Supporting Information

Rapid coating process generates omniphobic dentures in minutes to reduce *C. albicans* biofouling

Anna Waterhouse, PhD[1#], Daniel C Leslie, PhD[1], Kayla Lightbown[1], Daniel Antonoff[1], Shanda Lightbown[1], Nikolaos Dimitrakakis[1], Julia B Hicks-Berthet[1], Cheyene N Leslie[1], Michael Super, PhD[1], Donald E Ingber, MD[1,2,3,a] and Marc B Ackerman, DMD, MBA[4,5,a]

[1]Wyss Institute for Biologically Inspired Engineering at Harvard University, 3 Blackfan Circle, Boston, Massachusetts, USA.
[2]Vascular Biology Program, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts, USA.
[3]Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, MA
[4]Department of Dentistry, Boston Children's Hospital, Boston, Massachusetts, USA.
[5]Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts, USA.
[#]Current Address: Charles Perkins Centre, Central Clinical School, Faculty of Medicine and Health, University of Sydney, Australia, and Heart Research Institute, Newtown, NSW, Australia.
[a]Authors contributed equally

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MATERIALS AND METHODS

Sample Production

Molded polymethylmethacrylate (PMMA) resin chips were made using Caulk Orthodontic Resin powder added to the well of a 10-mm glass bottom dish (MatTek Corporation, Ashland,

MA). Caulk Orthodontic Resin Pink Liquid (3 drops) was added to the powder in the dish until the powder was fully saturated, and a glass cover slip was added to the top of the well. The samples were cured at 37 degrees C for 5 minutes and removed from the glass bottom dish.

Recently fabricated unused PMMA dentures were obtained from a local dental laboratory and cut into to 1 cm x 1 cm x 0.25 cm pieces using a hot wire cutter (Proxxon, Föhren, Germany), in order to test the application and function of TLP on the specific morphology of dentures.

Modification of Surfaces with TLP

Surfaces were coated with the tethered liquid perfluorocarbon (TLP) as described in Leslie *et al*[1]. with the following modifications. Samples were etched with 40% phosphoric acid gel (Henry Schein Inc., Melville, NY) for 20 seconds each side, instead of the plasma treatment used by Leslie et al. Resin chips were then irrigated with water and rinsed twice with 100% ethanol (Koptec, King of Prussia, PA). Samples were submerged in a silane solution that consisted of a 50%:50% ratio of 5% v/v tridecafluoro-1, 1, 2, 2-tetrahydroocytyl trichlorosilane (Gelest, Morrisville, PA) in anhydrous ethanol (Sigma, St. Louis, MO) and silane bond enhancer (Pulpdent Corporation, Watertown, MA, containing 5-15% methacryloxy propyl trimethoxy silane, 92% isopropyl alcohol and <1% acetic acid) for 1 minute, unless otherwise specified in the results and figure legends, where varying ratios of the two silane solutions were used. Samples were immediately rinsed with anhydrous ethanol (Sigma, St. Louis, MO), sterile water, and three volumes of 100% ethanol (Koptec, King of Prussia, PA), and then air dried.

A liquid perfluorocarbon (LP) layer was added by dipping the samples in wells of perfluorodecalin (PFD) (FluoroMed, APF-140HP (sterile, high purity), Round Rock, TX) for 30 seconds.

Sliding Angle

The angle at which a droplet of liquid begins to move across a surface (sliding angle) was measured for the samples using a manual goniometer (Thor Labs GN05/M). Samples were dipped in PFD immediately before measurement and then placed on top of the leveled goniometer. A 15-µL drop of 1 mM blue erioglaucine dye (Sigma, St. Louis, MO) was placed onto the surface of the sample and the goniometer was tilted until the drop of dye was observed sliding across the surface.

Scanning Electron Microscopy

Samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences, Hatfield, PA) for 1 hour, 1% osmium tetroxide in 0.1 M sodium cacodylate (Electron Microscopy Sciences, Hatfield, PA) for 1 hour, dehydrated in ascending grades of ethanol, and chemically dried with hexamethyldisilazane (Electron Microscopy Sciences, Hatfield, PA) in a desiccator overnight before being mounted and sputter-coated with a thin layer of gold/palladium and imaged on a Zeiss Supra55VP microscope with an accelerating voltage range of 3-5kV.

Fungal Quantification Assay

Candida albicans (*C. albicans*) was grown with sterile resin chip or denture samples in sterile 50-ml glass tubes. A colony of *C. albicans* was picked and grown in 5 ml potato dextrose broth (Teknova Inc., Hollister, CA) for 3 hours at 37 degrees C, shaking at 225 rpm. The culture was then diluted 1:100 in fresh potato dextrose broth and the samples were added to the 50 ml glass tubes. The pathogen was grown with the samples for 3 hours with shaking, and the media was aspirated and replaced with fresh potato dextrose broth. The media was changed in the same way every 24 hours for 6 days.

The Bactiter-Glo Microbial Cell Viability Assay (Promega Corporation, Madison, Wisconsin) was used to quantify the number of viable fungal cells. The protocol was followed as

stated in the Bactiter-Glo Microbial Viability Assay Kit with slight modification. The assay was modified to measure the quantity of viable bacteria on the chip samples. The Bactiter reagent was added to a well plate, enough to cover the chip, and was then incubated at room temperature in the dark for 5 minutes. The incubated Bactiter reagent (200 µL) was then transfered to an opaque 96-well plate and the luminescence was measured using a Synergy H1 Hybrid microplate reader (BioTek Instruments Inc., Winooski, VT). Luminescence values were then converted to concentrations using a standard curve of *C. albicans* grown in solution to an optical density of 0.8. The colony forming units (CFU) were quantified by spiral plating (Eddy Jet 2, IUL, Barcelona, Spain) and automated counting (Flash & Go, IUL, Barcelona, Spain).

Statistical Analysis

Data analysis in Figure 2 were performed using Prism (GraphPad software) one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. Data analysis in Figure 4 were performed using the statistical *R* language. Statistically significant differences between the two groups of surfaces (control group versus TLP coated group) were identified using Non-Parametric Mann-Whitney-U-Test. A *p*-value lower than 0.05 was considered statistically significant. Experiments were carried out with triplicate samples per condition for TLP function and fungal growth quantified by Bactiter-Glo. Single samples were used for scanning electron microscopy experiments which were performed at the same time as the fungal growth experiments. All experiments were carried out 2-3 times.

References

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