

Introduction

In nature, many organisms accommodate a complex living environment by developing attributes that enable both reversible retention and anti-fouling properties (1, 2). For example, tree frogs adhere to wet surfaces due to the hexagonal micro-and nanotopographies that represent their attachment mechanism (Figure 1).

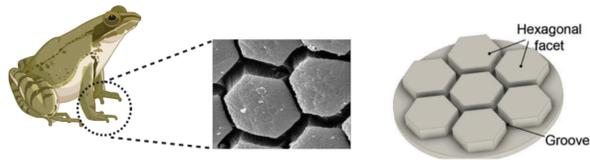


Figure 1. Hexagonal topographies – attachment system of a tree frog (1, 2).

The idea of replicating and producing naturally-occurring attributes is part of the field that is known as biomimetics. Biologically-inspired designs have found their purpose in the creation of super hydrophobic materials and wall-climbing robots. Applying the biomimetic principles and designs in prosthodontics, herein, we present the creative idea to overcome the shortcomings of complete dentures.

Background

- Edentulism represents a significant global public health burden. The number of patients requiring complete dentures is high and tends to increase despite improved measures for tooth preservation (3).
- According to the World Health Organisation, the prevalence of edentulism in the UK is high, with more than 46% of individuals, 65 years and older, being associated with the disability (4). Demographic growth might decrease the number of edentulous individuals, but the need for complete dentures persists (3).
- The problem of complete dentures remains their poor retention, as well as the attraction of common oral pathogens, predominately *Candida albicans* (5).
- Dental implants have offered the solution for complete denture shortcomings, and the clinical success of implant therapy is well-documented (6). Nevertheless, inadequate bone support, bisphosphonate therapy, uncontrolled medical conditions, and lack of financial support exclude implant therapy as an option. Therefore, complete dentures are still the therapy of choice (6).

Aim

Mimicking hexagonal topographies of a tree frog, both morphologically and dimensionally, we aim to solve a retention problem in complete dentures and reduce the formation of microbial biofilm.

Objectives and standards

Towards validating our aim, we fabricated acrylic specimens with various dimensions of hexagonal topographies (Figure 2).

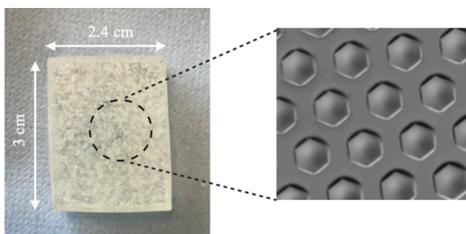


Figure 2. Acrylic specimen with hexagonal topographies (Digital microscope E500:X2000).

First, we examined the hydrophobicity of the acrylic surface with topographies. Second, we performed an adhesion test with acrylic specimens and artificial saliva. And finally, we quantified the formation of *Candida albicans* biofilm on acrylic discs with topographies.

Bioinspired topographies on a fitting surface of dentures should increase their retention, and create a hydrophobic surface that reduces the formation of *Candida albicans* biofilm.

- Side length (a) μm
- Edge to edge spacing (b) μm
- a/b

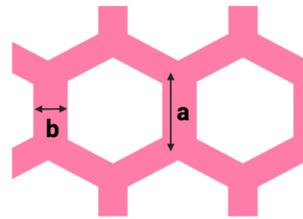


Figure 3. Parameters used to define hexagonal topographies on acrylic specimens.

Methodology

- Contact angle estimation was used to determine the hydrophobicity of acrylic specimens that contain topographies. A flat acrylic surface was a negative control.
- The adhesion test was performed with the Instron universal testing machine and tensile bond strength (TBS) was calculated.
- *Candida albicans* biofilm formation on acrylic discs was quantified by using crystal violet and XTT assays.

Results

- All specimens with topographies demonstrated a hydrophobic surface.
- Water droplet demonstrated a larger contact angle on a surface with topographies than on a flat surface, at the moment of placement (0min), and 30 minutes later (Figure 4).

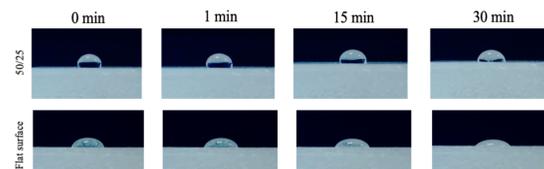


Figure 4. Contact angle change over 30 minutes.

- Water droplet had the largest contact angle value on specimen 50/25, $\theta=104.9^\circ (\pm\text{SD } 9.198)$.
- Contact angle of a droplet on a flat surface was $\theta=68.96^\circ$ (Table 1).

Specimen	0 minute	1 minute	15 minutes	30 minutes
25/2	79.06	74.9	66.56	56.53
25/5	98.66	93.06	79.83	69.76
25/10	97.73	91.93	79.1	71.16
25/25	97.06	95.23	83.12	70.9
50/2	82.73	78.7	73.7	65.06
50/5	89.9	81.86	76.8	67.13
50/10	90.56	83.1	76.23	66.93
50/25	104.9	102.93	93.8	81.53
Flat control	68.96	68.03	70.23	58.46

Table 1. Average contact angle values.

- Specimens with topographies did not exhibit larger TBS in comparison to the negative (flat) control.
- More *Candida albicans* biofilm was on the acrylic disc with topographies than on the negative control (Figure 5 A and B).

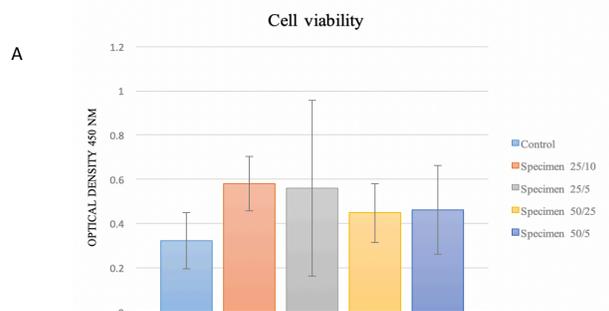


Figure 5A. Optical density readings for XTT assay. Bars represent standard deviation.

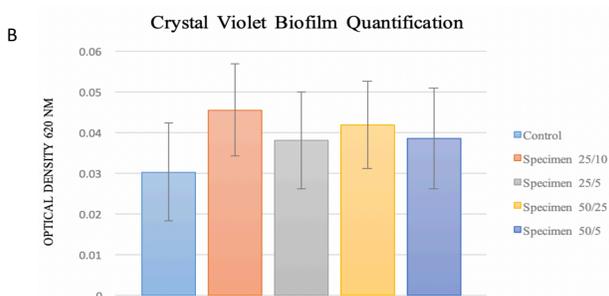


Figure 5B. Optical density readings for crystal violet assay. Bars represent standard deviation.

Discussion

- Patterned surface maintains the water droplet sitting on the apex of topographies due to the entrapped air in the grooves (Figure 6). This generates a hydrophobic surface.

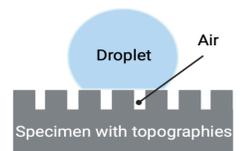


Figure 6. Droplet on a specimen with topographies.

- Water droplet had a larger contact angle when the micro-grooves were bigger, $b = 5 \mu\text{m}$, $10 \mu\text{m}$, or $25 \mu\text{m}$.
- When the distance between patterns approaches a zero size ($b=2 \mu\text{m}$), the specimen starts to demonstrate the characteristics of a flat surface (negative control) that is hydrophilic. On a hydrophilic surface, the water droplet has a smaller contact angle.

- Specimens with topographies did not demonstrate larger TBS in comparison to the flat surface. When two flat surfaces, which are hydrophilic, come into contact, the drop between them forms a meniscus (Figure 7A). The formation of a meniscus generates an intrinsic adhesion force and increases the TBS.

- While testing adhesion between flat surface and surface with topographies, the meniscus failed to form. It is because the flat surface attracts more saliva than a surface with topographies due to its hydrophilicity. Thus, saliva takes a shape of a parabola (Figure 7B), with a tip facing towards patterns and the detachment occurs. The wide base remains in contact with the flat surface.

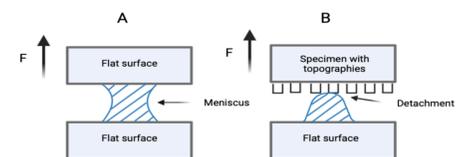


Figure 7. Formation of the meniscus (A) and detachment (B).

- The size of *Candida albicans* cells that were used in our project was $2 \mu\text{m}$. The distance between hexagons was larger (5, 10, 25 μm). This might explain why our topographies contained more *Candida* biofilm despite surface hydrophobicity.

Recommendations and actions

- From the current perspective, we could state three limitations with existing experimental strategies: a denture base material, dimensions, and designs of topographies.
- Softer and flexible materials could improve retention due to better adaptability to a variety of surfaces.
- Changing the dimensions of topographies could result in lower adhesion of microorganisms and increased tensile bond strength.
- Other designs, i.e. octopi suction cups, could hold promise for better retention as they would create a void between the material and substrate.

Conclusion

This research could be used as an optimization and a source of inspiration for future work. If we overcome existing limitations, we will get clinically relevant results, comparable to the other experiments in the field of biomimetics.

Biologically inspired complete dentures would not only solve the problem of retention in dentistry but also might be financially sustainable due to the lessened applications of widely used dental adhesives.

References

1. Bhushan B. Biomimetics: lessons from nature - an overview. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. 2009;367(1893): 1445-1486.
2. Rao P, Sun TL, Chen L, Takahashi R, Shinohara G, Guo H, King DR, Kurakawa T, Gong JP. Tough Hydrogels with Fast, Strong, and Reversible Underwater Adhesion Based on a Multiscale Design. *Advanced Materials*. 2018;30(32): 1-8.
3. Tyrovolas S, Koyanagi A, Panagiotakos DB, Haro JM, Kassebaum NJ, Chrepa V, Kotsakis GA. Population prevalence of edentulism and its association with depression and self-rated health. *Scientific Reports*. 2016;6(1): 1-9.
4. Petersen PE, Yamamoto T. Improving the oral health of older people: the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol*. 2005;33:81-92.
5. Al-Fouzan AF, Al-Mejrad IA, Albarrag AM. Adherence of *Candida* to complete denture surfaces in vitro: A comparison of conventional and CAD/CAM complete dentures. *The Journal of Advanced Prosthodontics*. 2017;9(5): 402-408.
6. Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. A long-term follow-up study of Osseointegrated Implants in the Treatment of Totally Edentulous Jaws. *The International Journal of Oral & Maxillofacial Implants*. 1990;5(4): 347-359.