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Interactions of *Staphylococcus aureus* with ultrasoft hydrogel biomaterials

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ABSTRACT

Ultrasoft biomaterials—polymers, gels, and human soft tissues with an elastic modulus less than ~100 kPa—are increasingly used in medical devices. While bacterial interactions (adhesion and biofilm formation) have been extensively studied on stiffer materials, little is known about how bacteria colonize ultrasoft materials as a nidus for infection. The goal of this work was to determine how material properties of ultrasoft hydrogels used for dermal fillers might affect pathogenesis of associated infections. We first synthesized a range of polyacrylamide hydrogels (PAAm) with moduli similar to clinically used dermal fillers and characterized the rheological, morphological and porous properties. We then developed a novel microfabricated insert to contain the PAAm in a flow system for quantification of bacterial adhesion and biofilm formation. The rate of adhesion and numbers of adherent *Staphylococcus aureus* on the surface of PAAm both decreased as the modulus increased. Adhesion was reduced by 3 logs (from $93 \times 10^4/cm^2$ to $0.083 \times 10^4/cm^2$) with increasing modulus (from 17 Pa to 654 Pa). However, the number of bacteria in the bulk was the highest within the stiffest gels. This trend was further amplified in subsequent biofilm studies, where interfacial coverage of biofilm decreased as the modulus increased, while the fraction of biofilm in the bulk was the highest within the stiffest gel. The results show significant differences in bacterial colonization of PAAm based on material properties, and reveal how the injection process may unexpectedly create discontinuities that provide a microenvironmental niche for bacterial colonization.

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1. Introduction

Ultrasoft biomaterials—polymers and gels with an elastic modulus less than ~100 kPa [1,2], —can be precisely tailored to match the non-linear mechanical properties of a biological matrix such as human soft tissue, enabling their use in diverse medical products such as injectable implants, cell scaffolds in tissue engineering, and drug releasing vehicles. Among these biomaterials, ultrasoft hydrogels are distinguished from soft hydrogels (100 kPa–1 GPa) [3] because they can be placed in the body by injection through a narrow bore needle. This unique property is the basis for their role in the rapidly growing use of dermal fillers to augment skin tissue [4]. Partial failure of skin elasticity can be caused by factors such as sun exposure, free radicals and trauma. Dermal fillers are tailored to match the elasticity of cutaneous tissue that is normally provided by collagen and elastin fibers. The optimal elasticity varies depending on the anatomic location and depth of placement. Materials with lower elastic modulus are generally matched to delicate tissue structures and may cause less pain when injected. Materials with a higher elastic modulus may be used in deeper injections or for bulkier or longer-lasting effects.

While injection of filler materials is not subject to the same infection risks as open surgery, injected materials share in common with implanted and indwelling medical device materials (Fig. 1) the potential for associated infection with persistent, antibiotic resistant organisms [5,6]. The time of onset for symptoms of infection can vary from shortly after injection to years later [7]. Although adverse event rates for some dermal fillers can be as high as 20% [8],

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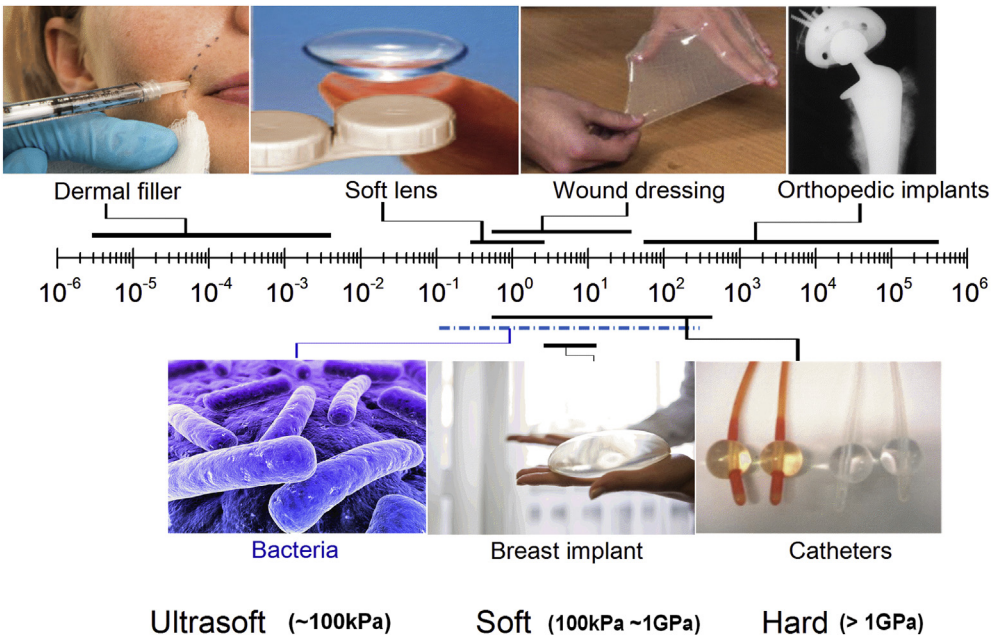

Fig. 1. Stiffness (Young's modulus, MPa) of some medical devices that are colonized by bacteria. In black print, from top to bottom, left to right: Dermal filler (0.02–3 kPa) [18]; Soft contact lens (0.2–1.5 MPa) [19]; Wound dressing (0.5–25 MPa) [20,21]; Orthopedic implant (5–300 GPa) [22]; Silicone gel-filled breast implant shells (2–12 MPa) [23]; Catheters: 0.4–300 MPa [24], [25]; For reference, bacteria (blue print, bottom left) have a stiffness of 0.1–200 MPa [26]; The modulus of dermal fillers was estimated using the classical relation between the Young's modulus and the shear modulus- E = 3G) [27]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

it is not known how many are related to bacterial colonization vs. aseptic origin (allergies, inflammatory response, etc.). The use of molecular techniques such as PCR and FISH has increasingly shown a link between colonization and adverse events. In one clinical case-control study, aggregates of skin bacteria such as *Staphylococcus epidermidis* and *Propionibacterium acnes* were identified in 53 out of 54 patients with severe adverse events due to filler injection, while none were found in 24 control subjects (with dermal filler injection but no adverse reactions) [9]. Longer lasting materials (semi-permanent or permanent) [10] and some injection locations [11,12], have also been associated with higher risk of infection. Treatment of these materials often requires a combination of surgery and prolonged antibiotic use, at significant cost and pain to patients [5,10,13].

To develop improved dermal fillers, it is important to understand the pathogenesis of infections associated with ultrasoft biomaterials. In contrast to the extensive knowledge about how bacteria can colonize hard and soft materials to become a persistent source of bioburden [14,15], less is known about bacterial interactions with ultrasoft materials. A recent *in vitro* study showed that *Pseudomonas aeruginosa*, *S. epidermidis*, and *P. acnes* formed robust biofilms in several dermal filler materials (hyaluronic acid gel, calcium hydroxyl apatite microspheres in carrier gel, and polyacrylamide hydrogel) [16]. *P. aeruginosa* biofilm in polyacrylamide gel was not susceptible to tobramycin at concentrations 200× the normal minimum inhibitory concentration (MIC) obtained from disc diffusion testing [17]. The same authors also showed that as few as 40 bacteria in filler materials could cause infection in a mouse model. The outcome was strongly material dependent.

Ultrasoft hydrogels have fundamental differences with stiffer materials that could potentially impact mechanisms of bacterial pathogenesis. The adhesion mechanisms may be different. Bacterial adhesion on stiffer materials relies on both chemical interactions (van der Waals, electrostatic and hydrophobic forces) and surface mechanical clues (roughness, patterns). In ultrasoft hydrogels, mechanical (viscoelastic) properties may play a more dominant role in bacterial interactions because of the high water content at the gel-medium interface. In contrast to the discrete solid-liquid interface of stiff materials, the topography of loosely crosslinked hydrogels presents a more gradual transition [14]. In addition, colonization may be impacted by the shift from a planar two-dimensional surface to a porous three-dimensional matrix. Many eukaryotic cells have different growth dynamics in a three-dimensional matrix when compared with planar surfaces [28] and bacteria may follow similar environmental cues. Finally, ultrasoft hydrogels are less homogeneous than stiffer materials [29]. Mechanical processes such as injection can further enhance this non-uniformity, resulting in unique spatial constraints and micro-environments— that may have unexpected impact on biofilm growth dynamics. The porous and heterogeneous structure may present an ecological niche for bacteria while hindering the response of larger immune cells.

The goal of this work was to determine how the properties of ultrasoft hydrogels affect bacterial adhesion and initial biofilm growth, two of the earliest stages in the bacterial pathogenesis of dermal filler associated infections. The work was divided into four stages: (i) We synthesized polyacrylamide hydrogels (PAAm) with elastic moduli similar to the range used for commercial dermal fillers, and characterized the rheological, morphological and porous properties. (ii) We developed a strategy to test bacterial adhesion and biofilm formation in PAAm using an aqueous flow cell with quantifiable shear stress. Due to the buoyant and fluid-like behavior of the gels, a novel microfabricated insert was developed to hold them in place for testing. The insert was integrated into a conventional flow system for studying adhesion and biofilm formation using confocal microscopy. (iii) The interaction of green fluorescent protein (GFP)-tagged staphylococci with the gels was quantified under flow, and (iv) biofilm formation was evaluated.