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# Strategies for the management of biofilm on silicone maxillofacial prosthesis – a review of the literature

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#### Abstract

Background and objectives: Maxillofacial prostheses are used in the rehabilitation of maxillofacial defects to restore function and improve aesthetics. Silicone elastomers are considered the most suitable maxillofacial materials for extraoral prostheses to date, due to their superior physico-chemical properties. They have recognised clinical problems such as biofilm formation which contribute to premature failure. The aim of this review was to describe the characteristics of biofilm formation on silicone used for maxillofacial prostheses and review different strategies of biofilm management for silicone maxillofacial prosthesis.

*Methods:* The MEDLINE database was searched in English using the determined search strategy and an additional search of the bibliographies of all full-text articles, selected from the electronic search, was also performed. A total of 12 articles met the inclusion criteria and were included.

Results: There are currently no clinical studies that investigate strategies of biofilm management for silicone maxillofacial prosthesis. The authors identified twelve in vitro studies that assessed novel strategies to enhance the biofilm resistance of silicone for maxillofacial prostheses or eliminate biofilm from the silicone surface. However, the successes of these novel strategies remain confined to strictly-controlled laboratory conditions. Conclusions: The existing literature does not allow for any further recommendations beyond the conventional strategies for biofilm management comprising mechanical cleaning with neutral soap and immersion in chlorhexidine solution. Progress has been made in the investigation and development of novel strategies to manage biofilm on maxillofacial silicone prostheses, but high-quality clinical studies are required to confirm the efficacy of these strategies.

#### Introduction

Maxillofacial defects may be acquired due to cancer, trauma or congenital diseases of the maxillofacial region (Ariani et al., 2013). Individuals living with maxillofacial defects often experience significant aesthetic impairments, functional limitations and psychological strain (Goiato et al., 2009). The rehabilitation of patients with maxillofacial defects often requires a multi-disciplinary approach and may be accomplished either surgically and/or prosthetically (Goiato et al., 2011a). Complete autoplastic repair of a defect through reconstructive surgery is usually the treatment of choice but it can be limited by factors such as the amount of tissue loss, vascular compromises, procedural complications or the psychophysical conditions of the patient (Visser et al., 2008; Ariani et al., 2013). When such limitations pose an issue, prosthetic rehabilitation with maxillofacial prosthesis becomes a favourable alternative and is often used in conjunction with surgery. These prostheses are made to match the colour of the surrounding tissue and retained using soft tissue undercuts, adhesives and/or percutaneous implants (Visser et al., 2008; Goiato et al., 2009). Patients rehabilitated with quality maxillofacial prostheses have reported significant improvements in aesthetics, function, psychological well-being and overall quality of life (Goiato et al., 2009).

Physicians from ancient Egypt and China were the first to restore parts of the face using waxes and resins but the first maxillofacial prosthesis by modern definition did not appear until 1575, when French surgeon Ambrose Pare developed obturators to close palatal perforations (Mitra et al., 2014). With the introduction of maxillofacial prostheses, researchers also began searching for the ideal maxillofacial material to satisfy requirements in functionality, biocompatibility, aesthetics and durability (Montgomery and Kiat-Amnuay, 2010). The ideal material is yet to be discovered but silicone (polydimethyl siloxane) elastomers have emerged as the most successful material (Mitra et al., 2014). First developed in 1946, silicone is a combination of organic and inorganic compounds with elastomeric properties (Mitra et al., 2014). It was trialled as a maxillofacial material in 1969 and quickly gained popularity over other materials due to its high tensile strength, good thermal and oxidative stability, low toxicity as well as the ability to produce very aesthetic prostheses (Hatamleh et al., 2016).

Despite the relative success of silicone elastomers as a maxillofacial material, it has several limitations such as discolouration, deterioration and biofilm formation which restrict the lifespan of prostheses to 6-24 months, depending on patient and prosthesis characteristics (Hooper et al., 2005; Karakoca et al., 2010; Ariani et al., 2013; Hatamleh et al., 2016). Just like other oral biomaterials, maxillofacial silicone can be readily colonised by microorganisms in the presence of warmth, moisture and nutrient-rich bodily fluids (Meran et al., 2017). The growth of microorganisms is further promoted by the acidic pH of facial skin in contact with the prosthesis, which ranges between 4.0 and 4.9 (Korting and Braun-Falco, 1996). The resultant biofilm is poly-microbial and comprises mostly common human commensal species found in the oral cavity and on the skin surface (Ariani et al., 2012; Murakami et al., 2013). Although most microbial species within the biofilm are commensal in nature, it is considered a major cause of the premature failure of maxillofacial prostheses and has also been shown to facilitate the discolouration and degradation of silicone (Ariani et al., 2013). Under predisposing conditions, opportunistic strains within the biofilm such as Candida species may begin to disturb the symbiosis between normal microflora and the host, leading to skin irritations and dermatitis (Ariani et al., 2012). Among immunosuppressed individuals, these opportunistic strains may cause recurrent or chronic mucosal infections as well as severe systemic infections (Garner et al., 2015). To overcome the biofilm challenges, microbiological research has investigated many innovative strategies to enhance biofilm resistance on maxillofacial silicone or eliminate the biofilm from the silicone surface. Frequent re-fabrication of maxillofacial prostheses is a significant burden for both the patient and the health care system so increasing the longevity of the prostheses via the management of biofilm is extremely desirable (Meran et al., 2018).

The aim of this review was to describe the characteristics of biofilm formation on silicone maxillofacial prostheses and review the different strategies of biofilm management for silicone prostheses. The study intended to identify the most effective protocol and provide recommendations for the care and maintenance of silicone maxillofacial prostheses.

#### Methods

The MEDLINE (PubMed) database was searched using a detailed strategy developed by the authors (Table 1). The search was conducted in February 2018 and all articles that met the following inclusion criteria were selected: (1) Articles written in English published from 1 January 1990 to 1 January 2018; and (2) Studies that investigated biofilm management on silicone maxillofacial prostheses. A total of 64 articles were retrieved by the search strategy and their titles and abstracts were independently screened by two authors (R.M. and L.M.) based on the defined inclusion criteria. Disagreements were resolved by discussion and if a consensus was not reached, the third author (K.L.) was invited to discuss the

Table 1. Search strategy for MEDLINE via PubMed

Search NO.	Search Terms
1	"Silicone" OR "siloxane" OR "polysiloxane" [All Terms]
2	"Maxillofacial" OR "facial" OR "obturator" [All Terms]
3	"Prosthesis" OR "prostheses" OR "implant" [All Terms]
4	"Biofilm" OR "microorganism" OR "microbial" OR "bacteria" OR "bacterial" OR "fungi" OR "fungal" [All Terms]
5	1 AND 2 AND 3 AND 4

article. Following this, the selected articles were obtained in full-texts and analyzed again for meeting the inclusion criteria. An additional search of the bibliographies of all full-text articles, selected from the electronic search, was also performed.

#### Results

From the 64 articles retrieved by the search strategy, 17 were selected for full-text evaluations of which eight were excluded after being assessed for eligibility. The manual search of the bibliographies identified three additional publications, bringing the total number of articles to 12 (Figure 1). All were *in vitro* studies published between 1994 and 2018. The basic characteristics of these studies, the strategies investigated, and the methods used to assess antimicrobial efficacy are listed in Table 2. Due to the large variation in the methods used to measure the antimicrobial effects and the variability of the data, a meta-analysis was not performed in this review. Instead, a case study qualitative investigation was attempted to identify the best biofilm management protocol for silicone maxillofacial prostheses.

### Characteristics of biofilms on silicone maxillofacial prosthesis

The formation of microbial biofilms is dependent on the physiochemical properties of the substrate and can be subdivided into four distinct stages, initiation, maturation, maintenance, and dissolution (Bazaka et al., 2012). Due to the inert nature of silicone used for maxillofacial prostheses, the initial adhesion of planktonic microorganisms is usually facilitated by the formation of a surface layer called conditioning film (Lorite et al., 2011). The conditioning film absorbs macromolecules from the surrounding environment and its composition typically includes lipids, proteins, polysaccharides and inorganic



Figure 1. Study Flowchart

Study	Country	Strategy Investigated	Efficacy Assessment
Pigno et al (1994)	United States	Incorporation of clotrimazole and nystatin	Disc diffusion
Shi et al (2008)	China	Disinfectant – recombinant human beta-defensin-3 (rHBD3)	Colony forming units (CFU) count
De Prijck et al (2010)	Belgium	Incorporation of nystatin, miconazole, tea tree oil and zinc pyrithione	Disc diffusion and CFU count
Kurtulmus et al (2010)	Switzerland	Altering polymerization duration	Candida and adherence assay
Zhou et al (2010)	China	Parylene coating	Cell count, XTT assay <sup>1</sup> , laser microscopy and scanning electron microscopy (SEM)
Cochis et al (2012)	Italy	Endophytes biosurfactants – from Robinia pseudoacacia and Nerium oleander	CFU count, XTT and MTT assay <sup>2</sup>
Garner et al (2014)	United Kingdom	Chlorhexidine nanoparticle (NP) coating	Cell proliferation assay
Ariani et al (2015)	Netherlands	Deionised water, antibacterial soap, essential oil-containing mouth rinse, 27% ethanol, chlorhexidine mouth rinse, and buttermilk	CFU count and live/dead staining
Guiotti et al (2016)	Brazil	Water and neutral soap, 4% chlorhexidine solution, Cymbopogon nardus and Hydrastis canadensis extracts	XTT assay and SEM
Khalaf et al (2017)	Malaysia	Surface coating of gypsum molds used in fabrication	CFU count and SEM
Meran et al (2017)	United Kingdom	Silver NP coating	Ethanol assay, light microscopy and SEM
Pinheiro et al	Brazil	0.12% chlorhexidine, 10% Ricinus communis solution,	CFU count

#### Table 2. Characteristics of included studies

<sup>1</sup>[2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenyl amino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay

<sup>2</sup> [3-(4,5-dimethyliazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium] (MTT) assay

salts (Lorite et al., 2011). "Conditioned" silicone surfaces exhibit changes in physiochemical properties that support microbial adhesion and aggregation (Busscher et al., 1997). Once the pioneer species have established onto the silicone surface, they begin to secrete insoluble exo-polymers that promote further microbial adhesion and form the extra-cellular matrix of the biofilm (Bryers, 2008). Mature biofilms are complex 3-dimensional microbial communities arranged to accommodate many micro-niches (Bryers, 2008). Bacterial cells within a biofilm community are far more resistant to the influence of external factors such as pH, temperature and antibiotics than planktonic cells of the same species (Costerton et al., 1999; Bryers 2008).

Any silicone maxillofacial prostheses fitted over the skin or mucosa surface will to some extent reduce ventilation, increase humidity, and compromise hygiene which facilitates rapid microbial colonisation and biofilm formation (Abu-Serriah et al., 2003). Persistent contact of the microbial biofilm with healthy mucosa or skin surface may lead to localized irritations and/or dermatitis, common among patients with limited dexterity or vision who make up the majority of maxillofacial prosthesis wearers (Ariani et al., 2012; Ariani et al., 2013). The exact composition of the biofilm on silicone maxillofacial prostheses varies between patients but studies have used conventional microbiological cultures and checkerboard DNA–DNA hybridization to confirm the presence of at least 38 species of bacteria and fungi found on both the prosthesis and the supporting tissues (Ariani et al., 2012; Pinheiro et al., 2018). Although many microbial species that colonise the silicone surfaces of maxillofacial prostheses are commensal species of humans, the biofilm challenges associated with silicone maxillofacial materials has become a recognised clinical problem with significant consequences for some patients (Goiato et al., 2011a; Ariani et al., 2012; Murakami et al., 2013). Scanning electron microscopy (SEM) analysis of the fitting surface of silicone maxillofacial prostheses revealed the ingrowth of microorganisms into silicone and its association with "bag like" deteriorations that could contribute to clinically-observed deteriorations (Ariani et al., 2012). Even after brushing with neutral soap and water which effectively removes any surface biofilm, SEM analysis shows residual micro-organisms buried within the irregularities on maxillofacial silicone (Reisberg and Habakuk, 1995; Goiato et al., 2011b; Ariani et al., 2012). Under favourable conditions, these residual microorganisms can rapidly recolonise the prosthesis surface, potentially causing recurrent infections.

## Strategies for management of biofilm on silicone maxillofacial prosthesis

Four main strategies for management of biofilm on silicone maxillofacial prosthesis were identified from the 12 articles included in this review: 1) laboratory procedures 2) incorporation of antimicrobial compounds 3) antimicrobial coatings and 4) use of disinfectants. Table 3 briefly summarises the main findings of the included studies and outlines the advantages and disadvantages of each of the four strategies. The quality of the included studies was not assessed due to the lack of randomised control trials as all studies were conducted *in vitro*.

#### Discussion

Despite recent advances in reconstructive surgical techniques, maxillofacial prosthodontics remains a fundamental aspect of the rehabilitation of maxillofacial defects. Although the silicone used for maxillofacial prostheses is an inert material, continuous exposure to nutrient-rich bodily fluids and favourable conditions created by the environment around the prosthesis are able to facilitate the development of mixed species biofilms. If patients cannot maintain meticulous hygiene of the prosthesis and surrounding tissues, they are likely to experience localised infections as well as discolouration and deterioration of the prosthesis (Abu-Serriah et al., 2001; Visser et al., 2008). This review has identified various attempts to manage microbial biofilm on silicone maxillofacial prostheses which could contribute to improving the lifespan of the prosthesis.

The most significant limitation of this review is the lack of relevant clinical studies within the literature. Although the findings of some in vitro studies seemed promising, they are based on very specific laboratory conditions that do not accurately represent in vivo environments. Without well-designed randomised control trials to confirm the efficacy of biofilm management strategies in everyday use, it is entirely possible that the complex maxillofacial environment may reduce or remove any antimicrobial effects. In the case of denture liners for example, many in vivo studies have failed to demonstrate antimicrobial effects for strategies that have proven effectiveness in vitro (Uludamar et al., 2011). Of the in vitro studies included, only one attempted to simulate a "dynamic" in vivo situation with a Modified Robbin Device (MRD) while the rest measured antimicrobial efficacy in "static" systems such as petri dishes or microtiter plates (MTP). In the MRD flow through system which mimics the constant flow of saliva in the oral cavity, antimicrobials incorporated into maxillofacial silicone demonstrated a significantly reduced antimicrobial efficacy than the "static" system. The reduced efficacy was most likely caused by the dilution or "wash away" effect of the water flow within the MRD, making the biofilm management

Strategy	Study	Results	Advantages	Disadvantages
Laboratory procedures	Kurtulmus et al (2010)	12-hours room-temperature polymerized silicone exhibited the least <i>Candida albicans</i> adherence	Does not involve antimicrobial agents Does not alter physio-chemical properties	Increases the burden on dental technicians Questionable efficacy <i>in vivo</i>
	Khalaf et al (2017)	Coating gypsum molds produced smoother, less porous silicone with reduced microbial adhesion		
Incorporation of antimicrobial compounds	Pigno et al (1994)	Incorporated clotrimazole inhibited fungal growth and indicated a degree of stability and longevity	Simple to implement Uses readily available antimicrobial agents. Does not depend on patient compliance	Questionable efficacy and longevity <i>in vivo</i> Development of resistant strains
	De Prijck et al (2010)	Incorporated antimycotics demonstrated good antifungal efficacy in static models but poor efficacy in dynamic models		
Antimicrobial coatings	Zhou et al (2010)	Parylene coating reduced <i>C. albicans</i> adhesion and aggregation	Does not alter physio-chemical properties Does not depend on patient compliance Does not induce antimicrobial resistance	Fabrication of coating is technique sensitive Questionable durability <i>in vivo</i> Development of resistant strains High cost of production
	Cochis et al (2012)	Endophytes biosurfactants from <i>R. pseudoacacia</i> and <i>N. oleander</i> caused a greater reduction in biofilm cell number and viability than chlorhexidine		
	Garner et al (2014)	Chlorhexidine NP coating inhibited <i>C. albicans</i> and demonstrated a controlled release profile		
	Meran et al (2017)	Silver NP coating inhibited <i>C. albicans</i> without appreciable adverse effects on human dermal fibroblast cells		
Use of disinfectants	Shi et al (2008)	rHBD3 exhibited antimicrobial activity against both <i>C. albicans</i> and <i>S. aureus</i> biofilms	Simple to implement Effectively eliminates microorganisms Mostly affordable and readily available for patients	May cause premature discolourations and deterioration of prosthesis Relies on patient compliance
	Ariani et al (2015)	Chlorhexidine solution demonstrated the highest reduction in CFUs under all conditions investigated		
	Guiotti et al (2016)	Water + neutral soap was the most effective against <i>C. albicans</i> and <i>Staphylococcus</i> aureus biofilms		
	Pinheiro et al (2018)	Immersion in chlorhexidine solution was most effective protocol in all conditions investigated		

#### Table 3. Strategies of Biofilm Management

strategy unreliable *in vivo* (De Prijck et al., 2010). Within the small volume of an MTP well however, higher relative concentrations of the antimicrobial could be achieved, which in turn kills planktonic cells and inhibits biofilm formation on the silicone specimens.

Another significant limitation of the in vitro studies included in this review was the use of planktonic cells or single-species biofilms, and specially-prepared silicone specimens to evaluate the antimicrobial efficacy of biofilm management strategies. Without the support of other microbial species within a biofilm community, planktonic cells or single-species biofilms demonstrate reduced microbial adhesion and antimicrobial agents can penetrate into the biofilm more readily to exert their effect (Cannon and Chaffin, 1999). Standardised silicone specimens with smooth surface topography also act to reduce microbial adhesion and their use does not simulate clinical conditions where silicone surfaces deteriorate to form surface irregularities (Ariani et al., 2012). The effect of these limitations on the results of in vitro studies cannot be accurately determined but it is entirely possible that they amplified the efficacy of the biofilm management strategies investigated, making their results unreliable in in vivo conditions.

From the results of the studies included in this review, it seems that microbial adhesion to maxillofacial silicones can be modified by controlling the processing temperature and duration of polymerisation (Kurtulmus et al., 2010). Coating the inner surface of plaster moulds with clear acrylic prior to packing the silicone also helps to reduce the surface irregularities on the prosthesis produced, which in turn reduces microbial adhesion (Khalaf et al., 2017). Although the efficacy of these strategies has not been verified in vivo, the concept does have a sound scientific background as the relationship between surface roughness and bacterial adhesion has been well-demonstrated in materials such as polymethyl methacrylate (Dantas et al., 2016). It is therefore logical to adopt laboratory procedures that minimise the surface roughness of silicone maxillofacial prostheses in order to reduce microbial adhesion and subsequent biofilm formation.

The incorporation of antimicrobial compounds into silicone elastomers to enhance biofilm resistance is not a recent invention and has already gained popularity in other areas of prosthodontics such as denture liners/ conditioners due to the advantage of not requiring patient compliance. Nystatin, miconazole, tea tree oil and zinc pyrithione have been incorporated into maxillofacial silicone and their antimicrobial efficacy measured under both "static" and "dynamic" in vitro conditions (Pigno et al., 1994; De Prijck et al., 2010). Although the results of included studies suggest the antimicrobial effect demonstrated may be insignificant in vivo, it has been possible to relate the antimicrobial effect to the total free fractions available for release and the subsequent concentrations of antimicrobials produced in the surrounding environment. Using current techniques of admixture or solvent-based impregnation, the only way to increase the free fractions available for release would be to increase the total dose of antimicrobials incorporated

into silicone. However, the dose cannot be increased indefinitely without altering the physiochemical properties of maxillofacial silicone. The risks of developing antimicrobial resistance is another concern that would limit the potential applications of this strategy to only patients at high risk of prosthesis-related infections such as those who are immunocompromised.

Regarding the use of disinfectants, several protocols have achieved positive results in terms of biofilm reduction. Immersion in chlorhexidine solution was the most effective protocol and is already widely used in all aspects of dentistry. Other novel plant-based disinfectants (Cymbopogon nardus and Hydrastis canadensis extracts) and essential oils were also investigated but they were consistently inferior than conventional methods such as chlorhexidine or brushing with water and neutral soap. An exception was the recombinant human β-defensin-3 peptide (rHBD3) which produced similar inhibitions of S. aureus and C. albicans as the sodium hypochlorite control after 30 minutes of continuous immersion. Despite the success of the disinfectants, none of the studies compared various concentrations of disinfectants and there is no evidence to justify the concentrations to use on silicone maxillofacial prostheses. Finding the ideal concentrations to provide sufficient disinfection with minimal risks could be an interesting point for further research as some disinfectants such as chlorhexidine and sodium hypochlorite are recognised causes of premature discolouration or deterioration of silicone maxillofacial prostheses (Goiato et al., 2011b). For the purpose of this review, mechanical cleaning with neutral soap and water was included in the category of disinfectants. Although its efficacy is well recognised, it has always been a controversial strategy as repeated brushing has been associated with prosthesis damage through abrasion and dissolution of pigments (Ariani et al., 2015). In some clinical studies investigating the prevention and treatment of denture stomatitis, mechanical disruption of the biofilm was reported to be more important that the use of antimicrobial agents, but these findings are unable to be generalised to silicone maxillofacial prostheses (Skupien et al., 2013).

Antimicrobial coatings for maxillofacial silicone are more recent strategies that incorporate both new technologies and existing materials. Of the four studies in this category, two explored the deposition of nanoparticles (1-100 nm diameter) on maxillofacial silicone specimens to enhance biofilm resistance (Garner et al., 2015; Meran et al., 2018). Chlorhexidine and silver nanoparticle coatings did not appreciatively alter the physio-chemical properties of the silicone and exhibited sustained antifungal action even when challenged by very high doses of C. albicans unseen in clinical situations (Garner et al., 2014; Meran et al., 2017). The other studies investigated the inhibition of microbial adhesion through the application of Parylene and biosurfactant coatings. Parylene (poly para-xylene) is a polymer used extensively in biomedical devices due to its biocompatibility, chemical inertness and thermal stability (Bourlidi et al., 2016). Biosurfactants on the other hand, are a structurally

diverse group of surface-active substances produced by various microorganisms. Both coatings successfully inhibited adherence of C. albicans but biosurfactants also exhibited additional anti-Candida activity that was higher than chlorhexidine solutions of the same concentration (Zhou et al., 2010; Cochis et al., 2012). Although these studies produced promising results, none reported the deterioration of the coatings. One must question the durability of antimicrobial coatings in vivo as they must resist the damaging effects of both mechanical and chemical hygiene protocols while maintaining the physio-chemical properties of the maxillofacial silicone. Like the incorporation of antimicrobial compounds, the longevity of the antimicrobial effects and the clinical relevance of these effects also remains unknown. Even if these coatings are effective, the high cost of production associated with nanoparticle and biosurfactant coatings would also be a critical limitation that prevents the widespread use of these strategies.

#### Current recommendations for patients

Research findings suggested that patients should remove their maxillofacial prostheses during the hours of sleep to relieve the underlying soft tissues and reduce moisture accumulation (Goiato et al., 2010). Hygiene procedures should be performed at least once a day before the patient goes to bed. A soft-bristle toothbrush could be used to gently brush all prosthesis surfaces and any retentive elements with warm water and neutral soap (Reisberg and Habakuk 1995). Immersion in disinfectants such as chlorhexidine could be an effective auxiliary method (once a week) to reduce risks of premature discolouration of the prosthesis (Goiato et al., 2010). Care must be taken to clean the adjacent soft tissues and with an implant-retained prosthesis, the most critical area to clean is around the abutments within the crevice (Allen et al., 2000). After cleaning, the prosthesis should be thoroughly rinsed in running water, dried and stored in a container away from light and heat (Goiato et al., 2010).

#### Conclusions

Within the limitations of this review, the current literature does not allow for any recommendation beyond the conventional strategies to overcome the clinical problems associated with biofilm formation on silicone maxillofacial prostheses. However, novel strategies of biofilm management are being studied and could potentially extend the lifespan of the prostheses. In future research, promising strategies should be studied further to determine their toxicity towards human fibroblasts and gingival epithelial cells. Their efficacy should also be examined in vivo and meta-analyses could be performed to compare them with conventional strategies. To strategically manage the biofilm formation associated with silicone maxillofacial prostheses and provide prostheses with improved lifespans, clinicians and laboratory-based researchers could collaborate closely in the development and validation of innovative materials and methods.

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#### Conflict of interest

The authors report no known conflicts of interest.

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