Delayed reaction

Dr Sotirios Foutsizoglou, on biofilm formation following dermal filler injections: facts and considerations

SYNOPSIS

Delayed reactions associated with dermal fillers have often been attributed to hypersensitivity reactions; however the evolving literature suggests that biofilms may represent an underrecognised cause and a challenging diagnosis to make. In this article, the mechanism of action for biofilm formation, diagnostic methods to recognise biofilms, timely treatment and preventative measures will be explored.

Implanted medical devices such as dermal fillers have the potential to elicit a reaction, ranging in severity from mild to life threatening with an onset of seconds to years. Reactions can be classified based on timing of their onset and are generally classified as either acute or delayed. Acute reactions and complications are usually related to either a product or injection technique and may include bruising, erythema, oedema, vascular occlusion, acute allergic reactions, infection etc. Delayed reactions can include late-forming nodules and granulomas. Although these complications have commonly been attributed to delayed immune responses, there is increasing evidence that these may be infectious in nature, arising either secondary to slow-growing bacteria, such as mycobacteria, or as a result of biofilm formation.[1]

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Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. Many bacterial species form biofilms, and their study has revealed them to be complex and diverse. Biofilm formation has been implicated in a number of diseases ranging from atherosclerosis to endocarditis and inner ear infections.

Bacteria capable of forming biofilms represent a source of infection due to their glycocalyx structure that shields these bacteria from the hosts' immune system and from administered antibiotics. Biofilms have been studied and are amply documented with implantable devices such as artificial joints and intravascular central venous devices.[2] More recently, it has been demonstrated that biofilms may occur with soft-tissue augmentation products.[3] These implantable soft-tissue devices also represent a foreign body, and, as their duration increases with newer generations of dermal fillers, there may be a greater risk of biofilm formation. This issue may be magnified by the ever-increasing popularity and use of dermal fillers.

SOFT-TISSUE REACTIONS

Hypersensitivity reaction is an inappropriate or exaggerated immune response. It may result in tissue injury by

- Release of vasoactive substances, cytokines, proteolytic enzymes
- Phagocytosis or lysis of cells
- Activation of inflammatory and cytolytic components of the complement system

Hypersensitivity reactions require a pre-sensitized state of the host and may be immediate or delayed.

Concern with Type I reactions to bovine collagen dermal fillers resulted in patients undergoing intradermal collagen injections to ensure the patient was not sensitive. The inconvenience of this pretesting contributed to the popularity of hyaluronic acid (HA) dermal fillers, which do not require hypersensitivity tests. Delayed reactions can take place weeks to years from the time of the initial injection.

Antigenic stimulation tracing back to the contaminants from the bacterial fermentation method of producing HA is one source ascribed to delayed reactions. Friedman and colleagues retrospectively examined the incidence of hypersensitivity reactions before and after a manufacturing process change for an HA filler.[4] Trace amounts of protein in the HA raw material were reduced approximately 6 times lower than the original raw material. To assess the impact of the process revision, the authors

Table 1. Gell and Coombs classification of hypersensitivity reactions

Type	Effector Mechanism	Typical Clinical Manifestations	Description
I(Immediate)	IgE	Urticaria, Anaphylaxis, Angioedema, Asthma, Atopic Dermatitis	Fast response which occurs in minutes. Provoked by re-exposure to the same antigen. Free antigens cross link the IgE on mast cells and basophils which causes a release of vasoactive biomolecules and cytokines. It can be local or systemic.
II (Antibody dependent)	IgM or IgG Complememt	Hyperacute graft rejection of an organ transplant, Coombs positive Haemolytic Anaemia, Hashimoto Thyroiditis, Anti-Glomerular Basement Membrane disease (e.g. Goodpasture syndrome), Myasthenia Gravis, Pernicious Anaemia, Acute Rheumatic Fever	They result when antibody binds to cell surface antigens or to a molecule coupled to a cell surface. The antigen-antibody complex activates cells that participate in antibody-dependent cell-mediated cytotoxicity (eg, natural killer cells, eosinophils, macrophages), complement, or both. The result is cell and tissue damage.
III (Immune complex mediated)	IgG	Serum Sickness, SLE, RA, Leukocytoclastic Vasculitis, Cryoglobulinemia, Pneumonitis, Post Streptococcal Glomerulonephritis	Antibody (IgG) binds to soluble antigen, forming a circulating immune complex. This is often deposited in the vessel walls of the joints and kidney, initiating a local inflammatory reaction. Type III reactions develop 4 to 10 days after exposure to antigen and, if exposure to the antigen continues, can become chronic.
IV (Delayed)	T-cells	Contact Dermatitis, Multiple Sclerosis, Allograft Rejection, Hypersensitivity Pneumonitis	T cells, sensitized after contact with a specific antigen, are activated by re-exposure to the antigen; they damage tissue by direct toxic effects or through release of cytokines, which activate eosinophils, monocytes and macrophages, neutrophils, or natural killer cells.

looked at adverse events before and after the change that was implemented in 1999 and found that hypersensitivity reactions were 5.9 times more frequent with the batch prepared using the original raw material than with the batch made with the lower-protein raw material. A recent review of reported hypersensitivity reactions speculated that most of the reported hypersensitivity reactions were likely due to an infectious process.[5]

Implanted foreign bodies can become infected during a procedure, typically with skin contaminants and may also be subject to colonization secondary to either direct or haematological spread of an infectious agent. For example, a recently published case report of a patient injected with an HA filler in the cheek described a firm nodule 3 months after the injection. Further investigation revealed the source of infection as coming from improper endodontic treatment of a tooth resulting in Enterococcus, Lactococcus, and Streptococcus bacteria in the area.[6]

The diagnosis of an infection associated with a filler can be challenging using conventional methodology. Some bacteria are difficult to culture because of their distinct slow-growing nature and are known as small colony variants. These represent a naturally occurring subpopulation of the normal bacteria (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescens, and Escherichia coli) and are often missed with traditional culturing techniques because of overgrowth of the more rapid-growing normal bacteria or because the culturing protocol does not allow sufficient time for incubation1. These organisms can be phenotypically distinct and take on a sessile nature with a decreased metabolism, allowing them to elude antibiotics. Small colony variants have been associated with persistent infections and are more resistant to many antibiotics.[7,8] Some, but not all, bacteria may also have the ability to secrete an elaborate extracellular matrix of exopolysaccharides that also includes HA. The bacteria adhere to one another among this matrix, allowing this sessile community to be protected. Sadashivaih and Mysore point out that a biofilm may exist in a dormant state and then become activated by external factors. Triggers can include disturbances to the local environment such as trauma, manipulation, and injections.[9]

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DIAGNOSIS

Diagnosis of a delayed reaction in soft-tissue augmentation patients requires a careful patient history. In some instances, patients have been injected with materials that are not approved or by an unlicensed practitioner. Patients also may simply be ignorant of what was injected, and it may be impossible to find out the exact nature and origin of the filler. The definite diagnosis of the reaction relies on a biopsy. Whenever possible, sufficient tissue should be sent to accommodate bacterial, fungal, and mycobacterial cultures. It is essential to work with a laboratory that is well versed in multiple testing modalities and has the ability to transport the specimens without external contamination or tissue degradation.

Establishing the diagnosis of biofilm formation is difficult. Special stains such as Gram, Fite, periodic acid–Schiff, and others may identify some infectious aetiologies, although their absence is not sufficient to rule out the diagnosis of an infection. Some organisms are of a slow-growing nature, and this must be considered or else their detection will be missed; appropriate cultures with sufficient incubation time are required.

In the absence of a positive culture, consideration should be

given to evaluation using molecular techniques such as polymerase chain reaction (PCR). Fluorescent in situ hybridization (FISH) may also be of assistance; the technology uses fluorescently labeled DNA probes or synthetic nucleic acid probes (e.g., peptide nucleic acids [PNA] with a backbone of repetitive units of N-[2-aminoethyl] glycine). Bjarnsholt et al [10] investigated 8 patients with reactions to injections of polyacrylamide gel who were found to be culture negative on biopsy. Using FISH with PNA probes, bacteria were detected in 7 of the 8 culture-negative patients. Interestingly, 6 of the 8 had an onset that began after 6 months.

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PREVENTION AND TREATMENT

The heightened awareness of biofilm formation has stimulated discussion around the injection technique, optimal antiseptic solution and the degree of sterility required. Rodriguez et al11 reported cases of Mycobacterium chelonae infections after cosmetic dermal injections with HA. A thorough investigation traced the root of the infection to the ice cubes used at the injection site; the organisms grown from the patient's culture matched the isolates from the faucets and water supply at the clinical site.[11] Mycobacteria are known to have a spectrum of growth rates from slow to rapid, and this may impact the timing of a clinical infection presentation.

Biofilm related infections are notoriously difficult to treat and may require amounts of antibiotics that are thirty two times higher than that required to kill planktonic bacteria.[12] There has also been an interest in 5-fluorouracil (5FU), a compound that impacts both DNA and RNA synthesis. It has been hypothesized that 5FU may be exerting its therapeutic effect by interacting with AriR, a regulatory gene that inhibits the formation of biofilm.

Interestingly, hyaluronidase, along with the broad-spectrum antibiotics, has been used in treating persistent infections associated with non-HA fillers. It has been suggested that hyaluronidase may break down a biofilm matrix allowing the immune system and antibiotics to penetrate into the infected field.[12]

CONCLUSION

History and histopathology are important parts of the investigation and subsequent treatment of delayed reactions. Although delayed reactions are often readily assumed to be hyper- sensitivity reactions, it is important to consider in the differential the possibility of an infectious process that may contain atypical organisms or biofilms.

Hyaluronic acid fillers can persist in the skin for a year or more, and it is likely that infection resulting from inadvertent bacterial inoculation or secondary to a transient bacteraemia may manifest as an increase in delayed reactions. Sterility around the injection site may help to prevent peri-procedural infections. Post-treatment antibiotic coverage may also help to prevent infection and reduce the incidence of delayed reactions1. Ultimately, the prevention of biofilm formation may be the next frontier in the management of infections related to implantable devices.[12] **AM**

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