplakias" or "white verrucous plaques," in contrast to the typically inflamed, red or red and white FBG lesions described by Gordon and Daley.[16] To the best of our knowledge, there is no report in the literature showing an association between foreign materials in the gingiva and the presence of white verrucous clinical lesions. On the microscopic level, foreign material was consistently observed in lesions exhibiting significant epithelial changes, such as verrucous hyperplasia with marked hyperorthokeratosis and, occasionally, even epithelial dysplasia. Given these clinical and microscopic findings, the specific objectives in this study were:

(1) To evaluate the clinical and histopathologic features of gingival lesions containing foreign material (GLFMs) retrieved from the POPL archives.

(2) To identify the chemical composition of the foreign material by using EDX.

(3) To challenge primary human gingival fibroblasts (HGFs) with SiO_2 microparticles as one may find in FBG, to evaluate the production of collagen type I (COL-1), the activity of matrix metalloproteinase 2 (MMP-2), and the secretion of inflammatory cytokines.

MATERIAL AND METHODS

Clinical and histopathologic investigations

After institutional review board approval (IRB Protocol #17-65), 102 GLFM specimens submitted to the POPL at the University of Pacific Arthur A. Dugoni School of Dentistry from July 2014 to August 2017 were retrieved from the files of the laboratory and included in the study. Cases that were microscopically diagnosed as amalgam tattoos were not included. The criteria for inclusion of the gingival biopsy specimen in the investigation followed Gordon and Daley's criteria[16] for microscopic diagnosis of FBG:

1. The presence of chronic inflammation in a gingival specimen.
2. The presence of foreign bodies in an area of inflammation.
3. The consistent localization of these foreign bodies in at least two serial tissue sections.

Sixteen cases were excluded from the investigation for the following reasons: (1) cases that had insufficient tissue for EDX analysis were excluded; and (2) if more than one biopsy specimen was found for the same patient and from the same location, the earliest biopsy was included and the subsequent biopsies were excluded to avoid the possibility of introduction of the particles during the surgical procedure or secondary to a denuded wound, and to

avoid counting the same lesion twice. Biopsy samples obtained from different gingival sites in the same patient were included. Thus, a total of 86 biopsy specimens were further investigated.

Thirty gingival biopsy specimens received by the POPL during the same period and which, under diligent light microscopic examination, showed no evidence of foreign material were used as controls.

Clinical records were reviewed, and information, including patient age and sex; location, color, and configuration of lesions; and clinical provisional diagnoses, was extracted. The localization of the gingival lesion in jaw segments was recorded as follows:

Maxilla, anterior segment: Teeth #6 to #11
Maxilla, posterior segment: Teeth #1 to #5 and #12 to #16
Mandible, anterior segment: Teeth #22 to #27
Mandible, posterior segment: Teeth #17 to #21 and #28 to #32

The biopsy samples had been fixed in 10% buffered formalin, processed according to standard procedures, and embedded in paraffin. After calibration to apply standardized criteria, 2 board-certified oral and maxillofacial pathologists (D.C. and L.F.) examined, under light microscopy, at least two 3- to 4-μm hematoxylin and eosin−stained sections of each case. The pathologists reviewed the sections independently and without reference to the original diagnosis. If a case showed microscopic evidence of epithelial dysplasia, the World Health Organization (WHO) 2017 classification of dysplasia was used; in this classification, "mild dysplasia is defined as cytologic atypia limited to the basal third, moderate dysplasia by extension into the middle third, and severe dysplasia by extension into the upper third."[17] The term *verrucous hyperplasia* was used for lesions exhibiting what has been previously described by Müller[18] as "atypical verrucous hyperplasia," and showing the following characteristics: surface epithelium exhibiting a corrugated or verrucous surface architecture, often a thickened orthokeratin layer.

Scanning electron microscopy and EDX

Paraffin-embedded sections of the specimen measuring 8 μm in thickness were mounted on a copper sheet substrate and heated at 70°C for 30 minutes. The sections were then deparaffinized overnight in xylene at room temperature. After deparaffinization, the specimens were air-dried for 2 hours, and the copper sheet with the specimen was mounted on the Al stub platform by using double-sided adhesive conductive carbon tape. Gold coating was performed for 60 seconds. An S-3000 N scanning electron microscope (Hitachi Science Systems, Tokyo, Japan) equipped with an EMAX Energy EX-250 EDX device (Horiba, Tokyo, Japan) was used to obtain EDX spectra on the prepared specimens, which were then analyzed by using EMAX software (Horiba, Tokyo, Japan).[19,20] In both point and area mode analyses, the data acquisition time was set at 60 seconds. A field-emission scanning electron microscope (JSM-7500 F; JEOL, Tokyo, Japan) was also used to acquire images of high magnification and resolution.

Cell culture

HGFs were purchased from American Type Culture Collection and cultured, as described in a previous study[21] by our group. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Carlsbad, CA) with 10% fetal bovine serum and 1% penicillin/streptomycin. For stimulation of the SiO_2 microparticles, cells were cultured in serum free OptiMEM and stimulated with low (62.5 μg/mL), medium (125 μg/mL), or high (250 μg/mL) concentrations of SiO_2 microparticles (0.1−10 μM) for 24 hours. HGFs between the 4th and 8th passages were used in all experiments.

Western blotting

Western blotting was performed with antibody against COL-1 (Abcam, Cambridge, MA), as described previously by our group,[22] after 24 hours of SiO_2 stimulation. The same membranes were stripped and reprobed with anti-glyceraldehyde 3-phosphate dehydrogenase (Abcam, Cambridge, MA) as a loading control.

Zymography

Zymography was performed to measure the activity of the gelatinase MMP-2, as described previously by our group.[23] After 24 hours of SiO_2 stimulation, the conditioned medium was collected and concentrated with a 10-kDa Amicon Ultra Centrifugal Filter (EMD Millipore, Bedford, MA), according to the manufacturer's instructions. Ten microliters of the conditioned medium were loaded on collagen zymogram gels containing 1% of COL-1. After electrophoresis, sodium dodecyl sulfate was eliminated by a 2.5% Triton X-100 solution in 50 mM Tris (pH 7.5; 2 × 30 minutes). Gels were rinsed with distilled water and then incubated for 24 hours in a proteolysis buffer containing 50 mM Tris, 0.5 M NaCl, and 2.5 mM $CaCl_2$ (pH 7.8) before staining with Coomassie blue solution. The resulting gels had "clear bands" that corresponded to the gelatinolytic activity of the MMP-2 against a blue background. The gels were imaged by using scanning densitometry with a ChemiDoc gel-documentation system (Biorad, Hercules, CA). Densitometric quantification was performed by using Image J software.

Enzyme-linked immunosorbent assay

Supernatants of the HGF stimulated with silica microparticles were collected, and enzyme-linked immunosorbent assay was performed to measure the concentration of CXCL8 (C-X-C Motif Chemokine Ligand 8), interleukin-6 (IL-6), and transforming growth factor-β (TGF-β) according to the manufacturer instructions (R&D Systems, Minneapolis, MN), and as previously described by us.[24]

Statistical analysis

For the clinicopathologic analysis, the 86 biopsy specimens containing foreign material and the 30 control samples were analyzed for potential differences in various clinical and microscopic findings. All data were considered category data, so the χ^2 test was used. Where small cell sizes were encountered as expected, Fisher's exact test was used. Clinicopathologic data were analyzed by using SPSS software, version 25 (SPSS Inc., Chicago, IL).

Data from the in vitro studies were presented as mean $\pm$ standard deviation. Statistical analyses were performed by using one-way analysis of variance (ANOVA). Experimental data were analyzed by using Prism 7 software (GraphPad, San Diego, CA).

RESULTS

Clinical findings

We characterized a total of 76 patients and 86 biopsy specimens (8 patients had 2 biopsy samples each and 1 patient had 3 samples). Patient age ranged from 9 to 87 years (mean age 58.7 years). The cases were found most frequently in the age group 51 to 70 years (50 patients). Most patients were women (46 [60.5%]). Differences in gender distribution between patients and controls were not statistically significant (Table I).

The color of the lesion was described in the majority of the cases. The vast majority of the GLFMs (63 cases [75%]) were described as white in color: 10 (12%) were described as red; 7 (8%) were described as a mixture of red and white; and in only 2 cases, the color of the lesion was not specified. The tendency for the GLFMs to appear as white clinically was highly significant in comparison with the controls ($P < .001$) (see Table I) (Figures 1A and 2C). With regard to the lesion's morphology, most lesions were described as plaques (36 cases [51%]) or verrucous plaques (8 cases [11%]), whereas the controls were more commonly described as "raised" lesions (23 cases [79%]). This

Table I. Demographic and clinical findings

	GLFM		*Cnt*		*P value*
Number of biopsy specimens	86		30		–
Number of patients	76		30		–
Male/Female ratio	30/46		7/23		NS
Mean age (years)	58.7		54.4		NS
Color of lesion	**GLFM (N)**	**GLFM (%)**	**Cnt (N)**	**Cnt (%)**	<.001
White	63	75	1	4	
Red	10	12	9	33	
Red and white	7	8	1	4	
Pink	3	4	15	56	
Brown	1	1	1	4	
Morphology	**GLFM (N)**	**GLFM (%)**	**Cnt (N)**	**Cnt (%)**	<.001
Plaque	36	51	0	0	
Raised	6	8	23	79	
Papillary	7	10	0	0	
Verrucous plaque	8	11	1	3	
Reticular/Striated	3	4	0	0	
Ulcerated	5	5	3	10	
Pebbly	2	3	0	0	
Flat	1	1	0	0	
Desquamative	0	0	1	3	
Macule	2	3	1	3	
Location	**GLFM (N)**	**GLFM (%)**	**Cnt (N)**	**Cnt (%)**	.04
Posterior mandible	42	49	9	30	
Posterior maxilla	33	38	11	37	
Anterior maxilla	8	9	5	17	
Anterior mandible	3	4	5	17	
Distribution	**GLFM (N)**	**GLFM (%)**	**Cnt (N)**	**Cnt (%)**	.003
Single	41	60	26	90	
Multiple	27	40	3	10	

P values refer to χ^2 tests of control versus experiments groups crossed with features of the patient demographic characteristics or lesions.
Cnt, controls; *GLFM,* gingival lesion containing foreign material; *NS,* not significant.

difference in morphologic appearance between GLFMs and controls was also highly significant ($P < .001$).

GLFMs were localized in the mandibular and maxillary posterior segments in 42 (49%) cases and 33 (38%) cases, respectively. Only in 8 cases (9%), the maxillary anterior region was involved, and in 3 (3%) the mandibular anterior segment was involved. The tendency for GLFMs to occur in the posterior mandible was statistically significant in comparison with controls ($P = .037$) (see Table I).

There was a significantly higher tendency for GLFMs to be described as a multifocal process or to present as "multiple lesions" in comparison with controls ($P = .004$) (see Table I).

Of the clinical diagnoses provided by clinicians in GLFM cases, the most commonly listed diagnoses were "lichen planus" (20 [23%]), "hyperkeratosis" (20 [23%]), "leukoplakia" (18 [21%]), and "papilloma" (5 [6%]).

Microscopic findings

The vast majority of GLFMs demonstrated some degree of chronic inflammation (75 [87%]), and only a few specimens (11 [13%]) showed chronic as well as acute inflammation. There was a statistically significant tendency for the studied specimens to show chronic inflammation, rather than a mixture of chronic and acute inflammation, in comparison with controls ($P < .001$) (Table II). In most cases, the inflammation was described as mild (25 [29%]) or moderate (36 [42%]) and distributed in a patchy pattern (48 [56%]) in the connective tissue. This patchy inflammation was, in the majority of cases, composed predominantly of lymphocytes and plasma cells, with occasional histiocytes, and showed a tendency to localize in the mid- or deep lamina propria (Figures 1B, 2A, and 3A). Foreign particles were consistently found intermixed with these

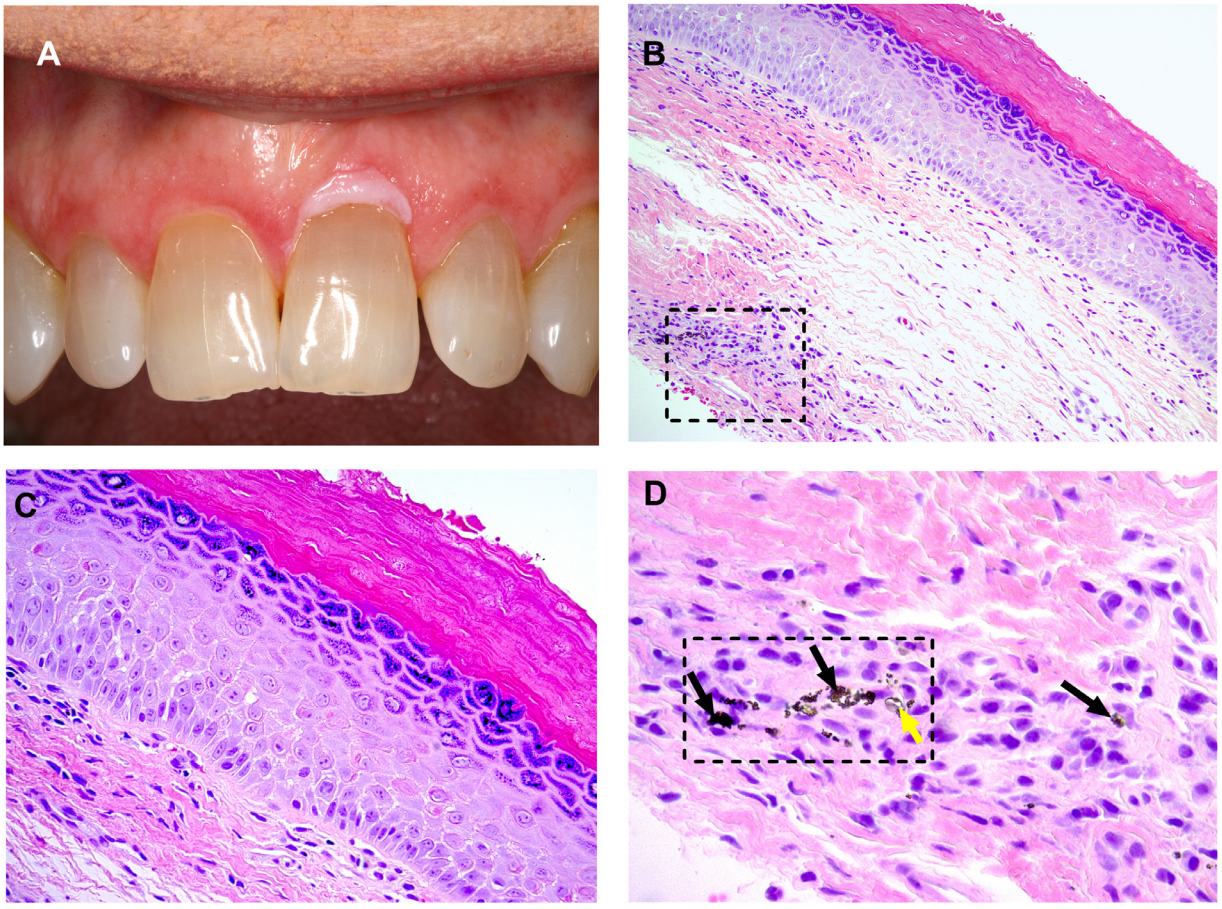

Fig. 1. **(A)** Clinical image showing a white lesion involving the facial marginal gingiva of tooth #9. (Courtesy of Dr. Reid Lester). **(B)** Representative photomicrograph showing hyperorthokeratosis and foreign material in the deep lamina propria *(square)* (hematoxylin and eosin [H&E]; original magnification × 200). **(C)** High-power view of the same case shows that the epithelium exhibits dyskeratosis, cellular discohesion, and nuclear pleomorphism limited to the lower one-third of the epithelial thickness—mild dysplasia (H&E; original magnification × 400). **(D)** High-power view of the square area of image B, reveals that the foreign material consists of very fine, blackish granules *(black arrows)* accompanied by slightly larger, colorless, refractile, crystalloid particles *(yellow arrow)* (H&E; original magnification × 800). *A high-resolution version of the image is available as eSlide: VM05618.*

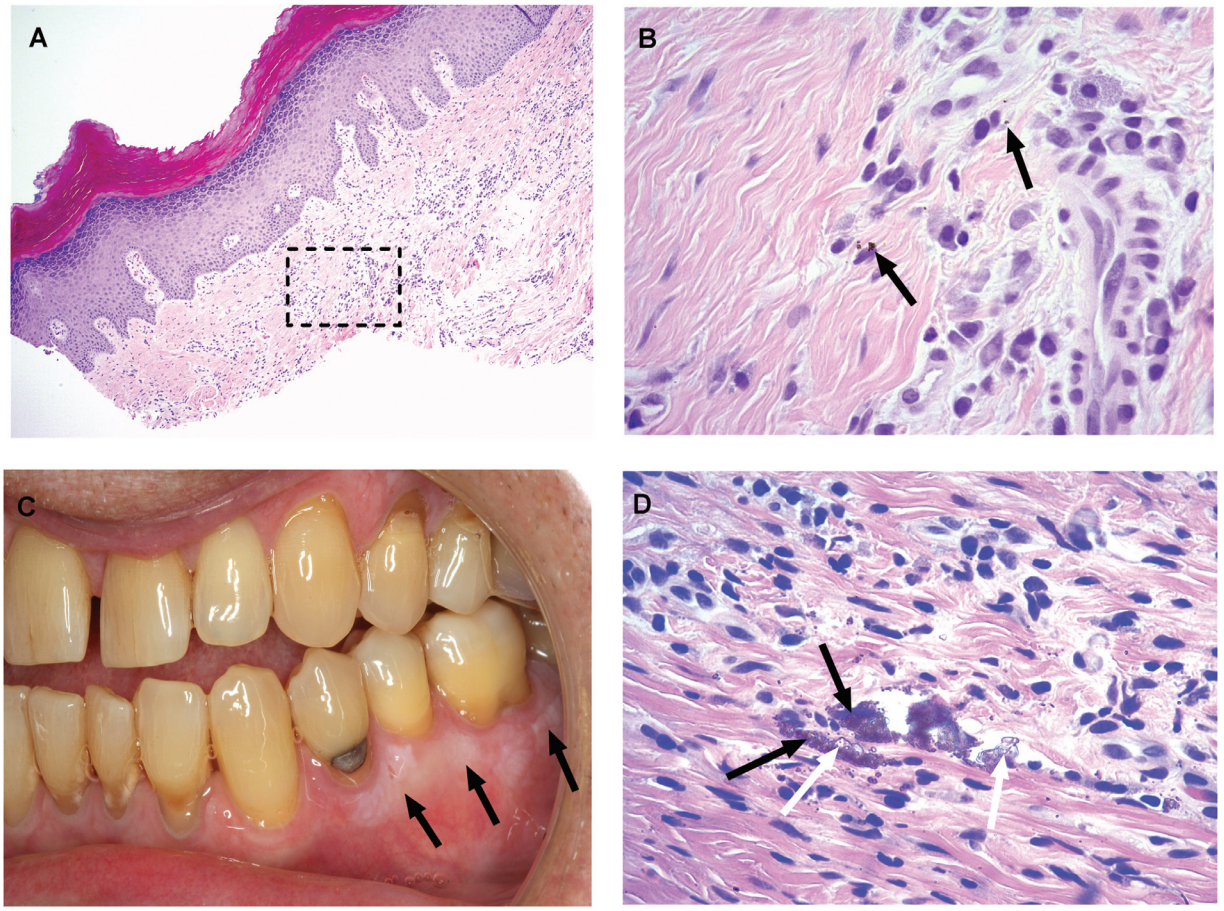

Fig. 2. **(A)** Representative photomicrograph showing the typical epithelial verrucous hyperplasia that was seen in the majority of the studied cases (hematoxylin and eosin [H&E]; original magnification × 80). **(B)** High-power view of the square area in A, which shows that the foreign material consists of very fine, opaque, blackish-gray granules *(black arrow)* that often might be very subtle and easy to overlook (H&E; original magnification × 800). *A high-resolution version of the image is available as eSlide: VM05617.* **(C)** Clinical photograph demonstrating white plaques involving the attached buccal gingiva of teeth #19 to #21 *(black arrows)*. Histopathologic examination of the corresponding biopsy specimen revealed verrucous hyperplasia with mild epithelial dysplasia and focal foreign material (photomicrographs not shown). **(D)** Representative photomicrograph of a more microscopically obvious foreign material composed of multiple blackish granules *(black arrows)* admixed with larger crystalloid particles *(white arrows)* (H&E; original magnification × 800). *A high-resolution version of the image is available as eSlide: VM05616.*

inflammatory cells and sometimes appeared to be within the cytoplasm of histiocytes or fibroblasts (Figures 1D, 2B, and 2D). Eosinophils were identified in 43 (50%) of the cases, and their presence was more common in the patients than in the controls ($P = .009$) (*black arrows* in Figure 3C). In 21 cases (24%), the inflammation was described as "lichenoid"; however, the presence of basal cell degeneration, with or without other features of lichenoid inflammation, was seen in 40 cases (47%). The presence of lichenoid inflammation and that of basal cell degeneration were both statistically significant ($P = .03$ and $P < .001$, respectively) (see Table II). Hyperorthokeratosis was found in the majority of the cases (58 [67%]), whereas hyperparakeratosis was seen in only 21 cases (24%). This tendency for GLFMs to demonstrate an increase in orthokeratinization was statistically highly significant ($P < .001$) (see Table II) (see Figures 1B, 2A, and 3A; Figures 4A and 5A). Fifty-one cases (59%) demonstrated verrucous hyperplasia of the epithelium (see Figures 2A and 3A). This trend was also highly significant ($P < .001$) (see Table II).

Another microscopic trend was the presence of epithelial dysplasia in the GLFM (Figures 1C, 3D, 4C, and 5B). Mild epithelial dysplasia was identified in 17 (20%) of the cases, moderate epithelial dysplasia was observed in 6 cases (7%), and severe dysplasia in 1 case. Compared with controls, the presence of epithelial dysplasia in GLFMs was also significant ($P = .01$) (see Table II).

With regard to the microscopic appearance of foreign particles, in 40 cases (47%), the particles appeared as minute, opaque, grayish-black granules (see Figures 1D, 2B, and 2D; Figures 3B 4B, 4D, and 5D). These granules

showed a tendency to aggregate and form small clusters containing multiple individual granules (*black arrows* in Figures 1D, 2D, and 5D). These granules did not polarize when examined under polarized light, and they were very fine and easily overlooked, especially when they were rare and localized within inflammatory infiltrates (see Figures 2B and 3B; Figure 5C). The majority of the cases (52%), however, showed a mixture of the pigmented granules with larger, colorless, translucent crystalline structures (*yellow, white, and green arrows* in Figures 1D, 2D, and 4D, respectively). These crystalline structures did polarize when viewed under polarized light. In 1 case, only crystalline refractile particles were present (Table III).

Table II. Microscopic findings

	GLFM (N)	*GLFM (%)*	*Cnt (N)*	*Cnt (%)*	*P value*
Type of inflammation					
Chronic	75	87	13	43	<.001
Chronic and acute	11	13	17	57	
Degree of inflammation					
Very mild	9	11	7	23	.04
Mild	25	29	2	7	
Moderate	36	42	13	43	
Severe	16	19	8	27	
Inflammation in a patchy arrangement					
Present	48	56	6	20	.001
Absent	38	44	24	80	
Inflammation in a diffuse arrangement					
Present	26	30	21	70	<.001
Absent	60	70	9	30	
Lichenoid mucositis					
Present	21	24	2	7	0.03
Absent	65	76	28	93	
Granulomatous inflammation					
Present	2	2	0	0	NS
Absent	84	98	30	100	
Eosinophils					
Present	43	50	7	23	.009
Absent	43	50	23	77	
Hyperparakeratosis					
Present	21	24	23	77	<.001
Absent	65	76	7	23	
Hyperorthokeratosis					
Present	58	67	5	17	<.001
Absent	28	33	25	83	
Verrucous hyperplasia					
Present	51	59	1	3	<.001
Absent	35	41	29	97	
Epithelial dysplasia					
Absent	62	72	30	100	.01
Mild	17	20	0	0	
Moderate	6	7	0	0	
Severe	1	1	0	0	
Basal cell degeneration					
Present	40	47	2	7	<.001
Absent	46	54	28	93	
Epithelial atrophy					
Present	30	35	8	27	NS
Absent	56	65	22	73	
Acanthosis					
Present	49	57	17	57	NS
Absent	37	43	13	43	
Desquamative changes					
Present	9	11	0	0	.06
Absent	77	90	30	100	

P values refer to χ^2 tests of control versus experiments groups crossed with features of the patient demographic characteristics or lesions.
Cnt, controls; *GLFM,* gingival lesion containing foreign material; *NS,* not significant.

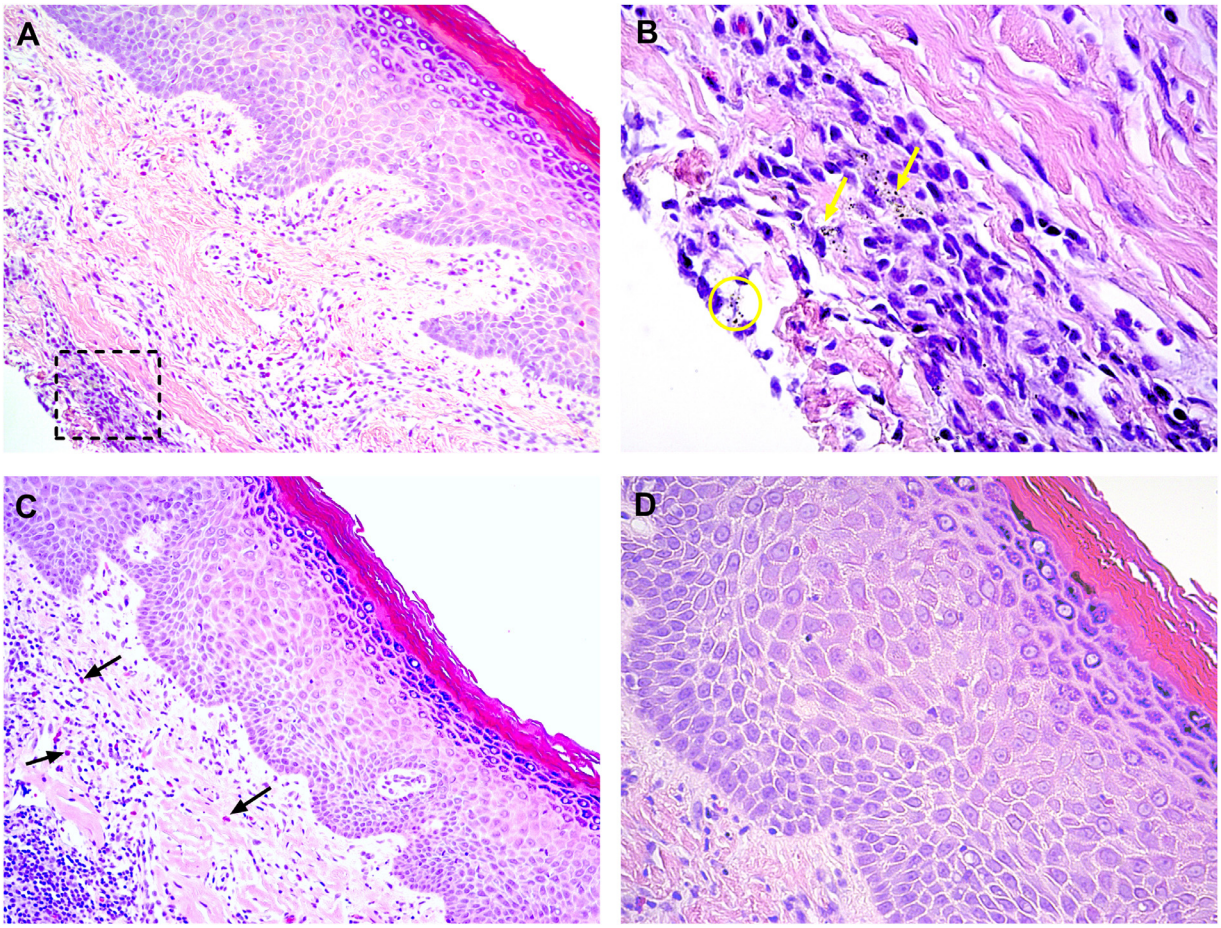

Fig. 3. Representative photomicrographs of one of the gingival lesions containing foreign material (GLFMs) that exhibited moderate epithelial dysplasia. **(A)** Low-power view shows hyperorthokeratosis, dyskeratosis, and cellular discohesion in the epithelium, as well as inflammation in the lamina propria, which is patchy and more concentrated in the deeper portion of the specimen *(square area)* (hematoxylin and eosin [H&E]; original magnification × 200). **(B)** High-power view of the square area in A, which shows chronic inflammation and the presence of several fine blackish granules *(yellow arrows and circle)* (H&E; original magnification × 800). **(C)** Low-power view of a different area of the same specimen, showing that the epithelium exhibits significant cellular discohesion, loss of polarity, and nuclear pleomorphism, whereas the lamina propria shows moderate inflammation composed predominantly of mononuclear cells, but showing occasional eosinophils *(black arrows)* (H&E; original magnification × 200). **(D)** High-power view of the epithelium in image C demonstrates that the dysplastic changes extend into the middle third of the epithelium—moderate dysplasia (H&E; original magnification × 400). *A high-resolution version of the image is available as eSlide: VM05614.*

Scanning electron microscopy and EDX of foreign materials

We identified foreign materials by visual inspection of the gingival specimen under scanning electron microscopy while searching for objects with distinctive texture that were incongruous with the surrounding biologic structures or with a raised appearance that did not resemble that of cells. A typical foreign body consisted of either single particles or clusters of smaller particles, some measuring less than 0.05 μm. As shown in Figures 6A to 6F, individual particles of smaller sizes formed densely packed clusters of aggregated particles. These particles appeared polyhedral, and the particle aggregates assumed a rough exterior texture as a result. Figures 6A, 6C, and 6E show the particles in low power, and a detailed high-magnification image of the same area can be seen in Figures 6B, 6D, and 6F. In some instances, the particles appear internalized into the underlying cellular structures (see Figure 6B). When subjected to EDX analysis, the foreign material (depicted in Figure 6B) yielded an elemental profile; a typical graph of detected elements is presented in Figure 6G.

For elemental profiles obtained by using EDX, gold, copper, carbon, and oxygen were not included in the final data analysis. Both carbon and oxygen are ubiquitous in biologic specimens. Carbon was also the component of the adhesive used to mount the target specimens. The

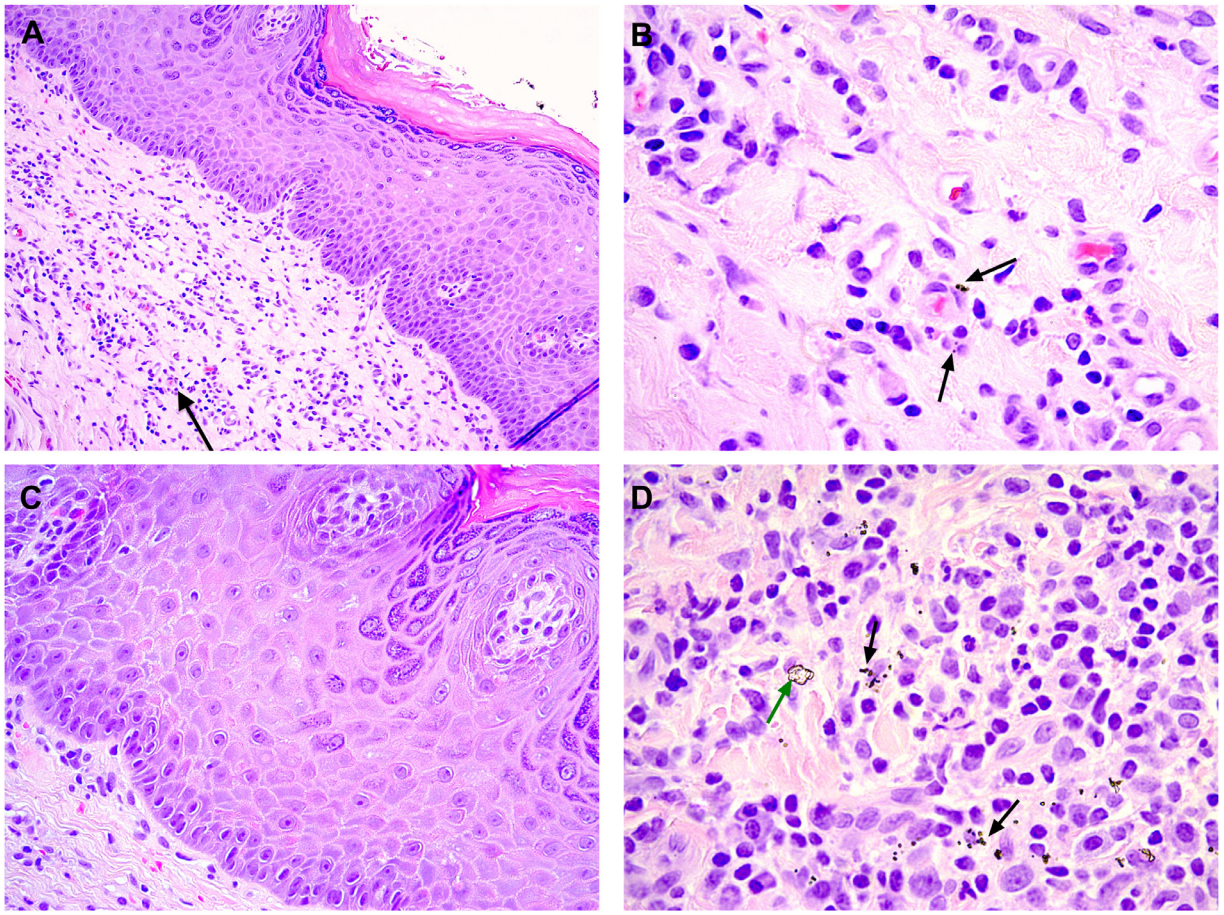

Fig. 4. Representative photomicrographs of one of the gingival lesions containing foreign material (GLFMs) that exhibited verrucous hyperplasia and mild epithelial dysplasia. **(A)** Low-power view shows verrucous epithelial hyperplasia and scattered chronic inflammatory cells in the connective tissue. The black arrow is pointing to an area where foreign material was identified (hematoxylin and eosin [H&E]; original magnification × 200). **(B)** High-power view of the area pointed to by the arrow in image A shows the subtle, rare foreign material granules identified in the connective tissue *(black arrows)* (H&E; original magnification × 800). **(C)** High-power view of the epithelium shows that the cells exhibit dyskeratosis, cellular discohesion, and large nuclei with prominent nucleoli which are limited to the lower one-third of the epithelium—mild dysplasia (H&E; original magnification × 400). **(D)** High-power view of a deeper area of the same specimen showing several fragments of foreign material composed of both blackish granules *(black arrows)* and larger crystalloid particles *(green arrow)* (H&E; original magnification × 800). *A high-resolution version of the image is available as eSlide: VM05615.*

abundance of gold could be accounted for by its use as the conductive coating of the specimens, and copper was the base material of the specimen mount. After disregarding those elements, Si was the most prevalent chemical element found in the studied specimens, with 81 of 86 specimens (94%) testing positive for the presence of Si. Other major elements detected in the foreign particles included Ca, Al, and Zn, all of which were found in more than half of the specimens studied (Figure 6H). Many of the major foreign body−associated elements were found to coexist with Si. The element with the greatest coexistence with Si was Ca, which was detected in 69% of all Si-positive samples (Figure 6I). Al was the second most abundant element, with detection in 67% of all Si-positive samples. Following Al, Zn, Fe, K, Na, Mg, Cl, Ti, and S were co-detected with Si, in descending order of detection rates (see Figure 6I). These data demonstrate the diversity of elements incorporated into foreign bodies.

A total of 146 Si-containing foreign bodies were identified microscopically in 81 specimens. The majority of the identified foreign bodies measured less than 10 μm in greatest dimension, but widespread size distribution was also noted, with the maximum dimension measuring between 60 and 65 μm (see Table III).

SiO_2 microparticles−induced effects in HGFs

Our in vitro findings demonstrated that COL-1 production was significantly higher in SiO_2-stimulated fibroblasts after 24 hours of challenge, irrespective of the

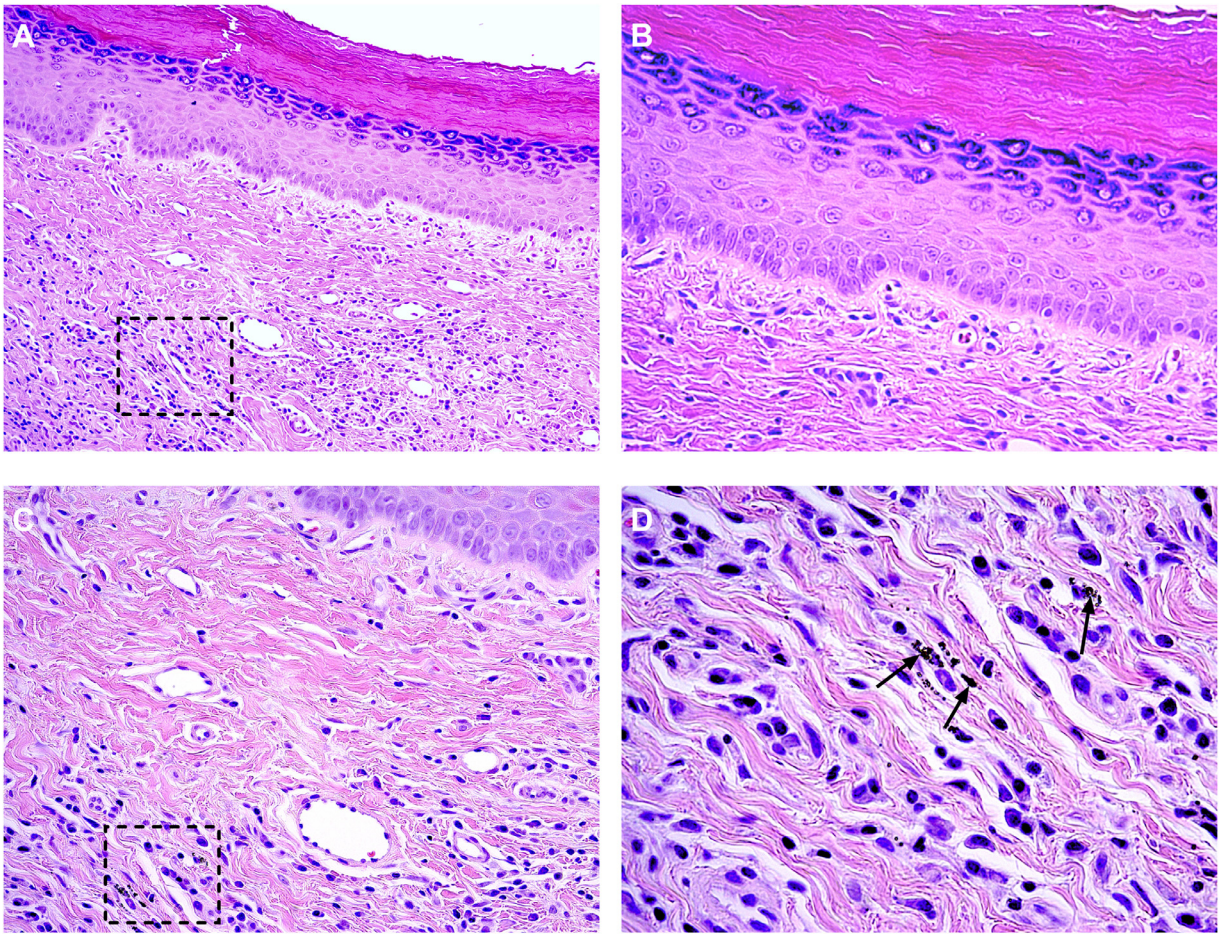

Fig. 5. Representative photomicrographs of one of the gingival lesions containing foreign material (GLFMs) that exhibited mild epithelial dysplasia. **(A)** Low-power view shows hyperorthokeratosis and atrophy of the spinous cell layer of the epithelium and scattered chronic inflammation in the lamina propria (hematoxylin and eosin [H&E]; original magnification × 200). **(B)** High-power view shows that the epithelium exhibits dyskeratosis, an increased nuclear-to-cytoplasmic ratio, and nuclear hyperchromatism, which are limited to the basal third of the epithelium—mild dysplasia (H&E; original magnification × 400). **(C)** High-power view highlighting that the connective tissue contains scattered chronic inflammation and minute blackish granules are seen adjacent to the inflammatory cells *(square area)* (H&E; original magnification × 400). **(D)** Higher-power view of the square areas in images A and C, which show in better detail the granules of foreign material *(black arrows)* (H&E; original magnification × 800). *A high-resolution version of the image is available as eSlide: VM05619.*

concentration of SiO_2 microparticles (Figure 7A). We detected higher proteolytic activity of MMP2 both in cell extracts (Figure 7B) and cell supernatants (Figure 7C) after 24 hours of challenge with the 3 concentrations of SiO_2microparticles (see Figures 7 B and 7C; Figures 7D, 7E, and 7F). The quantification of latent MMP2 (pro-MMP2) and active MMP2 and the ratio of active/latent MMP-2 are shown in Figures 7D, 7E, and 7F, respectively. We observed a peak of the MMP2 activity in the supernatant of cells stimulated with the medium SiO_2 concentration (125 μg/mL) (see Figures 7C, 7E, and 7F). With regard to inflammatory cytokine secretion, CXCL8/IL-8 (Figure 8A), IL-6 (Figure 8B), and TGF-β (Figure 8C) were significantly higher in a concentration-dependent manner. All inflammatory cytokines investigated in this study were higher, with the high concentration of SiO_2 microparticles (250 μg/mL).

DISCUSSION

Nomenclature and final diagnosis of GLFM

We analyzed 86 gingival lesions that microscopically contained particles of foreign material (GLFMs). Although the terms *FBG*[16] and *foreign body gingival lesions*[5] have been used in previous similar studies to describe these cases, in our experience and given the results of this study, the presence of foreign material can sometimes be concomitant with significant epithelial changes that are more clinically relevant than the inflammatory changes and therefore should take precedence in

Table III. Foreign material characteristics

Microscopic Appearance of the Foreign Materials Detected by Light Microscopy in 86 GLFMs		
Appearance	(N)	(%)
Opaque	40	47
Refractile (crystalloid)	1	1
A mixture of opaque and refractile (crystalloid)	45	52
Total	**86**	**100**

Size of the Total Number of Foreign Materials Detected by SEM in 81 Si-containing GLFMs		
Size (μm)	*(N)*	*(%)*
0–5	69	47
5–10	41	28
10–15	11	8
15–20	10	7
20–25	5	3
25–30	5	3
30–35	0	0
35–40	3	2
40–45	1	1
60–65	1	1
Total	**146**	**100**

GLFM, gingival lesion containing foreign material; *SEM,* scanning electron microscopy.

the lesion's final diagnosis. For instance, we found 24 biopsies containing foreign material that also demonstrated conspicuous epithelial dysplasia and were diagnosed initially as dysplasia with no mention of the foreign material present. We also found a high frequency of verrucous epithelial hyperplasia when foreign particles were present. Accordingly, we avoided the term FBG for the cases we studied as we feel that epithelial dysplasia and verrucous hyperplasia would be more clinically relevant diagnoses than foreign body gingivitis. Moreover, as previously considered by Gordon and Daley[16], it is possible that lichen planus or other erosive conditions may have allowed the introduction of the foreign material into the tissue; hence, foreign particles may be the consequence, rather than the main etiologic factor, in at least some of these GLFMs. Nevertheless, we do believe that the presence of foreign particles in a biopsy specimen should always be mentioned by the pathologist on the biopsy report for several reasons: (1) to raise awareness to the clinician of the possibility of their iatrogenic introduction during dental procedures; (2) to provide the clinician the opportunity to use clinicopathologic correlation to more definitively diagnose the patient's condition (e.g., lichenoid FBG versus lichen planus); and (3) for documentation purposes so that future studies could be performed to better elucidate the potential biologic effects of these particles. The aim of this study was not to specifically diagnose each of the GLFMs or to find a specific, unique diagnosis for such lesions but, rather, to better understand whether there are specific clinical and microscopic features that are consistently associated with the presence of the foreign material.

Clinical features

As in previous studies describing GLFMs,[5,16] most of our patients were females (60.5%). However, this was not statistically significant in comparison with the controls. The female predilection for both GLFMs and gingival lesions without any foreign material (i.e., controls) could be explained by the fact that females show an increased tendency to seek dental treatment and possibly to consent to a biopsy procedure, as previously proposed by Gordon and Daley.[16] Indeed, female patients report more dental visits compared with males in epidemiologic studies.[25] Moreover, hormonal influences may also predispose female patients to gingival inflammation, which is the most common etiologic factor for gingival lesions encountered in dental practice.

The mean age of patients with GLFMs was 58.7 years, with the age group 51 to 70 years being the most commonly affected. In contrast, Gordon and Daley[16] reported most cases in the age group 41 to 50 years, and Koppang et al.[5] found approximately equal occurrence in the age groups 41 to 50 years and 51 to 60 years. The higher incidence of GLFMs in middle-aged and older patients may be attributed to lifetime exposure to dental procedures and, consequently, higher exposure to dental materials. Moreover, our finding of a slightly older patient population affected by GLFMs, compared with Gordon and Daley's study findings published in 1997,[16] might be a consequence of the steady increase in the median self-reported dental visits from 58.5% in 1995 to 66.3% in 2008 by those patients 65 years of age and older in the United States.[25]

The vast majority (75%) of GLFMs were described by the referring clinician as "a white lesion." This is in contrast to Gordon and Daley,[16] who, in 61 studied cases, found that 52% of the lesions were described as "completely red" and 18% as "a mixture of red and white."[16] We believe that the difference in the clinical appearance of the GLFMs in our cases is because in Gordon and Daley's study,[16] only cases that were initially microscopically diagnosed as FBG were included. In contrast, in our study, we included gingival biopsy specimens, irrespective of their original diagnoses but still applying the same microscopic criteria used by those investigators (i.e., the presence of foreign bodies in an area of inflammation in the connective tissue and the consistent localization of foreign particles in at least 2 serial tissue sections). We acknowledge that in many of our cases, the foreign material was subtle and could have been easily overlooked, especially when encountered in a setting of more significant microscopic features, such as florid lichenoid inflammation or verrucous epithelial

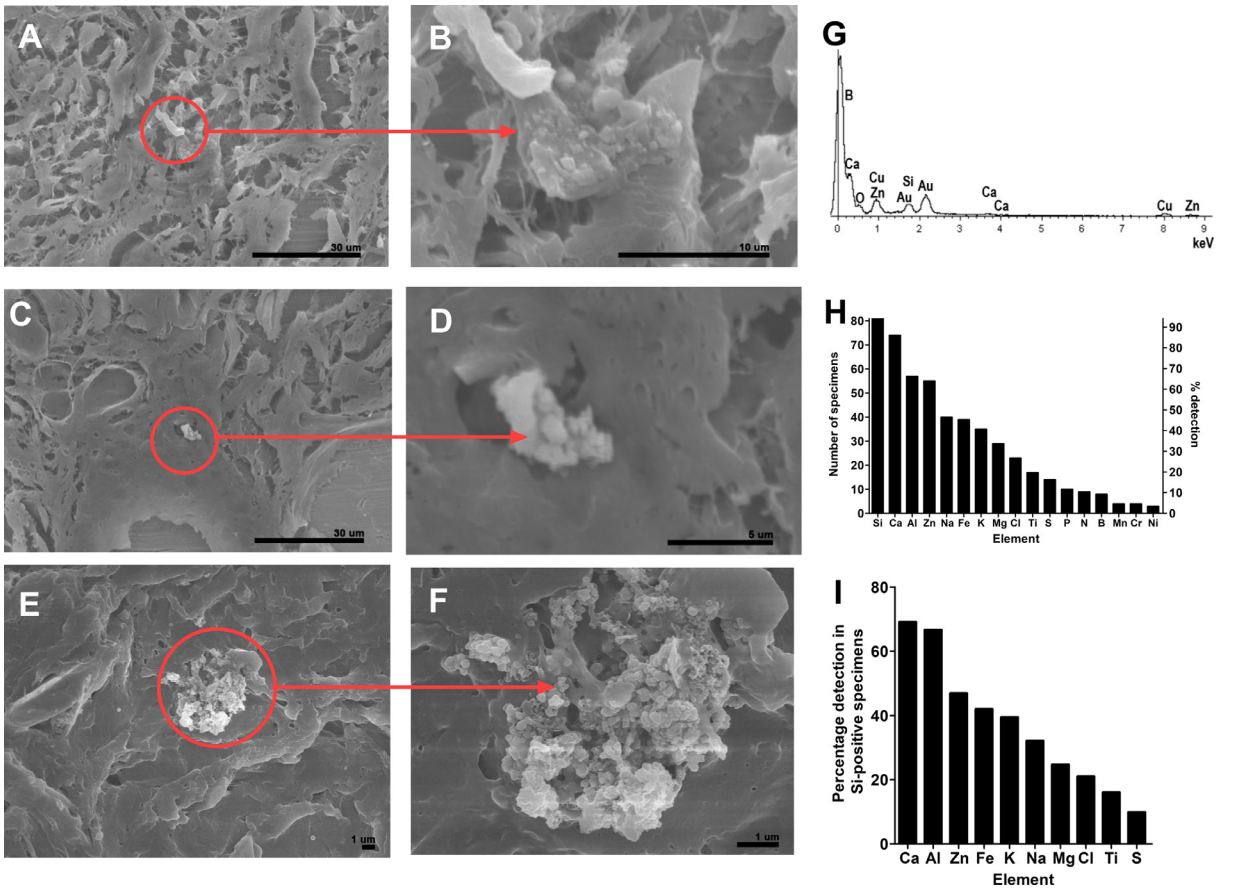

Fig. 6. **(A-F)** Representative low- and high-power micrographs of the foreign materials identified and targeted for the Energy Dispersive X-ray spectroscopy (EDX) analysis. The typical foreign material consisted of either single particles or clusters of smaller granules. **(G)** Typical EDX analysis graph showing the elements found in the foreign material depicted in image B. **(H)** Histogram showing the percentage of cases containing each of the elements detected in the studied cases. Silicon (Si) was the most commonly found element (81 cases). **(I)** The histogram shows that many of the major foreign material−associated elements were found to be coexisting with Si, especially Ca (69%) and Al (67%) in the same target area.

hyperplasia with dysplasia. Accordingly, in our study, we included cases that were not originally diagnosed as FBG and for which the presence of foreign materials would likely have been ignored if the particles were not intentionally searched for. Moreover, the pathologist might avoid diagnosing the lesions as FBG or even mentioning the presence of foreign material when the clinical lesion is described as a white plaque or leukoplakia (i.e., not clinically inflamed). Hence, in our opinion, the microscopic presence of focal foreign materials in clinical white plaques is likely underreported because the foreign material is intentionally or unintentionally overlooked by the pathologist.

GLFMs were more commonly seen in the mandibular posterior segment (49%), followed by the maxillary posterior segment (38%), and this is similar to the findings reported by Koppang et al.[5] Gordon and Daley[16] did not report a predilection for any anatomic site in their study. We agree with Koppang et al.,[5] who considered that the predilection for GLFMs to occur in the posterior segments of the jaws likely results from the higher frequency of dental procedures in these segments compared with the anterior regions of the jaws. Moreover, this site predilection supports the hypothesis that these foreign materials are most likely of a dental material origin.

There was a higher tendency for GLFMs to be multifocal processes or to present as "multiple lesions" compared with controls. We believe that there are 2 explanations for this finding: first, if the origin of the foreign material is a component of abrasive disks or of prophylactic paste and toothpaste,[3] then multiple areas of the dental arch where the patient receives dental treatment or typically uses the material, as in the case of toothpaste, will be susceptible to the inadvertent introduction of those particles; second, in some cases, we believe the foreign material is a consequence of disruption of the epithelial barrier caused by such

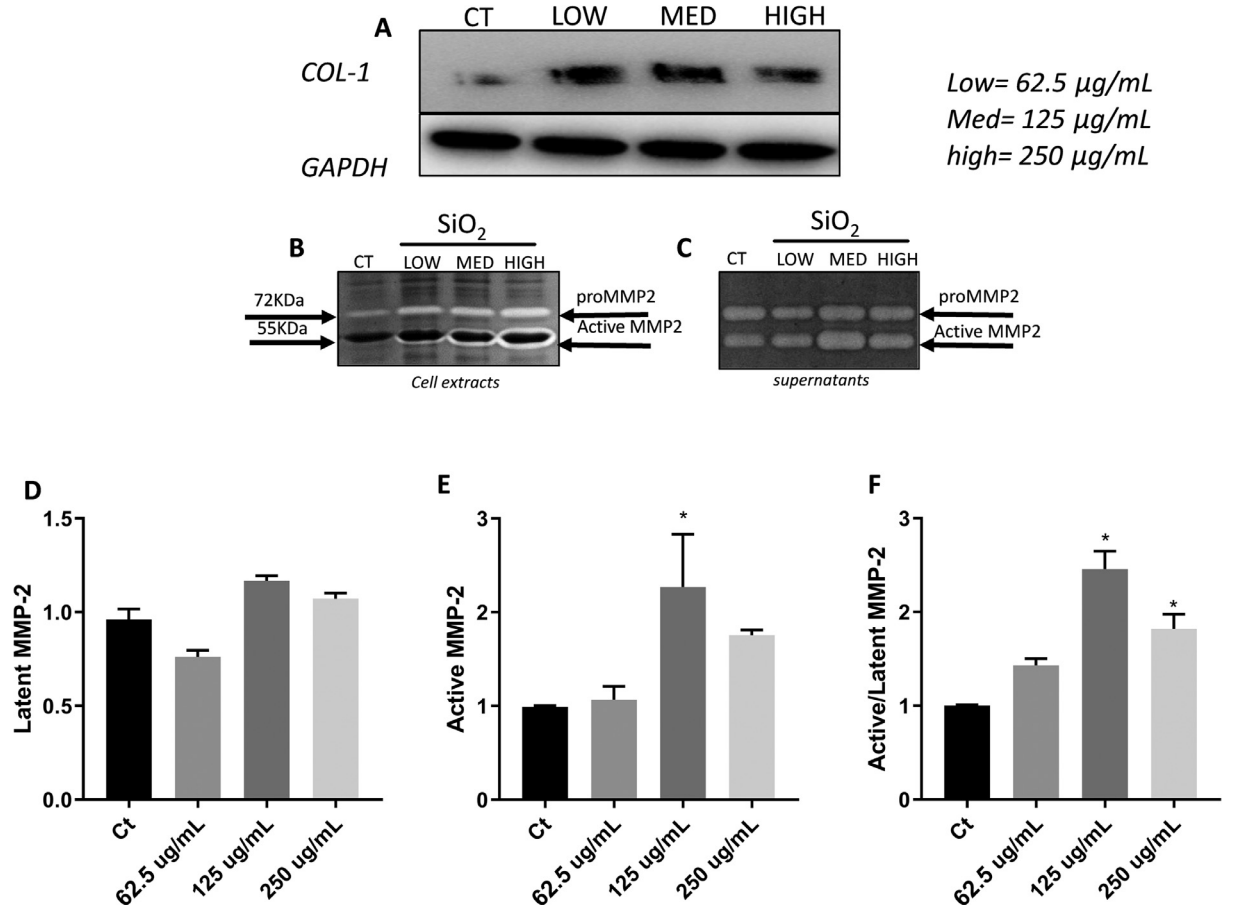

Fig. 7. **(A)** Collagen type 1 (COL-1) production in silica (SiO_2)−stimulated gingival fibroblasts after 24 hours of challenge with different SiO_2 microparticles concentrations. Proteolytic activity of matrix metalloproteinase 2 (MMP2) in **(B)** cell extracts and **(C)** supernatants after 24 hours for the different SiO_2 microparticles concentrations. Relative quantitation of latent **(D)** active **(E)** and active/latent **(F)** MMP-2 are shown. Results are shown as mean ± standard deviation (SD) and are representative of 3 independent experiments (*$P < .05$).

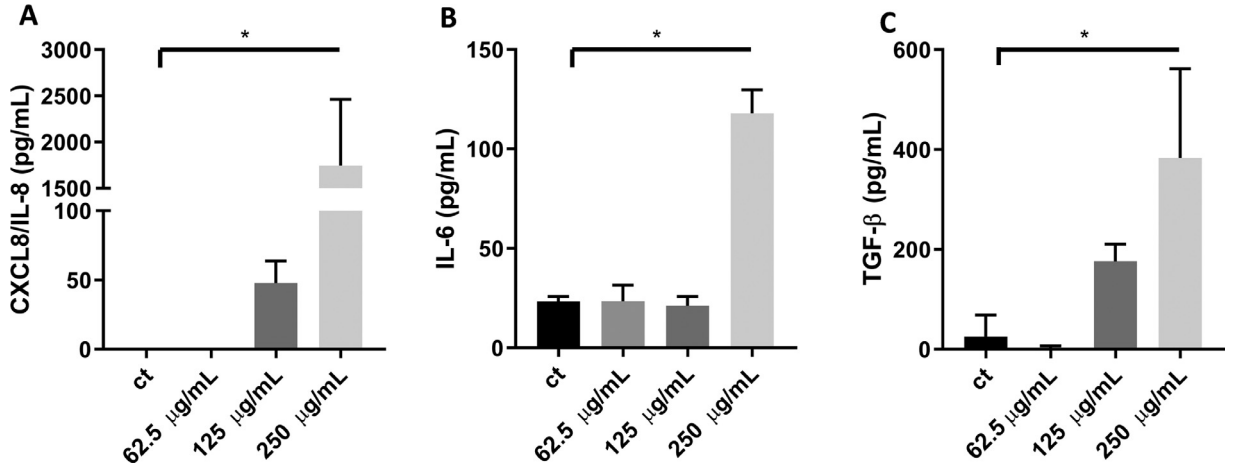

Fig. 8. CXCL8 **(A)**, interleukin-6 (IL-6) **(B)**, and transforming growth factor-β (TGF-β) **(C)** were measured by using enzyme-linked immunosorbent assay (ELISA) in cell culture supernatants after 24 hours of challenge with silica (SiO_2) microparticles. Results are shown as mean ± standard deviation (SD) and are representative of 3 independent experiments (*$P < .05$).

conditions as erosive lichen planus, which tends to involve the gingiva in a diffuse pattern, predisposing multiple areas of the gingiva to the introduction of the particles.

Microscopic inflammatory changes

Most of the GLFMs showed mild or moderate chronic inflammation in a patchy distribution in the connective tissue. The inflammation was, in the majority of cases, composed predominantly of lymphocytes and plasma cells, and the foreign particles were consistently found intermixed with these inflammatory cells and sometimes appeared to be within the cytoplasm of histiocytes or fibroblasts. This microscopic appearance alone indicates that these particles are not inert and that they tend to incite a chronic inflammatory response in the host. This was, indeed, corroborated by our in vitro findings, which are discussed later in this section. Gordon and Daley[16] included in their study only cases demonstrating chronic inflammation, and, similar to our cases, they found that the distribution of the inflammation was patchy in the majority of their cases (69%). Those investigators found the inflammation to be granulomatous in 48% of their cases and both lichenoid and granulomatous in 26% of the cases. We did include cases showing a mixture of chronic and acute inflammation in our study, as did Koppang et al.[5] because the presence of superimposed bacterial infection inducing a neutrophilic response is quite plausible and unavoidable in gingival lesions. Nevertheless, the acute inflammation in our cases was usually localized, superficially in the lamina propria, and the inflammation adjacent to the foreign material was consistently chronic in nature. Granulomatous inflammation was extremely rare in our GLFM cases (encountered in only 2 cases). Gordon and Daley[16] described the inflammation as only lichenoid in 48% of their cases, whereas we found lichenoid inflammation in 24% of our cases, and this trend was statistically significant in comparison with the controls in our study. There was basal cell degeneration with or without an overall lichenoid inflammation in 47% of our cases and in 51% of the cases in Gordon and Daley's study.[16] We hypothesize that this finding might have resulted from the presence of very fine particles of the foreign material in the epithelium, inducing a T-cell cytotoxic response against the keratinocytes. Indeed, we occasionally detected minute foreign particles in the cytoplasm of epithelial cells during both light microscopy and scanning electron microscopy examinations of the specimens (images not shown). In fact, an in vitro study found that silica nanoparticles are internalized by human oral epithelial cells.[26] The lipid bilayer of the cell membrane may form transient pores, which may facilitate nonspecific uptake of SiO_2 nanoparticles, or these particles may be internalized via endocytosis in the case of particles smaller than 200 nm or micropinocytosis in the case of submicrometer particles.[27–29] Alternatively, in some of these cases, the basal cell degeneration or the presence of an overall lichenoid inflammatory process in GLFMs may represent true lesions of lichen planus, and the presence of foreign material in the connective tissue is just a coincidental finding secondary to the disrupted epithelial barrier.

Characteristics and composition of foreign materials

Foreign material particles appeared as only minute, black-gray fine granules (47% of our cases) or most commonly as a mixture of these dark, fine granules and larger, crystalloid refractile structures (52% of the cases). In only 1 case, the foreign particles were composed exclusively of crystalloid refractile structures. This is in contrast to the observations by Gordon and Daley,[16] who found only opaque fine particles in 13% of their cases, all refractile in 44% of the cases, and a mixture in 43% of the cases. Similar to our findings, Koppang et al.[5] found that 31.8% of their cases demonstrated a mixture of black granules and crystalloid particles, and only 4.7% of their cases showed only crystalloid structures; however, 24% of their cases showed features compatible with amalgam because these investigators had included biopsy specimens diagnosed as amalgam tattoos in their study. Similar to those previous studies,[5,16] the fragments detected in our study were very small, mostly measuring 1 to 5 μm in greatest diameter. However, the fine, black-gray granules tended to aggregate and form larger clusters of granules, and therefore, only the greatest dimension of the aggregates were measured in some of the cases, rather than each granule being individually measured. Therefore, some of the foreign particles found in our study were actually in the nanoparticle size category (<0.1 μm). In fact, humans are frequently exposed to nano-sized particles that are added not only to dental materials but also to a vast number of consumer products, including food additives, ingredients in food packaging, and cosmetic articles (e.g., toothpaste, sunscreen, and lipstick). These nanomaterials are added to increase material properties, product quality, and ease of use.[30] SiO_2 nanoparticles were ranked among the most commonly used nanoparticles in a total of 580 consumer products that included nanoparticles, according to the Woodrow Wilson International Center for Scholars inventory.[31] The projections are that the number of products using nanomaterials will only increase, and the most updated Woodrow Wilson inventory now contains 1628 consumer products that are manufactured using nanotechnology.[32]

Si was the most prevalent chemical element in GLFMs, with 94% testing positive for its presence. Ca, Al, and Zn were also frequently found in our specimens. Si (61%) was also the most common element found by Gordon and Daley,[3] followed by Al (46%) and iron (43%). Koppang et al.[5] found silver (67%) to be more common in their specimens, followed by

Al (66%) and Si (61%). Silver was the most commonly encountered element in the latter study, likely as a result of the inclusion of amalgam tattoos in their investigation.

The element with the greatest coexistence with Si in our cases was Ca, which was detected in 69% of all Si-positive samples. Al came in at a close second, with detection in 67% of all Si-positive samples. Previous studies that performed EDX analysis of several commonly used dental materials found Si and Al to be among the most common elements of the studied dental materials.[3,5] This finding, combined with the frequency that dental materials are manipulated adjacent to the gingival margin, makes a strong argument that these foreign particles are iatrogenically introduced during professional or self-administered dental and oral hygiene procedures. The combination of Si and Ca in dental materials may be seen in temporary cement and pumice powder.[5] While the combination of Si and Al is indicative of sand from grinding wheel, abrasive disk, pumice, or prophylaxis paste. Si alone was typically found in carborundum powder or carborundum grinding disks.[5] In one study the majority of the foreign materials in the FBG cases were interpreted as either definitively of dental origin or of probable dental origin, usually abrasives.[3] We believe that SiO_2 is the major source of Si in the embedded foreign bodies, considering that, as previously stated, SiO_2 and silicates are widely used and found in many consumer products, including toothpaste and many dental materials.[33,34] This likelihood remains significant, even though it is not feasible to directly identify SiO_2 by using EDX, which does not distinguish Si-associated oxygen from oxygen found in biologic tissues.

Verrucous and dysplastic changes of the epithelium

The most important microscopic findings we detected in our GLFM cases was the presence of verrucous hyperplasia in 59% of the cases and epithelial dysplasia in 24 cases (28%), with both trends showing statistical significance in comparison with the controls. Gordon and Daley[16] reported that 59% of their cases showed hyperkeratosis and 57% showed acanthosis; however, neither verrucous hyperplasia nor dysplasia was reported in that study. In fact, to the best of our knowledge, this is the first time an association between the presence of foreign materials in the gingiva and the occurrence of verrucous hyperplasia or epithelial dysplasia has been reported in the literature.

The 2017 WHO definition of leukoplakia, which is the most common potentially malignant (i.e., premalignant) disorder of the oral cavity, is the following: "a clinical term used to describe white plaques of questionable risk, once other specific conditions and other oral potentially malignant disorders have been ruled out."[17] Many of our studied cases seem to fit this definition; indeed, in 21% of the cases "leukoplakia" was the clinical diagnosis provided by the clinician. Moreover, we found a great proportion of verrucous epithelial hyperplasia in our GLFM cases, and other studies have found that leukoplakia showing a verrucous architecture has an increased risk of progression to malignancy compared with nonverrucous or homogeneous leukoplakia.[35] In addition, although the majority of the verrucous hyperplasia cases that we identified did not demonstrate epithelial dysplasia, it has long been recognized that oral lesions showing verrucous epithelial proliferations of unknown cause (i.e., atypical verrucous hyperplasia), even without dysplasia, are a distinct subset of potentially malignant oral disorders.[18] Such verrucous hyperplasia is characteristically associated with the distinct, multifocal, and aggressive form of potentially malignant disorder known as *proliferative verrucous leukoplakia* (PVL).[18,36] PVL frequently involves gingiva, is more commonly seen in female patients older than 60 years of age and exhibits a strikingly high malignant transformation rate.[37,38] Patients with PVL usually do not have risk factors, such as tobacco smoking and alcohol abuse, which are typically seen in patients with other potentially malignant oral disorders.[36,39] Accordingly, given the clinical, microscopic, and demographic similarities between PVL and GLFMs, it is imperative that the presence of foreign material particles in lesions of PVL be investigated further.

The presence of epithelial dysplasia in 28% of GLFMs raises concerns about the potential genotoxic effects that these particles might exert in the oral epithelium. In most of the cases showing epithelial dysplasia, verrucous hyperorthokeratosis was also concomitantly found (see Figure 1C). According to the 2017 *WHO Classification of Head and Neck Tumours,*[17] oral epithelial dysplasia "is a spectrum of architectural and cytologic epithelial changes, associated with an increased risk of progression to squamous cell carcinoma." It is well established that carcinogenesis is a consequence of accumulation of DNA damage in a cell. Si was one of the elements most frequently found in previous studies[3,5] of GLFMs as well as in our present study. Si is usually found in dental materials and toothpaste as SiO_2 or silicates.[33,40] Although the effects of SiO_2 microparticles in the oral tissues is poorly understood, there are numerous studies on the effects of particularly crystalline SiO_2 in lung tissues. The body of evidence on the subject resulted in the IARC classifying crystalline SiO_2 as a group 1 carcinogen since 1997.[40] The genotoxic modes of action of crystalline SiO_2 microparticles in the lungs were summarized by Borm et al.,[41] who described the possible mechanisms as direct, indirect, or secondary. Direct, primary genotoxicity of particles is caused by a mechanism involving direct interaction of particles with genomic DNA. This may occur via oxidative attack by reactive oxygen species (ROS) at the particle surface or direct physical interaction

with DNA upon entry of the particles into the nucleus. Indirect primary genotoxicity has been described as the enhanced production of ROS by cellular constituents, such as mitochondria and membrane-bound nicotinamide adenine dinucleotide phosphate oxidases, in response to their interaction with foreign particles and/or through depletion of antioxidants, such as glutathione, within the cell. Secondary genotoxicity is driven by host inflammatory cells (macrophages and neutrophils). These cells are recruited to sites of particles deposition through elicitation of the inflammatory response and, in situations of a chronic inflammatory response, may lead to persistent oxidative stress and DNA damage in an environment containing various prosurvival and proliferation signaling molecules.[41,42] This may result in the accumulation of genetic defects, which may facilitate the transformation of cells into a malignant or potentially malignant phenotype.[41] Of these different modes of action, most studies seem to support the notion that the mechanism of crystalline SiO_2 carcinogenicity in the lung is a secondary, inflammation-driven one. It appears that impaired particle clearance leading to macrophage activation and persistent inflammation is the established mechanism for both silicosis and tumorigenesis in the lung.[12,41] In vitro studies have found that amorphous SiO_2 nanoparticles could penetrate into the cell nucleus of human buccal epithelial cells[26] and Hep-2 human laryngeal carcinoma cells.[43] Thus, SiO_2 nanoparticles can come in close contact with genetic material, and this raises the concern that these nanoparticles may exert direct undesirable genotoxic effects. In fact, another in vitro study found that chronic exposure to low-dose SiO_2 nanoparticles causes altered gene expression and induces malignant transformation of human lung epithelial cells via p53 signaling.[44]

The presence of SiO_2 is ubiquitous in dental materials and personal oral hygiene products. Consequently, development of silicosis of the lung has been reported in the dental laboratory personnel as well as in dental supply workers.[45,46] Nevertheless, the effects of either amorphous or crystalline SiO_2 deposition in the oral tissues of dental patients has not been well studied. Few studies have examined the effects of SiO_2 in oral tissues, even though these tissues are susceptible to these particles as confirmed in this and previous studies.[5,16] An in vitro study found that treatment of oral epithelial cell cultures with SiO_2 nanoparticles enhanced intracellular ROS levels and upregulation of the inflammatory genes tumor necrosis factor-α and IL-6 in a dose-dependent manner.[26] Nevertheless, the effects of continuous SiO_2-induced chronic inflammation and consequent persistent oxidative stress in the proliferation and possible carcinogenesis of gingival epithelium needs to be better elucidated.

In vitro inflammatory response of gingival fibroblasts

Because the presence of the foreign material containing SiO_2 in the majority of the cases was observed in the connective tissue, we hypothesized that gingival fibroblasts would be a biologically relevant cell type to study the effects of SiO_2 in vitro. Therefore, we measured the levels of COL-1 and the proteolytic activity of MMP-2, which are both involved in the dynamics of remodeling of the extracellular matrix. The increased expression of COL-1 and MMP-2 activity in SiO_2-stimulated gingival fibroblasts indicate that these cells responded to stimulation with SiO_2 microparticles, regardless of the 3 concentrations of SiO_2 used. Increased MMP-2 activity is an important regulatory step in the degradation of collagen[47] and the activation of COL-1 coincides with increased MMP-2 activity in many other models.[48,49]

In this study, SiO_2-stimulated fibroblasts demonstrated higher levels of inflammatory cytokines, such as CXCL-8, IL-6, and TGF-β. The effects of SiO_2-induced cytokine responses have been extensively studied in models mimicking lung inflammation, such as endothelial cells[50] or lung alveolar macrophages and fibroblasts.[51] It was reported that crystalline SiO_2 induced CXCL-8 release from human lung epithelial cells.[52] Previous work has suggested that IL-1β is an important triggering factor that determines SiO_2-induced release of several other cytokines.[50] Taken together, these results demonstrate the relevance of fibroblasts as the predominant cell type in the connective tissue in SiO_2-induced inflammatory responses in the gingiva. More importantly, our findings suggest the relevance of SiO_2 as a foreign material because it induces inflammatory responses in gingival fibroblasts, disturbing the homeostasis in the connective tissue and potentially causing pathologic changes.

CONCLUSIONS

This study characterized a large number of human gingival biopsy specimens containing foreign material. The presence of foreign material was significantly associated with clinically visible white plaques. Microscopically, the presence of foreign material was significantly associated with patchy chronic inflammation, basal cell degeneration, and the presence of verrucous hyperorthokeratosis and epithelial dysplasia. Our EDX results found that Si, which is usually found in nature as SiO_2, is the most frequent chemical element found in the foreign material. Importantly, our in vitro results demonstrated that gingival fibroblasts are activated by SiO_2 microparticles and respond with increased levels of inflammatory cytokines. These results, when viewed in light of the body of evidence in the literature demonstrating the deleterious effects of SiO_2 in human tissues, should raise awareness of the effects of persistent SiO_2-induced chronic inflammation in the gingival tissues.

DISCLOSURE

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PRESENTATION

The results of this study were presented as an oral abstract at the Joint International Association of Oral and Maxillofacial Pathologists and the American Academy of Oral and Maxillofacial Pathology Meeting, in Vancouver, Canada, in June 2018.

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