

**Bacteriophage Isolation from Denture Biofilm***Isolamento de bacteriófagos a partir do biofilme de dentaduras.**Aislamiento de bacteriófagos del biopelícula de dentaduras postiza.*

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ABSTRACT

Objective: this research evaluated if it is possible to isolate bacteriophages from dentures biofilm, and use it as an emergent way to control biofilm growing. **Method:** biofilm samples were collected from sixteen maxillary complete dentures in an aseptic zone by brushing their internal surfaces using a soft toothbrush. The biofilm suspensions obtained were added to standard strains (*Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*) and the presence of lysis was evaluated. **Results:** bacteriophages were not isolated from the denture samples. **Conclusion:** this study presented an opportunity to invest in a new method to detect bacteriophages from mature biofilms of complete dentures.

Descriptors: Denture, biofilm, bacteriophage.

RESUMO

Objetivo: este estudo avaliou se é possível isolar bacteriófagos a partir do biofilme de dentaduras e utilizá-los como uma ferramenta emergente no controle de biofilmes. **Método:** as amostras de biofilme foram coletadas de dezesseis próteses totais maxilares, em zona asséptica, escovando as superfícies internas de cada prótese com auxílio de uma escova de dente macia. As suspensões obtidas foram cultivadas junto a cepas padrão (*Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli* e *Pseudomonas aeruginosa*) e a presença de áreas de lise foi avaliada. **Resultados:** nenhum bacteriófago foi isolado das amostras de biofilme das dentaduras. **Conclusão:** este estudo apresentou a oportunidade de investir em novos métodos para detectar bacteriófagos em biofilmes de dentaduras.

Descritores: Denture, biofilm, bacteriophage.

RESUMÉN

Objetivo: este estudio evaluó si es posible aislar bacteriófagos a partir del biopelícula de dentaduras y utilizarlos como una herramienta emergente en el control de biopelículas. **Método:** las muestras de biofilm fueron recogidas de dieciséis prótesis totales maxilares, en zona aséptica, cepillando las superficies internas de cada prótesis con ayuda de un cepillo de dientes suave. Las suspensiones obtenidas fueron cultivadas junto a cepas estándar (*Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli* y *Pseudomonas aeruginosa*) y la presencia de áreas de lisis fue evaluada. **Resultados:** ningún bacteriófago fue aislado de las muestras de biofilm de las dentaduras. **Conclusión:** este estudio presentó la oportunidad de invertir en nuevos métodos para detectar bacteriófagos en biopelículas maduras de dentaduras.

Descriptores: Dentadura postiza, Biopelícula, bacteriófago.

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INTRODUCTION

The science of biofilms has been a field of intense discussion in the scientific research since these communities were described. Despite being common, biofilm formation is a complex process involving several steps. Firstly, it is necessary the surface conditioning by macromolecules originating from surrounding fluid resulting in microbial adhesion. Microorganisms have an initial adhesion and begin to produce signaling molecules, developing and producing a matrix of extracellular polymeric substances (EPS)¹.

The biofilm control has driven several studies in different areas of knowledge. There are physical-chemical methods for its removal², antimicrobial photodynamic therapy³, induction of biofilm detachment⁴, degradation of EPS matrix⁵ and quorum sensing inhibitors⁶. New investigations with the purpose of preventing biofilm formation had been reported through the development of non-adherent surfaces^{7, 8, 9} and specific bacterial inhibitors¹⁰.

Bacteriophages are viruses capable of limiting the bacterial overgrowth, without altering the local biological characteristics¹¹. The potential applicability of bacteriophages to control biofilm growth has already related¹². Over 20 years ago, clinical condition improvements of patients after using phage therapy in suppurative skin¹³ and urinary infections¹⁴ were reported. The therapeutic usage of bacteriophages in some European countries, such as Poland, is very usual and traditional, such as the Phage Therapy Unit, which develops research on phagotherapy¹⁵. Although the scientific literature is abundant in bacteriophage application to control infections and biofilms^{16, 17, 18}, gaps still exist and should be investigated and understood.

In view of the pathogenic potential of the denture biofilm and its implication in the health and safety of patients, strategies involving the bacteriophages usage are promising. In this way, the objective of this study was to standardize the

isolation of bacteriophages from mature biofilms of complete dentures.

MATERIALS AND METHODS

Firstly, this study was submitted for appreciation of the Institutional Review Board School of Dentistry of Ribeirão Preto (FORP/USP) - (Brazilian Ethics System register number, CAEE 14707413.4.0000.5419). It was started only after the signed informed consent form was submitted and agreed by the participants. The patients who were visiting the Complete Denture Clinic of FORP/USP were evaluated for participation. Complete edentulism patients wearing maxillary complete dentures produced with heatpolymerized acrylic resin and acrylic artificial teeth, both genders and any age were eligible. Exclusion criteria were dentures that had been used for less than 01 year, as well as the relined, repaired, or fractured ones. About the biofilm presence, it was considered dentures that presented scores equal to or greater than 01 of biofilm visible in five predetermined areas according to the Additive Index¹⁹.

Standard bacterial strains: *Escherichia coli* (ATCC 25922) - Ec, *Enterococcus faecalis* (ATCC 29212) - Ef, *Streptococcus mutans* (ATCC 25175) - Sm, *Pseudomonas aeruginosa* (ATCC 27853) - Pa and *Staphylococcus aureus* (ATCC 25923) - Sa were cultured in *Tryptic Soy Broth* (TSB) or *Brain Heart Infusion Broth* (BHI) - (HiMedia Laboratories Pvt. Ltda., Mumbai, India) at 37°C for 24 hours. The early exponential growth phase culture was adjusted at 625nm by using a spectrophotometer (Multiskan GO, Thermo Scientific Multiskan® Spectrum, MA, USA), giving a bacterial suspension of 10⁸CFU mL⁻¹ (absorbance ranging from 0.080 to 0.100).

To collect the biofilm, each denture was placed inside a Petri plate in a class II type A1 biological safety cabinet (Pachane, Pa 400-ECO, Piracicaba, SP, Brazil). The dentures were rinsed

with 10 mL phosphate buffered saline (PBS), and their internal surfaces were brushed for 2 minutes using a soft toothbrush (Johnson & Johnson, São José dos Campos, SP, Brazil). The biofilm suspension obtained was collected and vortexed (Phoenix®, AP56 model, Araraquara, SP, Brazil) for 2 minutes. Thereby, the suspension was centrifuged (5430R, Eppendorf, Hamburg, Germany) at 5000g at 4°C and 100 µL of the supernatant were added to 100 µL of bacterial inocula (*Sa*, *Sm*, *Ef*, *Ec*, and *Pa*). The mixture was thoroughly homogenized and incubated at 37°C for 20 minutes. After the incubation period, 4 mL of preheated (45°C) BHI (0.7% agar) were added to samples and the mixture (medium + samples + bacterial inocula) were seed on Petri plates (60x15mm) - (TPP; Trasadingen, Switzerland) with a base layer of BHI (1.5% agar). The plates were incubated at 37°C for 7 days and the presence of lysis zones were evaluated.

RESULTS

Biofilm samples were collected from sixteen maxillary complete dentures. The denture wearers were 4 men and 12 women with age ranging from 55 to 75 years (age mean: 64.5 years old).

All the plates showed abundant bacteria growth after incubating. However, the samples of mature biofilms from complete dentures did not demonstrate any presence of bacteriophages.

DISCUSSION

The lack of phages described from denture mature biofilms encouraged us to try a method to isolate and characterize it, considering the hypothesis that bacteriophages can be found wherever their hosts are present.

Since there are about 700 bacterial species inhabiting the oral cavity, and many of them are able to form biofilms^{20, 21}, the presence of bacteriophages in denture biofilms is a productive

field for scientific research. The scientific literature has been reporting that these viruses are present in saliva and oral biofilm²². Other authors have shown that endogenous phages can have an important role in human oral health, as well as in other ecosystems, limiting the bacterial overgrowth without changing local biological characteristics²³. However, studies involving isolation and characterization of bacteriophage from dental prostheses have not been reported yet.

Although the presence of phages in the oral cavity has already been reported²⁴, this study was not able to show it in denture biofilms. This result can be explained by the fact that acrylic dentures are mainly colonized by *Candida* spp. Besides, future researches are needed to enhance the bacteriophages detection in denture mature biofilm that had already been colonized with bacteria.

Even if the isolation of phage from the oral cavity has been reported for a long time, the literature does not present a solid method in order to guide a research in a laboratory. Szafránski and cols²⁵ reviewed 94 articles about isolation and application of phage therapy against oral cavity bacteria and showed that few studies were carried out. In view of this, only a small fraction of the available phages was isolated and characterized. Further studies are required to explore the phage activity, focusing on multi-species biofilms, application in animal models and possibly combining with other antimicrobial agents. The use of phages as oral biofilm controller should be evaluated, aiming at the development of affordable drugs or therapeutic products available for oral health care.

CONCLUSION

This study presented an opportunity to invest in a new method to detect bacteriophages from mature biofilms of complete dentures.

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COLABORAÇÕES

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CONFLITOS DE INTERESSE

Os autores não manifestam conflitos de interesses em esfera política, acadêmica, comercial, pessoal e financeira.

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