MAJOR ARTICLE

Bacterial Infection as a Likely Cause of Adverse Reactions to Polyacrylamide Hydrogel Fillers in Cosmetic Surgery

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Background. The etiology of long-lasting adverse reactions to gel fillers used in cosmetic surgery is not known. Bacterial infection and immunological reaction to the product have been suggested.

Methods. We performed a case-control study, with 77 biopsies and 30 cytology specimens originating from 59 patients with adverse reactions to polyacrylamide gel, and 54 biopsies and 2 cytology specimens from 28 control subjects with no adverse reactions. Samples from 5 patients and 4 controls could not be investigated for presence of bacteria owing to limited material. Samples from the remaining 54 patients and 24 controls were systematically examined for the presence of bacteria by culture, 16S rRNA gene sequencing, Gram stain, and fluorescence in situ hybridization.

Results. Bacteria, mostly normal skin bacteria such as *Staphylococcus epidermidis* and *Propionibacterium acnes*, were identified in bacteriologically investigated samples from 53 of 54 patients (98%), and in none of the 24 controls (0%). The bacteria were lying in small clusters, which in symptomatic lesions were detected up to 5 years postinjection.

Conclusions. Commensal bacteria of low virulence are capable of producing long-term infection in the presence of polyacrylamide filler in cosmetic surgery, possibly due to a biofilm mode of growth. Adequate skin preparation and use of sterile technique in these procedures are mandatory, but antibiotic prophylaxis prior to injection of nondegradable gels like polyacrylamide should be explored as well.

Keywords. foreign body infection; biofilm; polyacrylamide gel filler.

The widespread use of gel fillers for cosmetic applications is accompanied by a steadily increasing number of long-lasting adverse reactions [1–3]. According to the US Food and Drug Administration (FDA), serious adverse reactions are defined as fatal or life-threatening lesions and lesions requiring hospitalization for at least 24 hours or intervention to prevent permanent impairment [4]. Serious adverse reactions to fillers reported to the US FDA during the years 2008–2011 have tripled (from 457 to 1309) compared to 2005–2007 [4], and the most widely used fillers (hyaluronic acid hydrogels, longer-lasting collagen gels, and slowly degrading particulate gels) were also those responsible for the rise in these adverse reactions [4]. Long-lasting adverse reactions have mainly been reported in small series or case reports. In the only 3 clinical studies with long-term follow-up, these reactions were found in 17 of 251 (6.8%) patients injected with polyacrylamide gel [5], in 41 of 221 (18.6%) patients injected

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with slowly degradable poly-L-lactic acid gel [6], and in 5 of 111 (4.5%) patients injected with polymethylmethacrylate microspheres/collagen gel [7]. According to Ceresana Research, the filler market will grow by about 2.5% per year with huge expected global revenues of approximately US$22.5 billion in 2018 [8], and although incidence of gel injections is not registered and numbers and brands of gel syringes used are not publicly available, the high frequency of long-lasting adverse effects poses a considerable burden on healthcare systems.

Adverse reactions to fillers are predominantly characterized by indurations at the injected sites, which may be called lumps, nodules, swellings, or granulomas. The etiology of these is not clear. The fillers vary in composition and longevity, and although anti-inflammatory and other symptomatic drugs have been used in a high proportion of cases, albeit with varying efficacy [9–11], it may be impossible to treat these adverse reactions with anything but surgical intervention [12]. For hydrogels such as polyacrylamide gel (PAAG) and hyaluronic acid gel, we [3, 13, 14], and others, [15] have suggested bacterial infection—possibly in a biofilm mode of growth—as a potential cause of adverse effects. Bacteria have indeed been identified in 15 such lesions following PAAG injections [13], but a substantial large-scale investigation has not been done.

This case-control study presents a unique series of 107 samples from 59 patients with adverse reactions to PAAG and 56 samples from 28 controls. The samples were collected over 9 years and 3 months and systematically investigated for the presence of bacteria.

METHODS

Study Design

A multicenter case-control study was carried out from 19 September 2002 to 31 December 2011. Informed consent was obtained from all patients and controls (see below) to having their cell or tissue samples examined and used for scientific purposes under total anonymity.

The primary aim of the study was to investigate if adverse reactions to PAAG were associated with the presence of bacteria, and the secondary aim was to examine and compare the different types of cellular foreign-body response in these reactions.

Clinical Samples

Soft tissue biopsies and cell smears/fine-needle aspirates from 87 subjects (59 patients and 28 controls) were collected, primarily from the face (77%) and in particular the lips (53%). Data included age, sex, site of injection, time since latest injection, and treatment prior to the collection of specimens. Samples were mainly received from participating centers represented by authors V.B. (10 patients, 21 controls), M.J. (9 patients), and N.P. (3 patients), but also from 32 other physicians in Denmark, Sweden, Norway, Australia, The Netherlands, the United Kingdom, Austria, Germany, Italy, Spain, Portugal, the United States, and Iran, each contributing samples from 1–3 patients (in total 37 patients, 7 controls). The 32 cytology specimens, 30 from patients and 2 from controls, were either cell smears or fine-needle aspirates, arriving on glass slides. The 131 histology specimens, 77 from patients and 54 from controls, were either needle core or excision biopsies, arriving as cut sections on glass slides or as formalin-fixed tissue for paraffin embedding.

Samples from adverse reactions were collected from gel-injected sites with clinical symptoms such as swelling, pus secretion or firmness, pain, redness, warmth, and paresthesia.

Samples from control subjects were collected from similar sites, in connection with overcorrection or asymmetry, excision of nevi covering gel-injected areas, face-lift of areas that had previously been injected with gel, and scar revisions of previously gel-injected sites.

Diagnosis

Cytology and histology specimens were routinely stained with May-Grünwald-Giemsa (MGG) or hematoxylin-eosin (HE) for morphology, and Van Gieson/Alcian blue for connective tissue and gel, respectively. The primary criterion for determining bacterial etiology was histomorphological (ie, a positive Gram stain). However, whenever possible, additional 16S ribosomal RNA (rRNA) gene sequencing, culture, and fluorescence in situ hybridization (FISH) analysis from the same sites were done as well (see below).

Further histological sections were stained with Van Gieson/Alcian blue for connective tissue and gel, and with macrophage marker CD68 (clone pgm1) (Dako, Glostrup, Denmark) to illustrate the foreign-body response of the lesions. Six already MGG-stained cytology specimens with little material originating from 3 patients and 3 controls, and 3 already HE-stained glass slides of biopsies from 2 other patients and 1 control, could not be subjected to bacteriological analysis. As all samples were received in consultation, the pathologist (L.C.) was not blinded.

Tissue sections were also analyzed by sequencing of part of the 16S rRNA gene. DNA was purified using the Qiagen Blood and Tissue Kit (Qiagen, West Sussex, UK), and the first 500 bases of the bacterial 16S rRNA gene were amplified by polymerase chain reaction and sequenced on a ABI377 3130 × l sequencer using either the MicroSeq 500 16S rDNA Bacterial Identification System or the Big-Dye Cycle Sequencing Kit, both according to the manufacturer's guidelines (Applied Biosystems, Foster City, California). Bacteria were identified using the MicroSeqID database or BLAST (BLASTN 2.2.26, National Center for Biotechnology Information).

Deparaffinized tissue sections were also examined by FISH for identification of bacterial rRNA using a fluorescent-labeled universal peptide nucleic acid (PNA) probe directed against a

region universal for bacteria on the 16S rRNA [14]. Confocal laser scanning microscopy (CLSM) was used for visual verification. The FISH analyses were, if possible, combined with corresponding 16S rRNA gene sequence analysis. The molecular biologists (E.H., T.B., S.E.) conducting these tests were blinded with respect to patient data.

Grading

Each sample was initially graded for inflammation using routine histological stains and CD68 immunohistochemistry to identify macrophages/giant cells. Grading was as follows: normal—normal gel interspersed with a fibrous network; adverse reactions—grade 1, hyperacute inflammation: edema dominated by thick rims of swollen macrophages/giant cells and relatively few granulocytes; grade 2, acute/subacute inflammation: rims of normal sized macrophages/giant cells and many granulocytes; grade 3, chronic inflammation: fibrosis, thin rims of macrophages/giant cells, lymphocytes and occasional granulocytes; grade 4, late onset/de novo inflammation: rims of normal size macrophages/giant cells with many granulocytes surrounded by a normal gel network.

Clinical Information

Information on the site of adverse reaction, initial treatment prior to sampling, and time from injection to sampling was given by the treating physician.

Statistics

Time from injection to sampling for controls and for patients with different grades of adverse effects was compared using Mann-Whitney tests, and frequencies of bacteria-positive patients and controls were compared by Fisher exact test using GraphPad software.

RESULTS

A total of 77 biopsies and 30 cytology samples from 59 patients (8 men and 51 women) and 54 biopsies and 2 cytology specimens from 28 controls (3 men and 25 women) were investigated (Table 1). The hydrogel was predominantly the Aquamid product (Contura, Soeborg, Denmark [54 patients and 27 controls]). The few other subtypes were Interfall (Bioform, Moscow, Russia [1 patient with chronic inflammation and 1

Table 1. Overview of Patients and Controls, Samples, Detection of Bacteria by Different Methods, and Their Identity

		Patients				
Characteristic	Controls	Grade 1[a]	Grade 2[a]	Grade 3[a]	Grade 4[a]	Total
No. of subjects	28	13	19	21	6	59
Age range[b], y	27–78					18–86
No. of biopsies	54	11	19	39	8	77
No. of cytologies	2	10	14	3	3	30
No. of subjects inspected for bacteria	24	13	16	20	5	54
Bacteria by culture	0	3	4	2	1	10
Bacteria by Gram stain	0	13	15	18	4	50
Bacteria by 16S gene sequencing	0	4	6	5	2	17
Bacteria by FISH	0	3	2	8	4	17
Bacteria by any method	0	13	16	19[c]	5	53
Patients positive for bacteria by any method of those inspected, %	0	100	100	95	100	98
Bacterial species identified in each patient group						
Propionibacterium acnes		2	6	3	2	13
Staphylococcus epidermidis		2	4	4	0	10
Staphylococcus aureus		0	1	0	0	1
Streptococcus oralis		1	0	0	0	1
Streptococcus mitis		0	0	2	1	3
Streptococcus sanguinis		1	1	0	0	2
Burkholderia cepacia complex		0	1	0	0	1
Veillonella		0	1	0	0	1
Serratia marcescens		1	0	0	0	1

Abbreviation: FISH, fluorescence in situ hybridization staining.

[a] Grades of inflammation: 1, hyperacute; 2, acute/subacute; 3, chronic; 4, late onset/de novo.

[b] Age was not known for 2 patients.

[c] One sample negative by Gram staining, 16S sequencing, and FISH.

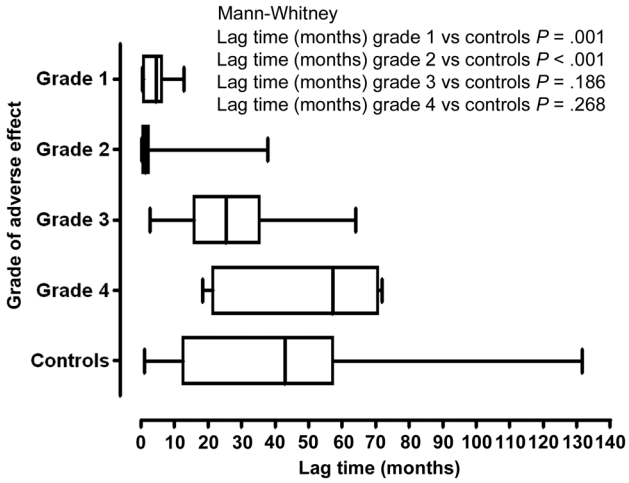

Figure 1. Lag time from gel injection to biopsy collection for the 4 grades of inflammation and for controls. As expected, lag time differences were seen among the 4 patient groups. The lag time for patients with grade 1 and grade 2 adverse effects was significantly shorter than for controls ($P = .001$ and $P < .001$, respectively).

control, both cytological samples]), and Bio-Alcamid (Polymekon, Brindisi, Italy [2 patients with chronic inflammation]). The age range was 18–86 years (mean, 46 years; median, 43 years) for patients and 27–78 years (mean and median, 44 years) for controls. Age was unknown for 2 patients. Patients and controls did not differ with respect to sex, age, size, site of gel deposit, or general health (data not shown). Time from injection to sampling ranged from 9 to 2161 days (mean, 492 days; median, 213 days) for patients with adverse reactions, and from 30 to 3947 days (mean, 1181 days; median 1292 days) for controls. For patients with grade 1 and grade 2 adverse effects, this lag time was significantly shorter than for the controls ($P = .001$ and $P < .001$, respectively, Figure 1).

At the time of tissue or cell sampling, all the patients had already been treated for their adverse reaction with several drugs including antibiotics. This was not the case for any of the controls.

Of all patients with adverse reactions to PAAG for whom bacterial analysis could be performed (n = 54), 53 (98%) showed the presence of bacteria, usually lying in small groups. No bacteria were detected in any of the 24 eligible controls ($P < .0001$, Table 1). The distribution according to grade of inflammation was as follows:

- Grade 1: Hyperacute inflammation (13 patients) was seen in biopsies and cell smears from patients who had been treated initially with steroids or large doses of nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce the initial inflammatory swelling. After a transient relief, symptoms returned and progressed with an excessive macrophage swelling, and although granulocytes were relatively few in numbers, fistulation with pus discharge ensued. Subsequent or simultaneous administration of antibiotics, generally fluoroquinolones, macrolides, and penicillins in standard regimes, had been ineffective. A positive culture was obtained in 3 of the patients, a positive 16S gene sequencing analysis was found for 6 patients, and a positive FISH analysis was found in 3. Gram-positive bacteria, generally lying in small groups, were seen by light microscopy in samples from all patients (Table 1). Eosinophils were not part of these or any other of the inflammatory lesions.
- Grade 2: Acute/subacute inflammation (19 patients) was generally seen in case of delayed start of antibiotic treatment or use of antibiotics with a narrow antibacterial range, usually penicillins (Table 1). For 3 patients, only prestained MGG cytology slides (n = 2) and prestained HE section (n = 1) were available and thus these patients were not eligible for bacteriological analysis. The remaining 16 patients tested positive for bacteria by at least 1 of the bacteriological tests (Table 1, Figures 2, 3, 5), and the biopsies displayed a distinct CD68 positive rim of macrophages surrounding the gel deposit (Figure 2).
- Grade 3: Chronic inflammation (21 patients) characterized by fibrosis (Figure 2) was seen after injection of large gel volumes, which had only given slight symptoms (usually paresthesia), present from the start (Table 1). Bacteria were detected in 19 patients by at least 1 of the bacteriological tests, 7 of the 9 being of the commensal species *Propionibacterium acnes* (n = 3) or *Staphylococcus epidermidis* (n = 4) (Table 1, Figures 2 and 4). One patient with a biopsy that had been examined by Gram stain, 16S gene sequencing, and FISH remained negative for bacteria. Another patient was represented only by a prestained HE slide of the biopsy and thus not eligible for bacteriological analysis.
- Grade 4: De novo inflammation (6 patients) was seen in patients who had been injected with PAAG years before and then suddenly presented with symptoms of acute infection in the injected area. A history of recent injury (ie, lip biting, recent filler injection, botulinum toxin injection, or dental nerve block) was reported for them all. From 1 patient only an MGG prestained cytology specimen was available; thus, this patient was not eligible for bacteriological analysis. Bacteria were detected in all remaining patients by at least 1 of the bacteriological tests (Table 1).

None of the samples from the controls showed any sign of inflammation.

Species of Bacteria

The following bacteria were identified by culture and/or 16S gene sequencing: *P. acnes* (13 patients), *S. epidermidis*/species

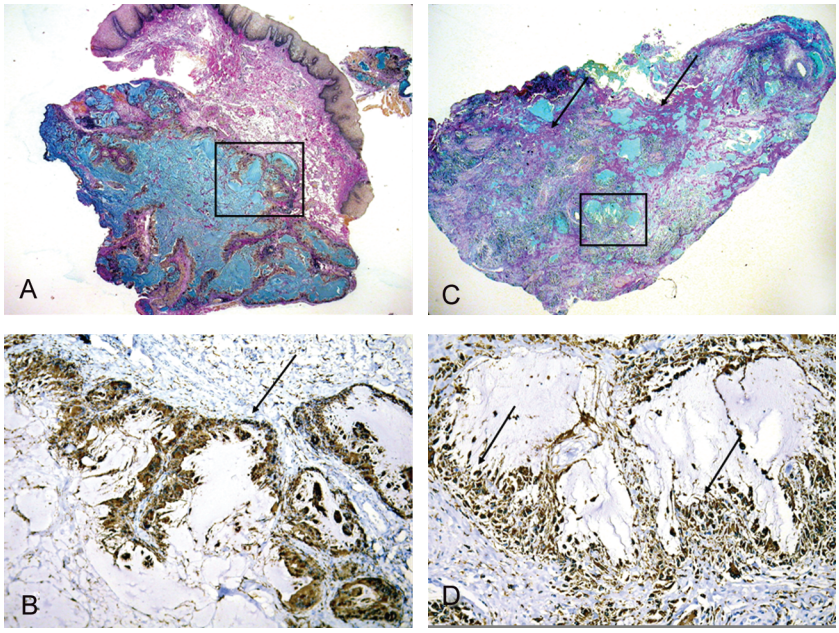

Figure 2. Grade 2 (*A* and *B*) and grade 3 (*C* and *D*) reactions in biopsies from lip and hand tissue seen after 3 weeks and 1 year, respectively. *Propionibacterium acnes* was found by culture in the early lesion and *Staphylococcus epidermidis* was found by 16S gene sequencing in the late lesion. The turquoise gel in (*A*) is surrounded by a rim of macrophages/giant cells, which in (*B*) shows dark brown positivity for CD68 (arrow). In the late reaction (*C*), the gel is split up by red fibrous tissue (arrows), but in some areas it is also surrounded by a rim of CD68-positive macrophages/giant cells (*D*). *A* and *C*, Van Gieson/Alcian blue ×60. *B* and *D*, CD68 (pgm1) immunostaining ×100.

[10], *Staphylococcus aureus* [1], *Streptococcus oralis/mitis/* species [3], *Streptococcus sanguinis* [2], *Burkholderia cepacia complex* [1], *Veillonella* [1], and *Serratia marcescens* [1].

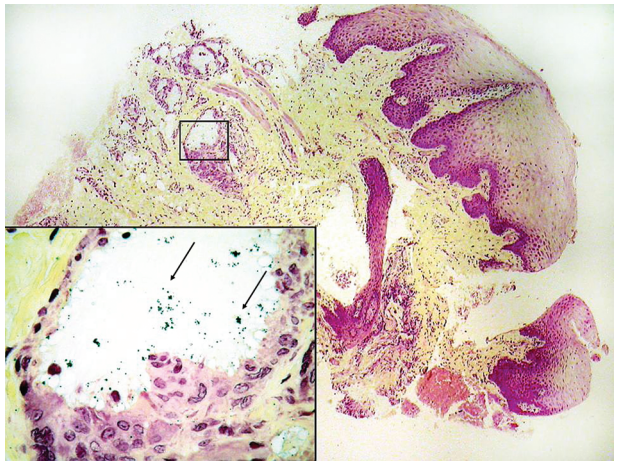

Figure 3. Lip biopsy with inflammation, grade 2, at 2 months with a few small gel pools surrounded by macrophages and containing many small groups of gram-positive bacteria identified as *Propionibacterium acnes* by 16S gene sequencing (inset, arrows). Gram stain ×60; inset: Gram stain ×400.

Staphylococcus aureus was the only bacterium associated with clinical symptoms already at day 2 after the gel injection. In all other cases, clinical symptoms did not become apparent until after at least 9 days. Bacteria detected by Gram stain were invariably gram-positive cocci or short rods (Figure 3), and bacteria detected by FISH were the same (Figures 4–6). A possible correlation between the species of bacteria and the type of inflammation, detection method, or time from injection to debut of symptoms could not be investigated, because the numbers of samples with identified bacterial species were small (Table 1).

DISCUSSION

It is widely assumed that adverse reactions to gel fillers are caused by product–tissue incompatibility and can be treated with steroids [16]. However, our results with PAAG have shown that infection as a possible cause is probably severely underestimated.

The study had its limitations with respect to material and hence the extent to which the samples could be examined for bacteria, but the message was clear: Foreign bodies predispose for infection, which is difficult to treat [17], and despite the fact that PAAG is a constantly undulating hydrogel that

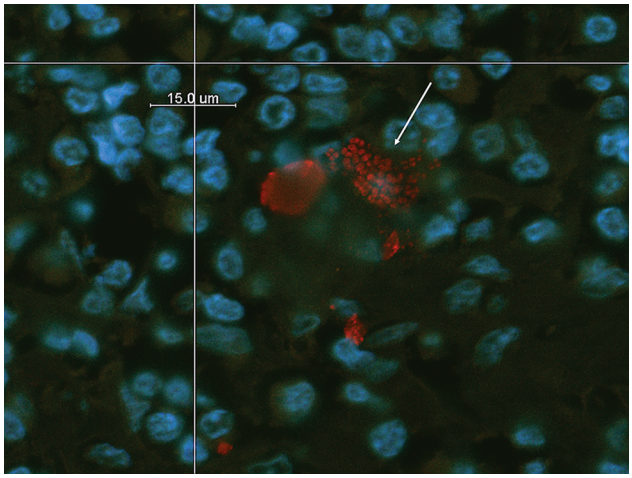

Figure 4. Three-dimensional confocal laser scanning microscopy (CSLM) of a biopsy from a grade 3 reaction following gel injection into the cheek 2 years previously. Peptide nucleic acid fluorescence in situ hybridization staining was used to visualize aggregates of bacteria (small red microspheres; arrows). The large blue dots represent DAPI nuclear counterstain, which was included to illustrate the predominating mononuclear cell type in these lesions. *Staphylococcus epidermidis* had been identified by 16S gene sequencing. CLSM magnification ×1000.

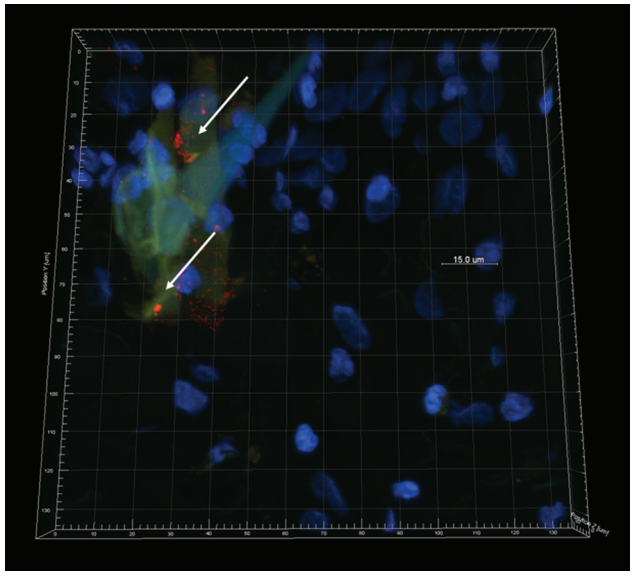

Figure 5. Three-dimensional confocal laser scanning microscopy (CLSM) from a grade 2 lesion occurring 9 days after gel injection into the glabella. Peptide nucleic acid fluorescence in situ hybridization staining visualization of bacteria (red) lying in irregular formations within an opaque yellow-green material, probably polyacrylamide gel (arrows). *Propionibacterium acnes* had been identified by culture and 16S gene sequencing. CLSM magnification ×1000.

exchanges its 97.5% water molecules with those of the surrounding tissues [18], histomorphological signs of infection with an increased foreign body reaction were present in all adverse reactions of any type, and with only 1 exception bacteria were demonstrated in all samples subjected to bacteriological analysis. It is therefore highly likely that bacteria are the cause of these adverse reactions, residing within the gel as part of an ongoing inflammatory process that may persist for years (Table 1, Figure 1) despite antibiotic treatment. Bacteria are known to amplify the immune response to biomaterials [19, 20], which in this study was seen as rims of macrophages/giant cells around gel pools (Figures 2 and 3). Although cultures of the majority of samples were negative, bacteria were identified by 1 or more of the other methods (Gram stain, FISH, or 16S gene sequencing; Table 1).

In biomaterial-associated infection, bacteria are present on the biomaterial surface as biofilms [16], as well as in the surrounding tissue and even intracellularly [20–22]. The survival rate of microorganisms in a biofilm is high, even when exposed to antibiotics [17], as is the case with bacteria in periimplant tissue in experimental biomaterial-associated infection [22]. Irrespective of the clinical presentation—lump, nodule, granuloma or cystic lesion—the bacteria were invariably seen lying in microcolonies (Figures 3–6).

The mechanism behind the severe clinical symptoms and characteristic microscopic features as observed in patients with

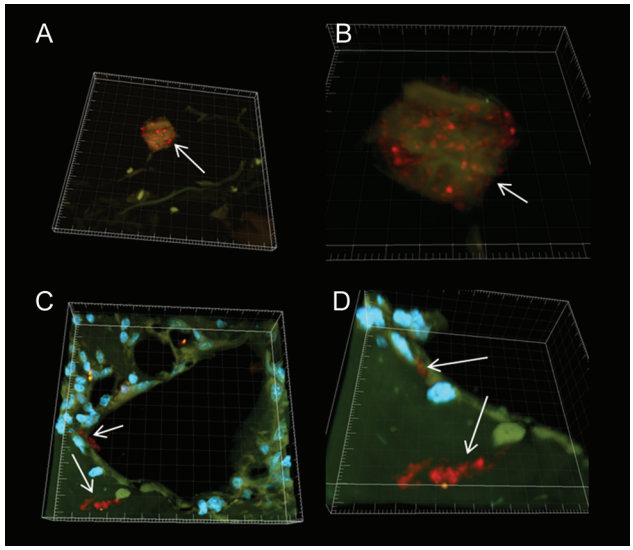

Figure 6. Aggregates of bacteria (*Propionibacterium acnes*) in a lip biopsy showing a grade 2 lesion 6 months after gel injection (arrows). This biopsy showed many macrophages, which were identified by their round, large blue DAPI positive nuclear dots by 3D confocal laser scanning microscopy. Frame (*B*) is an enlargement of frame (*A*), and frame (*D*) is an enlargement of frame (*C*). The slightly opaque yellow-green material is polyacrylamide gel. *A* and *C*, ×600. *B* and *D*, ×1000.

grade 1 inflammation having received anti-inflammatory drugs remains to be studied in more detail. These drugs suppress the inflammatory response of host tissue and cells [23, 24], indeed causing initial reduction of the swelling reported for the treated patients. They also give rise to a pathological overshoot or a reactive excessive foreign-body reaction, reported after accelerated withdrawal of anti-inflammatory drugs [23]. This overshoot has most likely been responsible for the excessive swelling of tissue and cells seen in the hyperinflammatory reactions.

In conclusion, PAAG stands out as a valuable gel filler with an excellent tissue integration but also with an inherent risk of supporting expansion of a population of contaminating bacteria, which most likely enter a dormant state allowing them to survive antibiotics and cause chronic infection. Because adverse reactions reported for other types or brands of fillers are at least equally prevalent, it cannot be ruled out that infection may occur with those fillers as well.

In view of the strongly expanding use of gel fillers with increasing longevity for cosmetic application, prospective trials on the efficacy of prophylaxis with appropriate antibiotics to prevent infection are warranted to avoid serious morbidity for patients and increasing healthcare costs. However, skin preparation and sterile technique performed in these procedures is of utmost importance as well. Antibiotic prophylaxis alone will not overcome inadequate sterile techniques.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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