The Ever-Changing Role of Biofilms in Plastic Surgery

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Summary: The goal of this article is to present a brief background of biofilms and how they pertain to plastic surgery. Of particular interest are how biofilms affect breast prosthesis and their subsequent complications. The authors also present information on how biofilms are involved in soft-tissue filler complications. After a brief review of the pathophysiology of biofilms and their effect on plastic surgery, the authors propose a revised algorithm to assist in guiding the plastic surgeon when faced with complications that involve biofilms that involves more rapid diagnosis and treatment using polymerase chain reaction technology. This article is a review of recent literature. (*Plast. Reconstr. Surg.* 133: 865e, 2014.)

Biofilms are prevalent and pervasive in the medical field, costing greater than $1 billion annually and being responsible for up to 80 percent of all infections[1,2] (Fig. 1).[3] This relative omnipresence is relevant with regard to indwelling medical prostheses, as in capsular contracture with breast implants and delayed complications with soft-tissue fillers. Recent advances in the use of polymerase chain reaction allow for rapid identification of biofilm microbes and for polymerase chain reaction culture-directed antibiotic therapy as opposed to empiric coverage. Using polymerase chain reaction, a more expeditious identification of an infectious microbe can lead to faster treatment and thus could lead to lower overall medical costs and improved outcomes. Although most accept their existence, the impact of biofilms on the medical field has yet to be fully appreciated.

Characterized as a microbial community that has produced a polymeric matrix that is irreversibly adherent to both living and nonliving surfaces, biofilms account for 99.9 percent of all microbial biomass on earth.[4] Biofilms are among the main sources of contamination in water, medical prostheses, and catheter-related infections.[5] Although biofilms were originally conceptualized in 1978, visual observation proved more elusive until the application of the scanning electron microscope.[6]

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Existing within this structurally heterogeneous polymeric matrix, bacteria are able to communicate through a process termed quorum sensing, which has been shown to be instrumental to biofilm production and differentiation.[7,8] Through this cell-to-cell signaling, biofilms seemingly act as an independent organism responding in kind to stimuli, growing, and maintaining homeostasis.[9] This polymeric matrix, however, not only exists as an environment for bacteria but may also impede phagocytosis.[10] Furthermore, biofilms act as a facilitating medium for extrachromosomal DNA plasmids that may confer antibiotic resistance up to 1000 times greater than planktonic bacteria[2,11,12] (Fig. 2).[13]

The biofilm life cycle can be characterized by stages of attachment, growth, and detachment[14] (Fig. 3).[15] Biofilms are established when free-floating bacteria adhere to living or inert surfaces and become sessile. Their formation can be rapid, as microcolonies can be detected within 8 to 10 hours after infection.[16,17] The biofilm matrix demonstrates progressive cell layering, resulting in an average cell thickness of 50 to 100 μm, and serves as a medium for the transfer of exogenous solutes, nutrients, and oxygen.[12,18] Although the microbial burden resulting from biofilm detachment has not been well established in a clinical setting, biofilms are known to passively detach by erosion and sloughing and actively seeding microcolonies and single planktonic cells.[5]

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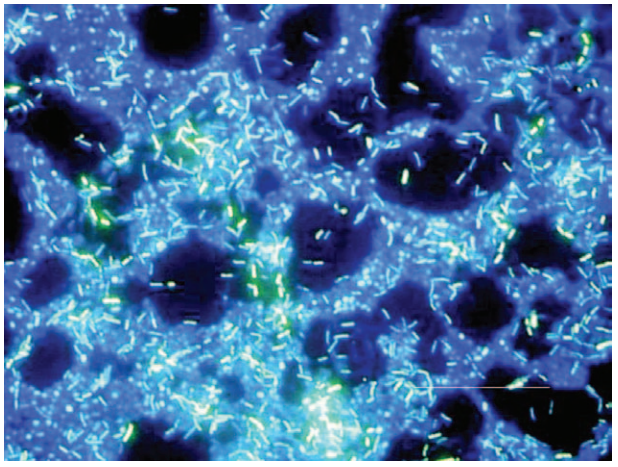

Fig. 1. Polymicrobic biofilm examined by epifluorescence microscopy. *Scale bar* = 20 μm. (Reprinted from Donlan RM. Biofilms: Microbial life on surfaces. *Emerg Infect Dis*. 2002;8:881–890. Figure is public domain and no permission to reprint is required.).

CLINICAL APPLICATIONS

Clinically, specimen culture is currently considered the criterion standard for microbial identification; however, typical specimen culture is inadequate for the positive characterization for biofilms. Current specimen culture is biased toward the identification of planktonic organisms that grow well on commonly used laboratory media. Biofilms, in contrast, require specific media for growth that are not commonly found in the hospital laboratory setting. Furthermore, the matrix of a biofilm must be broken apart to release the underlying microbes. Although novel approaches such as sonication were effectively used to release these microbes from the polymeric matrix, the resulting cultures were highly sensitive and the overall process was prone to technical error.[19] In addition, standard culture results sometimes take days to yield a result, often too late to contribute to critical decision making when dealing with surgical infections.

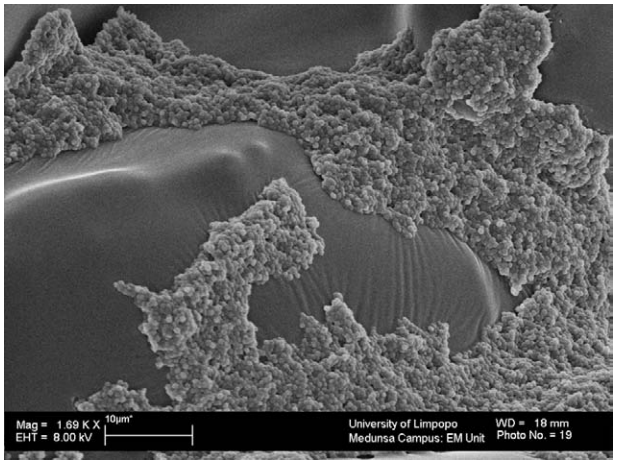

Fig. 2. A scanning electron micrograph illustrating the appearance of biofilm on a silicone surface. (Printed with permission from van Heerden J, Turner M, Hoffmann D, Moolman J. Antimicrobial coating agents: Can biofilm formation on a breast implant be prevented? *J Plast Reconstr Aesthet Surg*. 2009;62:610–617.)

Critical to successful treatment of any complications is the identification of any biofilms present so that culture-directed antibiotic coverage can be administered. One method, polymerase chain reaction, works by amplifying a single or a few copies of DNA over many orders of magnitude, which thereby generates millions of copies of a particular DNA sequence. Allowing for rapid identification of genes responsible for biofilm synthesis, such as *icaA*, *icaD*, and *atlE*,[20] polymerase chain reaction is able to obtain results in a matter of hours. Another benefit of polymerase chain reaction is the value-added ability to prescribe relevant antibiotics targeting organisms ranging from methicillin-resistant *Staphylococcus aureus* to *Pseudomonas* in lieu of empirically directed antibiotics.[21]

Biofilm formation on prostheses, however, presents similar problems in diagnosis and treatment in many disciplines, including orthopedic surgery, cardiothoracic surgery, neurosurgery, and oral surgery. In addition to rapid polymerase chain reaction identification, current methods of rapid diagnosis for prosthetic joint infections include automated ribotyping, matrix-assisted laser desorption ionization coupled with time-of-flight analysis mass spectrometry, and polymerase chain reaction–electrospray ionization based on nucleotide ratios.[22] Rapid polymerase chain reaction identification techniques are currently being used for identification of biofilms on neurosurgical implants such as external ventricular drains.[23] Identification techniques of biofilms in periodontal implants are similar to the aforementioned scanning electron microscopy and polymerase chain reaction–based methods.[24]

Plastic surgery can similarly benefit from the advent of rapid polymerase chain reaction techniques and methodologies to help in the treatment of complications arising from biofilms and implants. Management of recurrent capsular contracture in breast augmentation and a biofilm reaction to soft-tissue fillers are two examples of how rapid polymerase chain reaction technology can, within hours, contribute to a clinical algorithm for care that can lead to targeted antibiotic treatment.

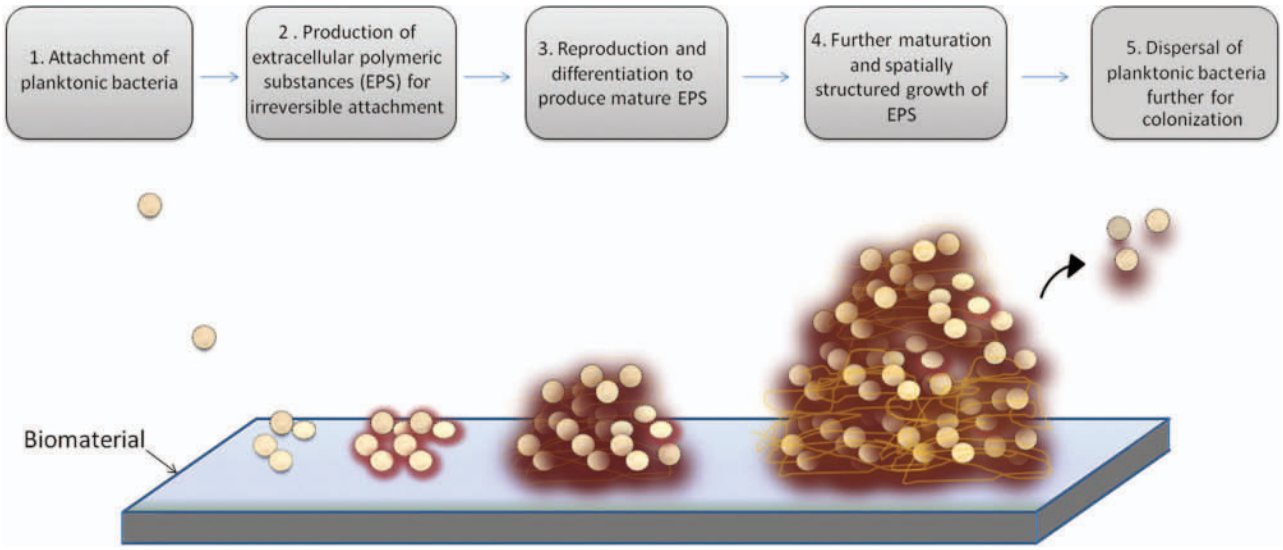

Fig. 3. Schematic depiction of the biofilm life cycle. *1*, Bacteria individual cells populate material; *2*, extracellular polymeric substance is produced and serves as a scaffolding or glue to hold biofilm together; *3*, attachment becomes irreversible; *4*, biofilm architecture develops and matures; *5*, bacteria can convert from sessile biofilm to planktonic form to seed new infections. (Per-Reprinted with permission from Nyame TT, Lemon KP, Kolter R, Liao EC. High-throughput assay for bacterial adhesion on acellular dermal matrices and synthetic surgical materials. *Plast Reconstr Surg.* 2011;128:1061–1068.)

BIOFILMS AND BREAST AUGMENTATION

Background

Augmentation mammaplasty is among the most commonly performed aesthetic plastic surgery procedures in the United States.[25] The formation of a fibrous capsule around the implanted material is a normal part of healing; however, if this fibrous capsule contracts and thickens, capsular contracture is said to have occurred.[26] Capsular contracture can lead to firmness, induration, discomfort, or contour distortion and has been cited as the most prevalent complication of augmentation mammaplasty.[27] Although infection has been hypothesized as a potential cause of capsular contracture for the past three decades, the detection of a subclinical infection in the form of biofilms has only recently been confirmed.[21,28,29] As part of the endogenous breast flora,[30] *Staphylococcus epidermidis* was implicated as the cause of the detected biofilms.[24] Through the use of a porcine model, Tamboto et al. have demonstrated a link between subclinical infection, biofilm formation, and capsular contracture. In this porcine model, the authors inoculated various implant sites with biofilm-forming *S. epidermidis* and noted a statistically significant association between inoculation and capsular contracture development—a fourfold increased risk of developing contracture (Fig. 4).[31] Jacombs et al. have shown in a similar porcine model that the use of an antibiotic-impregnated mesh can reduce bacterial access to breast implants at the time of surgical insertion and may subsequently protect against subclinical infection and capsular contracture.[32]

Prevention, Diagnosis, and Treatment

Preventative measures for avoiding biofilm infiltration in augmentation mammaplasty involve the use of rigorous aseptic technique, triple antibiotic (bacitracin-cefazolin-gentamicin) irrigation in the mammary pocket, and bloodless dissection.[33] Additional potential measures to avoid exposure to common breast flora (e.g., *S. epidermidis*) or other bacteria include the use of the Keller Funnel medical device (Keller Medical, Inc., Stuart, Fla.), which has been reported to have a 27-fold decrease in skin contact compared with digital insertion with smooth gel implants.[34] Recent experimental trials have shown that antibiotics such as aminoglycosides and fluoroquinolones were effective against *Staphylococcus* biofilms and could be considered for use postoperatively.[35] With that said, the rate of complications have been shown to be not significantly different when cephalosporins were used postoperatively.[36]

Complications with breast implants and soft-tissue fillers should be addressed in a measured, algorithmic manner. Currently, capsulectomy is the treatment of choice for most surgeons when addressing capsular contracture. The plastic surgeon may consider sending the capsule or a

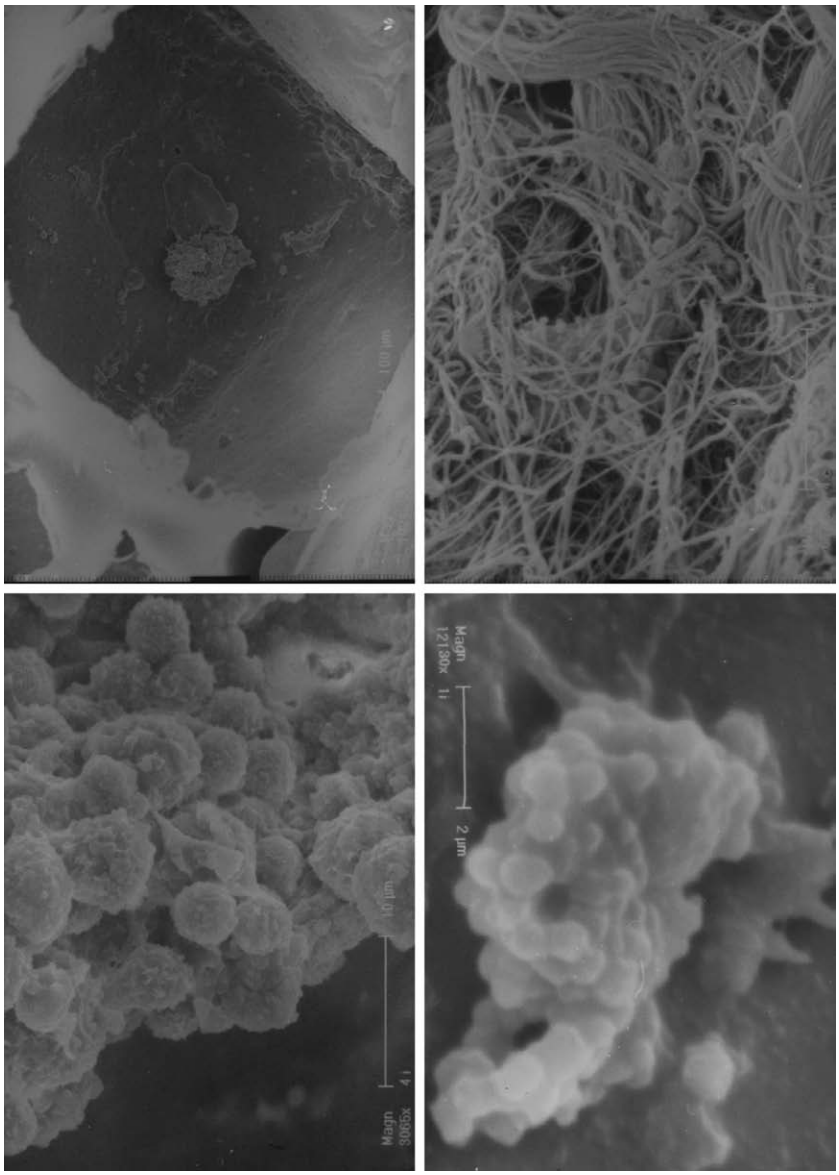

Fig. 4. Scanning electron micrographs of biofilm. (*Above*, *left*) Low-power micrograph showing biofilm attached to the surface of a prosthesis. (*Above*, *right*) High-power electron micrograph showing biofilm within capsular contracture. (*Below*, *left*) High-power electron micrograph showing biofilm attached to the surface of a prosthesis. (*Below*, *right*) Detail of biofilm clump attached to the surface of a prosthesis. [Reprinted with permission from Tamboto H, Vickery K, Deva AK. Subclinical (biofilm) infection causes capsular contracture in a porcine model following augmentation mammaplasty. *Plast Reconstr Surg.* 2010;126:835–842.]

granuloma found within the capsule for polymerase chain reaction processing for biofilm identification, which could prove useful in treatment and/or prevention of capsular contracture if reimplantation were to occur. Because we do not know the cause of capsular contractures, this may help elicit whether infection or a biofilm is the cause, especially in recurrent capsular contracture.

BIOFILMS AND FILLERS

Background

Although injectable soft-tissue fillers for facial rejuvenation and reshaping have been increasing in popularity, the rise of biofilm-related complications associated with these fillers will concurrently increase.[37] Soft-tissue fillers, which have been classified by their duration of effect as temporary,

long-lasting, semipermanent, and permanent,[38] have been tested extensively and have rare, minor side effects.[39] Most soft-tissue filler complications have typically been associated with technique-related or procedural errors and not the products themselves.[40] Despite their superb safety profile and the relative rarity of adverse reactions,[41] soft-tissue fillers (with the exception of autologous fat) are foreign to the body and are thus a potential attachment point and source of biofilm formation and subclinical infection—a mechanism of delayed foreign-body granuloma formation.[42]

The granuloma formation rate, however, is variable based on the type of filler used. For 6-month fillers, the literature indicates a granuloma rate of one in 2500 for hyaluronic acid. For fillers with a longer period of persistence such as Radiesse (Merz Aesthetics, Inc., Greensboro, N.C.) and Sculptra (Valeant Pharmaceuticals International, Inc., Bridgewater, N.J.) granuloma rates are indicated to be approximately one in 500.

The onset of complications in relation to the injection of soft-tissue fillers is distinguished by their timing: early complications occur within 14 days and are typically characterized by an inflammatory response. Late complications occur between 14 days and 1 year and typically involve the formation of a granuloma. Delayed complications occur greater than 1 year after the injection and are associated with biofilms.[29]

Early complications associated with soft-tissue fillers are the most common complications and may be seen immediately after injection. The traumatic puncture effects related to injection can lead to erythema and swelling and have been noted in up to 80 percent of cases.[43] Injection-site necrosis can occur because of intraarterial injection, especially in the supraorbital or angular artery.[44,45] Allergic reaction may also occur in the early stages after injection. Visible or palpable nodules, deemed "angry red bumps," are characteristic of delayed erythema in the sites of injection, which may be caused by hypersensitivity, infection, or foreign-body reactions.[46]

Late and delayed complications of soft-tissue filler injection are related to the formation of foreign body granulomas, which are composed of an inflammatory infiltrate including lymphocytes, plasma cells, neutrophils, eosinophils, and multinucleated giant cells, representing the body's reaction to inert foreign bodies.[44] Foreign body granulomas typically appear anywhere from 6 to 24 months after injection and occur at a rate ranging from one in 100 to one in 5000 patients.[47,48] Although the characteristics of the foreign-body granuloma are usually specific to the type of filler used, diagnosis has been clinically based.[38] The impact of biofilms on foreign-body granulomas is unclear at this time and should be pursued as an avenue for future research.[49] The infectious cause as it relates to foreign-body granulomas, however, has been supported by various reports[40,50] (Fig. 5).[51]

Prevention

Prevention of soft-tissue filler biofilm formation should include a good patient history to determine whether any previous fillers have been used and to ascertain any information on bleeding disorders, immunocompromised states, or previous infections. As with preventative measures for breast implants, aseptic technique should be followed, and chlorhexidine should be used to prepare the patient to use the residual antibacterial effects. Further preventative

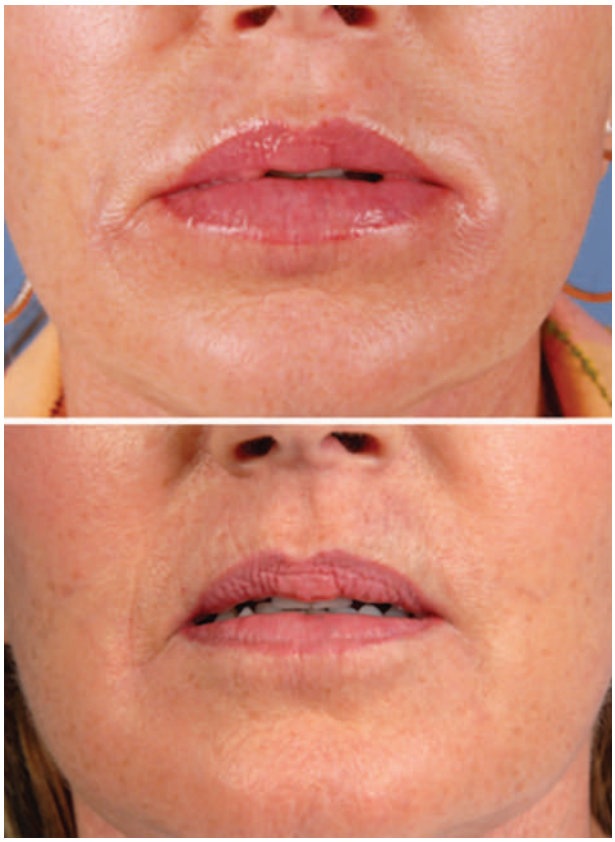

Fig. 5. Delayed complication of filler injection. (*Above*) The patient exhibits nonfluctuant inflammation following injection of hyaluronic acid to the lips. (*Below*) Appearance of the patient 6 months after algorithmic treatment. (Reprinted with permission from Rohrich RJ, Monheit G, Nguyen AT, Brown SA, Fagien S. Soft-tissue filler complications: The important role of biofilms. *Plast Reconstr Surg*. 2010;125:1250–1256.)

measures include the use of prophylactic antibiotics, especially when semipermanent and permanent fillers are used, and the use of smaller gauge needles to minimize trauma and access for bacteria. In addition, patients should avoid wearing makeup 8 hours before surgery and immediately after injection.[51]

There are known risk factors associated with the creation of biofilms that should be actively avoided. Injecting into an inappropriate plane is the most common error committed.[52] Deep planes are better for injection than superficial ones, especially for longer lasting fillers. Injection during active acne or other infections should be avoided, and injections near the lips are at high risk for biofilm formation because of the closeness of the oral flora.[45] Furthermore, stacking of fillers and large-volume injections have been related to granuloma formation and more inflammation.[53]

Diagnosis and Treatment

As with complications arising from augmentation mammaplasty, biofilm identification using polymerase chain reaction should lead to treatment in an algorithmic fashion. Empiric antibiotics can be started pending polymerase chain reaction results (macrolide and quinolone).[38] In the case of soft-tissue fillers, identification of the type of filler used followed by determination of wound fluctuance is the recommended method for dealing with complication. If the wound does prove fluctuant, it should be needle-drained and cultured.[47] If targeted antibiotics do not ameliorate the complications and hyaluronic acid was not used, intralesional high-dose steroids should be considered.[31] If hyaluronic acid was used, however, hyaluronidase should be used.[54] The last step to be considered is excision (Fig. 6).

CONCLUSIONS

Although biofilms have proven difficult to detect and equally difficult to eradicate, recent improvements in detection techniques, innovations in prevention, and research on eradication work to establish a potential algorithm for preventing and dealing with subclinical infection. Special attention must be paid to prevention, avoidance of various risk factors, detection, and management of the complications if and when they arise.

In this article, we propose a method of detection that will possibly lead to a quicker diagnosis of an offending infectious agent, allowing for faster treatment times. Faster turnaround times with regard to microbial identification and targeted antibiotic therapies are the goal.

An area of contention is practicality. How readily available is polymerase chain reaction technology outside of the major academic centers and how cost-effective is it? As is true with most newer technologies and methods in medicine, cost is usually high and availability is limited. However, with time, we hope to see these methods available to all plastic surgeons whether in community centers or large academic centers. We also hope to see research advancing with regard to antibiotic penetration of biofilms.

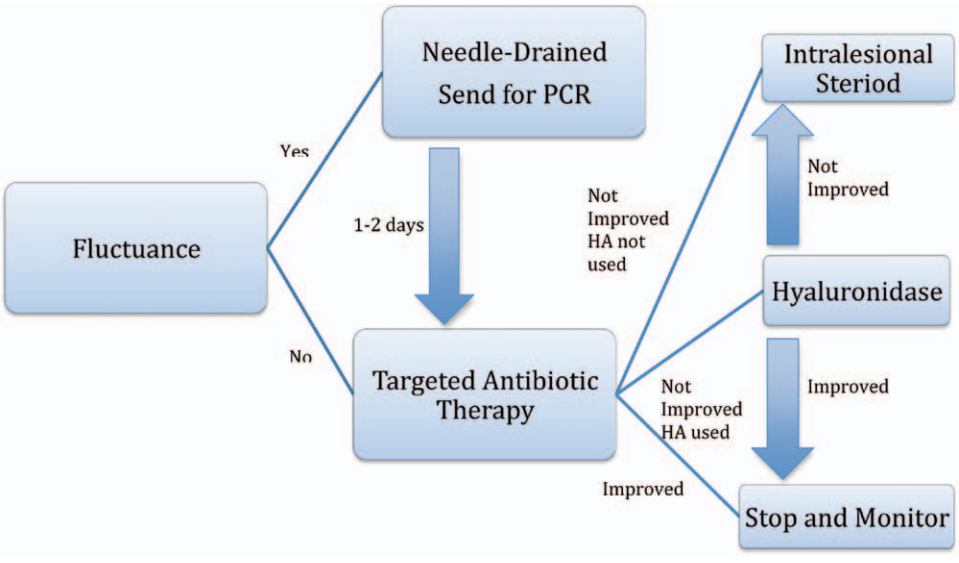

Fig. 6. Management algorithm of late and delayed complications associated with biofilms. *PCR*, polymerase chain reaction; *HA*, hyaluronic acid.

Biofilms are ubiquitous in our lives and yet have proven difficult to identify and eradicate. Although their existence has been accepted by most, their significance and impact have yet to be fully appreciated in plastic surgery. Recent advancements in polymerase chain reaction technology and a recent increasing knowledge base have laid the groundwork for future research in this exciting and ever-growing field.

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